A history of embryonic stem cell research: Concepts, laboratory work, and contexts

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A HISTORY OF EMBRYONIC STEM CELL RESEARCH: CONCEPTS, LABORATORY WORK, AND CONTEXTS

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AFHEA

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Declarations

I declare that the research for this thesis was carried out by myself (Cheryl Lancaster) under the supervision of my primary supervisor (Andreas-Holger Maehle) in the Department of Philosophy, Durham University. This thesis has been composed by myself and is a record of work that has not been previously submitted for a higher degree.

Dr Cheryl Lancaster

I certify that the work reported in this thesis has been carried out by Cheryl Lancaster, who, during the period of study, has fulfilled the conditions of the Ordinance and Regulations governing the Degree of Doctor of Philosophy.

Prof A-H Maehle

Abstract

This thesis focuses on the history of embryonic stem cell research, spanning in particular the nineteenth and twentieth centuries. As yet, there has not been a comprehensive history of embryonic stem cell research carried out, which is a particular aim of this thesis. The first two chapters consider the conceptualisation of the stem cell, and the development and diversity of relevant disciplines and their establishment in the twentieth century; in particular, this covers heredity, genetics, embryology and development. This is illustrated through the use of experimental embryology, or 'fantastical experiments', that were proposed in the nineteenth century, and carried out in the twentieth. The third chapter considers the theoretical and practical links between cancer and embryonic cells. The fourth and fifth chapters explore the isolation and culture of murine and human embryonic stem cells, focusing on the social, political, and economic factors affecting stem cell research, and the motivations behind the isolation of embryonic stem cells in the 1980s and 1990s. The sixth chapter queries whether the history presented suggests that a new stem cell concept is emerging.

There are three questions that this thesis aims to answer. Firstly, what are the (historical) social and political influences that affect (embryonic) stem cell research? Evidence presented suggests that this has occurred from the nineteenth century, and continues today. Secondly, this thesis queries the importance of cell fate, and cell fate studies, to embryonic stem cell research. Since one of the two abilities of stem cells is the ability to differentiate, cell fate and studies of cell fate are central to developing a stem cell concept, and may also be influential in changing that concept in the future. Lastly, this thesis asks which paradigms have affected embryonic stem cell research throughout its history. In particular, the genetic paradigm is shown to be influential from the early twentieth century onwards. More recently, it has been proposed that stem cell research needs to undergo a paradigm shift, from the stem cell entity view, to the stem cell state view. This is also explored through the thesis, with the aim of generating a better understanding of stem cells for future researchers.

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In particular, I want to thank my primary supervisor Prof Holger Maehle for his enthusiasm and guidance from our very first meeting (just as I was coming to the end of my first Durham degree!). At times, getting a biomedical scientist to think like a historian must have been rather trying, but I'm sure you've succeeded!

Lastly, I would very much like to thank my examiners, Prof Greg Radick, and Dr Staffan Müller-Wille, for their extremely useful suggestions for improvements to this work; this has been invaluable in significantly improving this thesis, and to help me in highlighting the key arguments I wanted to make.

Completeing this second PhD has been a fantastic journey, and one that I hope will lead me to many more.

C Lancaster
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Contents

List of Figures

INTRODUCTION

1. General introduction

As yet, there has not been a comprehensive history of embryonic stem cell (ESC) research carried out, with studies into the history and philosophy of stem cell research being distinct (with only little integration), and covering finite sections of stem cell research. A particular aim of this thesis is to generate a more comprehensive overview and discussion of ESC research. Histories of other types of stem cell have been produced, in particular haematopoietic stem cell research (discussed below). Other studies that have considered ESC research have focused on ethics or legislation, particularly as this varies greatly from country to country.

In order to provide a more extensive overview of ESC research, this thesis aims to further analyse several facets of ESC research that are generally known (such as the isolation of murine ESCs in the early 1980s, and human ESCs in the late 1990s), but from a previously under-considered aspect, or in an effort to examine some of the wider questions currently being asked in the history and philosophy of biology more generally. The thesis begins with an exploration of the conceptualisation of the cell, and especially the ESC. Chapter 2 also begins in the nineteenth century, and explores the relationship between heredity and embryology, genetics and ESC research, up to the twenty-first century. The third chapter focuses on a comparison of cancer and embryonic development from the nineteenth to the twentieth century; the recognised parallels are still discussed at length in current biological science. An immediate development of the mid-twentieth-century experiments which showed comparisons between cancer and stem cell biology was the work of Gail Martin and Martin Evans, who utilised their experiences with embryonal carcinoma cells (ECCs) to isolate and culture the first ESCs from mice in the 1980s; the fourth chapter therefore considers how political, social, and economic factors affected scientific research in the UK and USA in the 1970s and 1980s. The fifth chapter takes a different approach, exploring the reason for the seventeen-year delay between the isolation of mouse and human ESCs. Lastly, Chapter 6 integrates history and philosophy to consider whether a new stem cell concept is emerging in the twenty-first century.

Through this thesis, the aim is to provide some answers for three research questions in particular. Firstly, what is the role of social and political context in stem

cell research? As already highlighted, society can affect science, and science can affect society[1]; the one aim of this thesis is to demonstrate that there is indeed a 'two-way street' for a science (i.e. stem cell research), with science influencing society, whilst society also influences science. Stem cell research may be a particularly interesting way of studying this phenomenon, since it is so intimately linked with notions of individual and state development.

The second question asks what the importance or significance of cell fate research is. This, arguably, is linked to the first question, and its interest in social, political, and individual development. It has been argued previously that actual research has been driven by a greater need for understanding of cell fate, particularly at the beginning of the twentieth century[2]; this thesis will ask whether this hundred-year old approach is still appropriate for stem cell research today.

Moreover, this ties-in with the third research question: what is the role of paradigms in research? This thesis demonstrates that there are clear links between, for example, stem cell research, embryology, evolution, and so on. There was a key phase of development for both heredity and embryology research in the late nineteenth and early twentieth centuries, then genetics and stem cell research from the mid-twentieth century onwards, which appears to have deeply linked these areas of research. It is argued through this thesis that stem cell research carried out from the mid-twentieth century onwards was carried out under the significant influence of a genetics paradigm. Also explored in this thesis is the demonstration that stem cell research up until the twenty-first century has been interpreted on the assumption that stem cells are entities. Arguably then, experimental design has been based on the genetics paradigm, whilst results have been interpreted based on the stem cell entity paradigm (although of course there is some cross-over). This thesis asks whether this is still a useful approach to stem cell research in the twenty-first century; whilst the importance of genetics for our understanding of stem cells is still significant, it may not be the only method on which to base experimental design. Likewise, assuming that stem cells are entities may not be the only way of interpreting experimental results.

[1] Bensaude-Vincent, 2009; Wilson, 2011.
[2] As described in, for example, Maienschein, 1978.

Approaches

This thesis uses a variety of methods to demonstrate the wide-ranging approaches that can be taken to the history of ESC research. Included in the research chapters of this thesis are approaches from social history, cultural history, the history of ideas, and participant observation, for example. Considering the three research aims of this project, this thesis may also be moving towards the science and technology studies (STS) approach, particularly as STS is generally concerned with contemporary science, and is interested in scientific practice 'in action'[3]. This said, although there is discussion of contemporary stem cell research throughout this thesis, and a clear interest in how the research was carried out at the bench, there is also historical discussion of prior stem cell studies. The importance of archival materials may appear essential to such an endeavour, however these resources were generally unavailable for this project. This project focuses in particular on research, and the work carried out in laboratories. This means that, for much of the period explored in this thesis (i.e. the late twentieth and twenty-first centuries), there are no archives available[4]. Where enquiries were made, researchers were either reluctant to share laboratory notebooks, were still using these notebooks, or, universities that held such notebooks could not release them (reasons for this varied slightly, however were generally based on the requirement that the researcher who made the notes needed to have died before these could be released, and even then would have needed to have died many years prior to the notes being released). This made the study of every-day experiments almost impossible. For this reason, the project makes use of scientific publications as its window to the laboratory. This is a relatively under-used resource in itself, particularly for more recent research; this is likely due to the complex, specialised nature of the research papers now published, which, arguably, are now only accessible to those who understand the language and terminology used, and the way in which scientific publications are created. Since I have prior experience of both of these facets, it seemed logical to make use of these publications as primary sources for this research. The usefulness of looking at published papers as a source

[3] For example, see Asdal, 2012 p 380.

[4] Earlier periods covered in this thesis, particularly in Chapters 1 and parts of 2 and 3, of course would have archival material more readily available. However, due to the nature of these sections of the thesis, secondary literature (based on archival research) is adequate. In addition, it is unlikely that this researcher's current grasp of the German language would be at a standard high enough to refer to many untranslated primary sources from the nineteenth century.

for understanding knowledge production in the sciences has been highlighted by Hannah Landecker (see below). The original research articles published in ESC research and related disciplines have been utilised extensively in this project. In part, this has been a way to circumnavigate the issue above regarding laboratory books, and another that could arise with oral histories (such as misremembering, or telling a well-rehearsed story – see below). Secondly, as noted by Ohad Parnes, "published accounts may be no less useful for the reconstruction of an implicit investigative pathway than the unpublished research notes"[5]. Since the laboratory notebooks are not available, Parnes would argue that the published papers are potentially just as useful for understanding research development.

Hannah Landecker (an anthropologist), also makes use of this somewhat neglected historical resource in *Culturing life: How cells became technologies*, particularly focusing on the 'materials and methods' sections of scientific publications. This has been referred to as an "infrastructural approach", and is successfully used as a technique throughout *Culturing life*: to follow methods of researchers[6]. The 'materials and methods' sections of scientific papers have also provided some of the primary sources utilised in this thesis, since there is a continual focus on laboratory stem cell research, of which the materials and methods used are of significance. Using these sources, Landecker composed a history of techniques for enabling tissues to survive and grow *ex vivo*, juxtaposing theory and practicality, science and society, to "emphasize that these accounts…are not just 'popular' renditions of science but ways that scientists themselves narrate assumption-altering, philosophically disturbing technical change in their practices and objects"[7]. This has also been acknowledged in this thesis, where scientific publications have been utilised to develop narratives. Alongside this, a further range of commentators have been examined to generate a more comprehensive stem cell history than what scientists alone could provide. For example, the influence of the biotechnology company Geron is clear in the history of hESC isolation and culture; the work of Michael West in establishing links with James Thomson and John Gearhart has clear significance for the narrative produced (see Chapter 5).

[5] Parnes, 2003 p 135.
[6] Littlefield and Pollock, 2011 p 611.
[7] Landecker, 2007 p 161.

The context of the scientific work carried out, experimental results, and the history of ideas are approaches used throughout this thesis to emphasise the importance and/or influence of the social context (and scientific cultural context) in the development of the framework for ESC research from the nineteenth century onwards. The use of personal memories, and how such memories can be used to construct past events, has been utilised in various types of cultural history, and is often represented in various media, such as films and books[8]. In the history of science, this approach is particularly popular, although often the memories of those recorded are often those perceived as 'leaders' in their field, as opposed to the 'everyday' scientist. As early as the 1960s, this was argued to be problematic, since histories would no longer be constructed by "hanging" histories off the "pegs" of "great men"[9]. In this thesis, although there are many recognisable names mentioned throughout, the focus as been on the work produced, and the studies carried out, rather than using individuals as the starting-point for discussion. The purpose of this approach is to move away from the potentially whiggish style of history that may be generated by centring attention on the achievements of individuals, towards exploring how and why any researcher would carry out the studies described in this thesis. Oral histories could have been another way of obtaining further insight, however runs the risk of fueling the celebration of individuals. Nevertheless, it was initially believed that this would have been able to provide useful material for this thesis. Unfortunately, most of those contacted said that they would not be willing to be interviewed, or were unavailable, or simply did not respond to a request. Contact was only established with one researcher mentioned in this thesis, and that is Gail Martin. Martin was kind enough to respond to a written, email questionnaire, and some of her responses were used in Chapter 4.

From this approach, follows the relevance of social history to the history of science. Again, social, political, and economic contexts were demonstrated to be relevant to the history of science in the early twentieth century, particularly in the European and American, 'philosophically-informed' approach to the history of ideas[10]. I am inclined to agree with John Dunn with regards to the history of ideas; Dunn stated that the history of ideas should incorporate "the histories of particular

[8] For example, Confino, 1997.
[9] The Science News Letter, 1963 p 134.
[10] For example, see Fox, 2006 p 414.

intellectual practices, of science, history, political theory, economics, theology, etc."[11]; I have made use of political, social, and economic contexts throughout this thesis to more accurately situate the context in which scientific research was occurring. In some situations, there are clear relationships between political, social, and economic factors, and ESC research. This is similar to the cross-disciplinary approach (falling under the category of STS) as carried out by Landecker, who provided a valuable insight into cell culture, whilst enabling appreciation for the usefulness of inter- or cross-disciplinary work in the (historical) study of biological science, its techniques, people, theories, and objects.

Chapter 1, discussing the history of the conceptualisation of the ESC, particularly makes use of approaches from the history of ideas. For example, the political situation in nineteenth-century Germany affected the reception of research published by various researchers. The popularity of these publications affected which terms and ideas became incorporated into the concept of the cell, and the ESC. In Duncan Wilson's *Tissue culture in science and society*, Wilson focuses on tissue culture in Britain, however there is relatively little comparison between the development of tissue culture in Britain (and the public reception of this technique) and other countries or regions. In particular it would have perhaps been useful to include some comparison with Germany, since up until the mid-twentieth century, this region of Europe was considered to be at the forefront of biology, as demonstrated by the significant numbers of high-quality, influential publications produced in German at the time, as described in Chapter 1 of this thesis. Chapter 2 considers the development of disciplines relevant to ESC research, and therefore particularly makes use of approaches from the history of ideas in scientific culture. This examination of discipline development then also implicitly requires a history of technology; one way in which different disciplines emerge is not only intellectually, but also following the development and integration of new technologies (and specialist techniques) into scientific research. Chapter 3, considering the links between cancer and embryonic development through the nineteenth and twentieth centuries, also makes use of the history of ideas approach to look at how theories on cancer development paralleled with ideas on embryogenesis.

[11] Dunn, 1968 p 100.

Chapter 4 especially focuses on the political and economic affects on scientific research direction, specifically drawing on the history of politics in the USA and UK. This chapter uses the isolation and culture of mouse ESCs to look at the interaction between the public, politics, and economics. These connections have been made with regard to other related topics in the history of biology (such as the STS approach taken by Wilson in *Tissue culture in science and society* [see below]), although have not yet been discussed with regard to a specific episode in ESC history. Although Chapter 5 examines the history of ESCs via critical analysis of the motivations and goals behind the isolation and culture of human ESCs in the 1990s, there is a clear requirement for the social, political, and economic factors to be considered, as well as the scientific context (i.e. the work carried out in the laboratory), for the motivations and goals of human ESC isolation to become apparent. Again, although some personal accounts have been previously used to construct the event of human ESC isolation, such as Thomson's version provided by Parson[12], Chapter 5 of this thesis demonstrates that it is only when these are considered alongside other contexts that the more comprehensive history emerges. It then becomes possible, in this instance, to add further evidence supporting the position taken by Michel Morange (that the motivations and goals of mouse ESC isolation were different to those of human ESC isolation).

Just as I argue that Wilson uses tissue culture as an object to describe the interplay between science and society, Melinda Bonnie Fagan's *Philosophy of stem cell biology: Knowledge in flesh and blood* uses stem cell biology to review studies in the philosophy of science[13]. Fagan carefully and accurately described the science (of stem cell biology), as well as clarifying new methods in the philosophy of science, and Wilson carefully and accurately described the science (of tissue culture), as well as moving towards a useful new method in the history of science. This perhaps indicates the successful development of a useful new approach, integrating history, philosophy, anthropology, and sociology of science. Making use of such an integrated approach, Chapter 6 combines history of science with philosophy of science to examine whether stem cells are entities or whether stemness is a state. The

[12] Parson, 2004.
[13] Fagan's book also contains some references to cell culture, and the philosophical considerations of understanding cultured cells and tissues as model organisms, and these cultures as part of larger model experimental systems.

approach taken in this chapter is particularly useful, since it enables further analysis of historical events to be carried out based on the preceding philosophical arguments. For example, in Chapter 6 of this thesis, theories from historical ESC research are examined alongside more recent research. The advantage of Wilson's (STS) approach notwithstanding, his work appears to suggest that tissue culture stands for the whole of science, which of course is untrue. Although, as Wilson highlights, some have claimed that such work opened up a public discussion about entities rarely considered outside of a laboratory, Wilson's book demonstrated that such a discussion has been open for almost a century. Although some generalisations can be made, it is important to consider the context of tissue culture in biology and science as a whole. This is a factor that has been considered throughout this thesis, but which is most clear in Chapter 6. Lucie Laplane's discussion of the philosophy of cancer stem cell biology is geared firmly towards generating a way of thinking about cancer stem cells that is useful to those working in the laboratory, researching not only the biology of cancer stem cells, but also those who want to exploit the existence of cancer stem cells to produce more effective therapeutics (a more detailed review of Laplane's work is included below). This approach has a clear appeal to biologists, and especially those who are interested in finding new ways to carry out stem cell research, or to think about how stem cells function *in vivo*. This thesis also aims to draw on this recent work in the philosophy of stem cell research, integrating history and philosophy to, like Laplane, generate a study that will appeal not only to historians or philosophers, but to biologists as well. This is most clearly done in Chapter 6, which examines how the history of stem cell research can inform the philosophy, and practice, of experimental research in the future.

2. Situating this thesis

Research into the history and philosophy of stem cells, stem cell science, and stem cell research is a relatively recent trend, which has been focused on particular nuances. It is important to note here that the *ethics* of stem cell research has been given far greater consideration than the history (and, to an extent, the philosophy); although it is impossible to refer to stem cell research (especially embryonic stem cell research) in the past two centuries without discussing ethical considerations, this will

not be the focus of this thesis. Instead, where ethical arguments are relevant, they are used to generate historical and philosophical context, and inform discussion.

The historical consideration of stem cell research has increased significantly since the isolation and culture of hESCs in 1998, making this field a twenty-first century topic of interest. Several researchers, particularly historians, have written on topics either on stem cell research specifically, or associated areas. Work on topics associated with stem cell research have generally been carried out since the twentieth century, and more complete works have been produced. For example, on the history of tissue culture, Wilson has produced a history of British tissue culture in the twentieth century, whereas Hannah Landecker has published on the history of North American cell culture (from an anthropological perspective) (both of these texts are reviewed in further detail below). Likewise, several larger scale studies of embryology, experimental embryology, and developmental research have been produced; established scholars in history of biology have been involved in such studies, such as Jane Maienschein. Maienschein for example has published several books on these topics (two of which are also further discussed below).

The history of stem cell research has, as yet, not been the topic of such in-depth study. This is not to say however that no histories of stem cell research exist; these have been restricted thus far to book chapters and research articles. Several scholars have published work of particular relevance to this thesis, such as Ariane Dröscher, Christina Brandt, A-H Maehle, Alison Kraft, and Melinda Cooper[14]. Dröscher's, Maehle's, and Brandt's research has been focused on the early history of stem cell research, and in particular how 'the stem cell' was established, both conceptually and linguistically. Dröscher's work especially influenced the decision to include such a discussion in the first chapter of this thesis, establishing how the *Stammzelle* was conceived as a term and a concept. As demonstrated in Chapter 1, the history of this early, theoretical work heavily influenced the approach taken by researchers, as the stem cell concept was developed, and how it would fit-in alongside Cell Theory, evolutionary theory, and developmental biology. Melinda Cooper's work focuses on later research in stem cell history, and in particular how experimental research was carried out in the laboratory. Cooper's historical assessments of both teratoma research and haematopoietic research have been useful examples to follow

[14] Such as Brandt, 2012; Cooper 2009; Dröscher, 2002 and 2012; Kraft, 2009 and 2011; and Maehle, 2011.

for their methodology and approach (although teratoma research was also highly relevant to ESC research, as highlighted in Chapter 3). Likewise, Kraft's work has also focused on haematopoietic stem cell research. Kraft is especially interested in stem cell research carried out during the Cold War period, into the late twentieth century. Kraft is currently working on a book concerning the emergence of the stem cell from the therapeutic view, focusing on bone marrow transplantation and the potential for regenerative medicine. *The scientific, clinical, and commercial development of the stem cell: From radiobiology to regenerative medicine* is expected to be published at the end of 2017. What has been published specifically on the history of stem cell research so far then has pinpointed specific aspects of research, such as the initial conceptualisation, evolution and selection of terminology, and discrete histories of particular laboratory research. In this thesis, I aim to add to these topics individually, and also bring them together to generate a 'bigger picture' view of the past two centuries of ESC study.

As previously mentioned, the history of biomedical and biological research has not existed in isolation, and has, in the twenty-first century in particular, become entangled with considerations from philosophy of science. Very recently, Melinda Bonnie Fagan and Lucie Laplane have published books specifically on the topic of the philosophy of stem cells. Fagan's book is a highly philosophical work, applying well-known approaches in the philosophy of science to stem cell research. This includes, for example, utilising logical methods to produce models, describing stem cell capabilities or properties. Laplane's 2016 book focuses more specifically on cancer stem cells; the philosopher Laplane worked in laboratories alongside cancer stem cell biologists, which has clearly influenced the approach she has taken.

In order to further establish how this thesis fits into current research in 'stem cell studies', six recent books will be considered in more detail, including books on the history of teratoma research, cell and tissue culture, stem cell policy, and the philosophy of stem cell science. These books were selected since they focus on specific aspects of research also relevant to this thesis (Sornberger's *Dreams and due diligence*; Fagan's *Philosophy of stem cell biology*) or because they take a similar methodological approach to topics closely linked with this thesis (Wilson's *Tissue culture in science and society*; Landecker's *Culturing life*; Laplane's *Cancer stem cells*). Other works are on topics directly related to the subject matter presented in this thesis (Maienschein's *Whose view of life?* and *Embryos under the microscope*;

Gottweis, Salter, and Waldby's *The global politics of embryonic stem cell research*; the edited volume *Differing routes to stem cell research*).

Dreams and Due Diligence

In the late 1950s and early 1960s, two researchers began working together to investigate the potential usefulness of radiation therapy for those with blood cancers. These researchers, Ernest McCulloch (1926-2011) and James Till (1931-), when carrying out their routine experiments, would happen across an unexpected result that, one could argue, was the beginning of experimental stem cell research. As part of their investigations, Till and McCulloch would irradiate mice, resulting in the death of bone marrow cells (those responsible for generating red and white blood cells). Transplanting new bone marrow cells from a healthy donor should, Till and McCulloch hypothesised, re-populate the bone marrow with healthy cells, resulting in re-population of healthy cells in the blood system. Unexpectedly, when dissecting recipient mice, McCulloch observed that the donor cells had not only re-populated the bone marrow, but had also settled in the spleen. The donor cells had generated tumours, resulting in growths in the spleen. The tumours contained cells descended from single donor cells; Till and McCulloch decided to refer to these cells as 'colony-forming units' - i.e., cells that could form colonies of other cells (note here the borrowing of the term 'colony' from bacteriology). By isolating and culturing these colony-forming units, Till and McCulloch isolated and cultured the first stem cells *in vitro*.

For this reason, Till and McCulloch are recognised for having made a crucial step towards the enormous field that is stem cell research today. Till and McCulloch's finding has been rewarded with both the Lasker and Gairdner awards, indicating significant recognition amongst their peers. Their project however seems to be little-known outside the area of cell biology, and it has been implied that this was due to Till and McCulloch's work having been overlooked by the Nobel Prize committees[15]. Their work had received a small flurry of interest again following the death of McCulloch in 2011; writing in 2012, researcher in pathology, Paul Moorehead, admitted that he had only recently heard of the research results of Till and McCulloch, which he referred to as somewhat "embarrassing", comparing this to

[15] Sornberger, 2011.

"a geneticist who had never heard of James D Watson, Francis Crick, and Rosalind Franklin!"[16].

In particular, this appears to be a significant issue for veteran science journalist Joe Sornberger, who has written to date the only book based solely on the isolation of stem cells by Till and McCulloch. (McCulloch published his own book in 2003, *The Ontario Cancer Institute: Successes and reverses at Sherbourne Street*, that would refer to the finding of colony-forming units, alongside a history of the institute and other stories from the researchers who had worked there.) Although Sornberger's book was published by an academic publisher (University of Toronto Press), it is not a text written in an academic style, nor, apparently, to appeal to an academic audience. It appears to those who read Sornberger's book that the aim is to raise awareness of the work of Till and McCulloch, but this approach leaves something to be desired; for example, the chapter entitled 'Little Fame…No Nobel' does little more than complain about the lack of this Prize for his fellow Canadians.

Although Sornberger's aim is clearly to provide Till and McCulloch with the recognition they deserve, a few further insights can be gained from *Dreams and due diligence*. For example, there are efforts to refer to some of Till and McCulloch's students and colleagues, placing them in context alongside other relevant Canadian science research leaders. Sornberger's strength as a science journalist is also observed in his useful, accessible descriptions of Till and McCulloch's scientific work. The latter half of the book, again highlighting it's expected readership to be amongst the general public, is concerned with general topics in stem cell research - twenty-first century developments, ethics, regenerative medicine, stem cell therapies. This helps the reader to put the work of Till and McCulloch in context to some degree (Sornberger is insistent on reminding the reader of the grand leading pioneers of Canadian research), but latter chapters could be more explicitly relevant to the observation of colony-forming units.

Dreams and due diligence suffers somewhat in its structure, since it moves constantly between different topics (although the single agenda of the book is apparent throughout), and insists on constantly reminding the reader of the differing personalities of the protagonists; not something particularly unusual for researchers, who have a variety of backgrounds and personalities! This appears to be an important

[16] Moorehead, 2012 p E989.

point for Sornberger, even providing the title of the book (McCulloch's 'dreams' and Till's 'due diligence'); Sornberger fails however to elaborate on why this is of such significance for the groundbreaking work that Till and McCulloch carried out. In fact, it appears that both had a similar approach to their research, believing that the application of methods from the physical sciences would bear fruit in the biomedical sciences.

Sornberger's aim to highlight the relevance of Till and McCulloch's work is noble; as previously noted by Moorehead, there are few working in highly relevant fields today that appreciate the importance of the first recognition of stem cells in the laboratory. In the context of this thesis, Till and McCulloch's work is highly relevant; their work was the first to enable any stem cells to be isolated and cultured *in vitro*. This was a significant step in making stem cells experimentally available to researchers, and therefore the first step beyond learning through theory and observation alone.

It was Till and McCulloch's work that provided the initial influence for this project; a development of my Masters' dissertation querying whether the concept of the stem cell changed after their isolation[17]. The notion of the stem cell concept is explored further in this thesis, both historically (Chapter 1) and in its current state (Chapter 6).

Viewing life

Drawing on her background in the history of biology, and in particular embryology and evolutionary developmental biology ('evodevo'), Jane Maienschein highlights how current discourses in these disciplines have been forged through the twentieth century in *Whose view of life? Embryos, cloning and stem cells* (2003). Maienschien has since published a further book, *Embryos under the microscope: The diverging meanings of life* (2014), which focuses on human embryos, embryology, and development. Each of Maienschein's books demonstrate that historical concepts remain influential in the twentieth century. Maienschein showed that despite some scientific researchers believing that they may have developed a new technique or new idea, much of these notions had been previously conceived; this perhaps suggests that scientists would benefit from knowing more about the history of their fields. For

[17] Lancaster, 2009.

example, Maienschien highlighted that Aristotle studied development by using chick embryos, suggesting an early form of preformation theory (discussed in further detail in Chapter 1 of this thesis, in its eighteenth- and nineteenth-century context).

The motif that runs through Maienschein's works is the movement from theoretical, to observed, to experimental subject. The 'observed' stage of embryogenesis (and, to a point, stem cells) came into its own following the development of microscopy; this thesis identifies some of this research in Chapter 1 and Chapter 2, as microscopy develops and more can be learned about the cell and its components. As noted above, Sornberger believed that Till and McCulloch's research, that moved the stem cell from the observed to the experimental, was the stuff of Nobel Prizes; this was previously explored in my MA dissertation project, which queried whether the concept of the stem cell changed after Till and McCulloch's work which resulted in stem cells being available for experimentation. The conclusions of this project have been more fully realised through this later project, cumulating in the examination of the stem cell paradigm in Chapter 6.

Not only does Maienschein show how scientific antecedents discussed and considered 'modern' ideas, she also noted the mirroring of discussions regarding such innovations between historical discussions and discourses that are occurring in the early twenty-first century. For example, Maienschein noted the debates over recombinant DNA technologies and their uses in the 1970s; these, claimed Maienschein, parallel some of the discussions of stem cells and cloning in the late twentieth century and early twenty-first century. Maienschein observed the importance of the popular press in such debates, and how influential the press is in forming the opinion of the public (highlighting reports such as one on the cloning of Dolly in the *New York Times*, which included comments about cloning Jesus). A role for public opinion becomes clear again in Chapter 4 of this thesis, where it is clearly shown how public opinion can influence economic policy, influencing funding availability for scientific research.

This is just one example of how Maienschein demonstrated how context-dependent science and scientific research is; for the general reader, it may help to reveal how influential the general public and the mass media (and politicians) are on science policy, research, and its application. Maienschein is well-placed to accurately comment on this too, being a previous science advisor to Arizona's

congressman[18]. This theme runs clearly through *Whose view of life?*, with the first half presenting various historical debates, and the latter half focusing on more current, controversial, topics. In this thesis, I also aim to show how important historical context is to the practice of science through Chapters 1 and 2. Later in this thesis, and especially in Chapter 4 and 5, it becomes clear how influential social, ethical, economic and political contexts are as well.

Maienschein discussed some of these contexts with particular reference to more controversial techniques, such as somatic cell nuclear transfer (SCNT), defending freedom of scientific endeavour. The importance of ethical discourse is of course highlighted by Maienschein, who rightly asserted that any reduction to an 'ethics versus science' argument is unsatisfactory, since the issues discussed are far more complex than this basic reduction allows for. In this thesis, although there is not a specific focus on the ethics of stem cell research (this is a topic covered many times by historians, scientists, and philsophers in the recent past), the role of ethical considerations and its effects on stem cell research are a consideration. Just as Maienschein highlighted this through discussion of SCNT, this thesis demonstrates that ethical considerations had a role in the isolation and culture of hESCs in the 1990s, for example (Chapter 5).

A criticism of Maienschein's books is that there could have been more on social, economic, and political perspectives, although these are not entirely lacking. For example, Maienschein criticised the lack of public debate concerning funding and patents (with reference to projects in genetics and genomics), which would certainly have benefitted from social and economic discourse. For example, Chapters 4 and 5 of this thesis, exploring mESC and hESC research respectively, demonstrate clearly that the research carried out was directly affected by the social, economic, and political contexts of the 1980s and 1990s. Maienschein argued that embryo research in particular should be about science and research, all but ignoring the various other approaches that are important, such as patents (and capitalism in general for scientific and medical research), and women's rights (in the context of IVF, for example). Scientific research, particularly biomedical research, cannot exist in a vacuum away from the social, political, economic, and ethical contexts such as those highlighted in this thesis.

[18] Löwy, 2005.

Despite what may be argued to be a lack of context, Maienschein produces extremely useful histories, lacking the presentist mirage often accumulated (perhaps unconsciously) by some when referring to topics that are still current in biological and biomedical sciences research today (such as Sornberger, referred to above, perhaps). Maienschein asks questions of historical experiments and theories such as 'what experimental tests were devised?', and 'why is our hypothesis plausible?' in order to help her readers understand the scientific context of the discourses and research occurring. Dipping slightly into the waters of philosophy of science, Maienschein also demonstrates how experiments (and experimental design) can be reliable or fallible, using specific examples of historical work in developmental and embryological research. This flows from discussion about how prevailing theories can became sterile, then outdated, and not in keeping with more recent experimental results. Such theories are displaced by new ideas, better at explaining any newly-observed phenomena. This is also demonstrated throughout this thesis, occurring throughout the two centuries of stem cell research covered across the research chapters.

As noted above, Maienschein referred to several steps in research, the first three being the hypothetical, then the observed, and then the experimental. The experimental embryo arose at the turn of the nineteenth and twentieth centuries, in the laboratories of, for instance, Wilhelm Roux, Hans Driesch, and TH Morgan; all such work features in Chapters 1 and 2 of this thesis. The differences between the theoretical, observed, and experimental stem cell are demonstrated to be relevant in this thesis, which highlights the transitions between these three. It is interesting to note when researchers recognise the shift between the theoretical, the observed, and the experimental. In this thesis, it is argued that researchers are still interpreting their experimental results as if they were still working under the paradigm of the theoretical or observed stem cell. Maienschein developed this idea further however, adding another four steps. Following the hypothetical, the observed, and the experimental is the inherited embryo, a phase ushered-in by the increase in heredity and genetics research in the mid-twentieth century. This is more closely considered in the second chapter of this thesis. The inherited embryo was swiftly followed in the 1960s by the computerised embryo, where improvements in software and computing power enabled computer modelling to demonstrate embryonic and evolutionary development. The sixth step was the visualisation of the human embryo for the first

time, which occurred in the 1970s, as Patrick Steptoe and Robert Edwards worked towards fertilising a human egg *in vitro*, which could then be re-implanted for *in vivo* development. The final step identified by Maienschien is the constructed, or engineered, embryo. Like embryonic development, the constructed embryo was initially theoretical – Jacques Loeb attempted to create such an embryo in the early twentieth century – before becoming realised in the late twentieth century, when researchers such as Beatrice Mintz and Ian Wilmut carried out their studies on mammalian embryos (generally under the umbrella of 'cloning' research)[19], again noted in Chapter 2 of this thesis.

Cell and tissue culture

Two books of especial interest have been written on the history of cell and tissue culture: Hannah Landecker's *Culturing life: How cells became technologies* (2007)[20], and the more recent book by Duncan Wilson, *Tissue culture in science and society: The public life of a biological technique in twentieth century Britain* (2011). *Culturing life* has a US-focus, and Wilson's book helps to include more history of the British contribution to early cell and tissue culture. Although now generally considered to be a somewhat regular, even mundane technique in current biomedical and biological science, the survival and proliferation of cells outside the body was once regarded as ground breaking, and as Andrew Reynolds has observed, many of the "more recent biotechnologies would be impossible without it"[21]; this of course includes the ESC research discussed in this thesis. These books are so important in the context of this thesis since they have influenced the overall approach. In particular, this includes the use of scientific publications as primary sources (as in Landecker) and highlighting the important role of society in scientific research (as in Wilson).

According to Wilson, there are not always two opposing sides of 'the public' and 'science'. As he observed in his introduction, "the scientists who used tissue culture were only one group in a dynamic network that also comprised journalists, authors, documentary makers, anti-vivisection and pro-life groups, bioethicists,

[19] Huistra, 2015.
[20] *Culturing life* won the 2008 History of Science Society's Suzanne J Levinson Prize.
[21] Reynolds, 2011 p 149.

lawyers and politicians"[22]. Covering a century of tissue culture – from Ross Granville Harrison's (1870-1959) experiments with neural cells in 1907 to the first artificially created replacement organ transplants in 2006, and modern art installations using animal cells cultured on scaffolds – Wilson used tissue culture to show the evolving relationship of science and the public, with a focus on Britain. Other previous works have considered the relationship between science and the public, including popularisation of science; initially it may be troublesome to see what Wilson can add to this. For example, science popularisation has been studied since science first became a professional pursuit, and is part of an arsenal we can use to examine the relationship between science and society[23]. It is Wilson's specific approach however that is the novelty in *Tissue culture in science and society*; far from going back over well-trodden ground of examining the relationships between science and the public via the popularisation of science, Wilson brings together history, anthropology, and sociology to investigate the changing public attitudes to science.

Just as functioning society required every individual to have their inter-dependent roles and their place (the butcher, the draper, the councilor, the grocer), the same was considered for tissues of the organism. This is demonstrated to be the case in the historical study of the stem cell concept (Chapter 1), particularly in Germany. Wilson also noted the 'cell state' effect on US and British thinking; in the first chapter of this thesis, the popular USA cell lineage studies are mentioned; this fits neatly into Wilson's appraisal of the period. The Great Depression of the 1930s had led to significant unemployment and unprecedented shifts in society, and its problems were equated with the uprooting of tissues from their organised, specific place in the organism (i.e. attempts to maintain them in the laboratory)[24]. Such a shift led us to consider how science is affecting societies, and how the public view of science is socially constructed. The biomedical sciences constitute one area where research can have obvious effects on society. Wilson has been shrewd to select tissue culture as an object to examine how such construction might take place; arguably, this thesis demonstrates that stem cell research is also a suitable lens through which to view the effects of science on society, and perhaps even more clearly, the effect of society on scientific research. As has been previously highlighted by Roger Cooter and Stephen

[22] Wilson, 2011 p 3.
[23] Bensaude-Vincent, 1988; 2009.
[24] Willmer, 1935.

Pumfrey (1994), it is not always possible to separate the production and consumption or communication of science, and Wilson supports this idea.

Is Wilson investigating the socioconstruction of science, or considering the technoscientific construction of societies? The former, suggests Bernadette Bensaude-Vincent, has been "thoroughly investigated" in the past by historians of science, and what is needed now is an examination of the technoscientific[25]. In *Tissue culture in science and society*, Wilson works towards the latter. There is some detail regarding the origins of scientific knowledge, such as the laboratory, however Wilson has clearly attempted to observe the effects of the increasing construction of societies by science and technology. This approach has also influenced the approach taken in particular sections of this thesis, especially in Chapters 4 and 5, where the interactions between science and society are shown to be significant for scientific research.

In the latter half of the book, where it is most relevant, Wilson managed to hint at the notion of multiple 'sciences' and multiple 'publics'. Wilson highlighted the importance of several relevant parties (such as politicians, ethicists, artists, researchers), all arguably on the 'science' side, as well as several groups of 'publics' (journalists, educated lay persons, voters, recipients of certain medical treatments, for example), with some falling into multiple categories. In her work, Bensaude-Vincent preferred the term 'citizens' to 'the public', in order to highlight the more active role of individuals in society[26]. I believe Wilson's intention is the same: to demonstrate the role of science and scientific research in the maturation of modern Western democracy. Here then is further evidence that Wilson has contributed to an exploration of the technoscientific construction of British society. All such parties are also shown to have significant roles in the history of ESC research, as evidenced throughout this thesis, and in particular through Chapters 3, 4, and 5.

Although the beginnings of cell culture technique development lay in the late nineteenth century, Landecker also takes the work of Harrison as her starting point; Harrison's 1907 publications report on the survival of tissue fragments in his laboratory for several weeks. Harrison demonstrated that cells could live *ex vivo* for some time, which was an interesting novelty amongst the scientific community; novelty is probably the most appropriate term here, since few knew what to do with

[25] Bensaude-Vincent, 2009, p 365.
[26] Bensaude-Vincent, 2009.

this new research tool, including Harrison himself. Eventually of course, researchers would find a use for the new technique of 'tissue culture'. Harrison's hanging drop method was good for maintaining (and differentiating) individual neurons, but other methods were needed to culture other cell types *en masse*, as Alexis Carrel (1873-1944) would attempt to do at the Rockefeller Institute. Culture conditions enabling (or encouraging) both self-renewal and differentiation would become key for ESC research.

Tissue culture in science and society explained how tissue culture became a 'high profile tool' in the 1920s and 1930s, with a specific focus on the Cambridge Research Hospital (CRH) and scientist Thomas SP Strangeways. Development of tissue culture methods reflected a shift towards experiment in biological sciences – no longer was biological study based solely on observation of natural phenomena (as noted by Maienschein). Not only could biological materials now be experimented on, tissue culture warped the view of organisms - whole bodies were now seen as a stack of raw materials that could be removed and re-used elsewhere – the opportunity to control nature was on the horizon.

In 1929, the new Director of the re-named Strangeways Research Laboratory (from the CRH), Honor Fell, continued the science communication tradition, and made attempts to explain the uses and benefits of tissue culture to the general public. The mixed results of her efforts could have been a combination of journalists wanting a good story, and scientists being unable to provide this; as David Knight says, the day-to-day work of scientists can be rather dull[27]. Despite this, ex-British Prime Minister (1902-1905) and President of the British Association for the Advancement of Science (1904), Arthur Balfour, emphasised the economic and cultural importance of science and research in interwar Britain[28]. This is demonstrated in Chapter 4 and Chapter 5 of this thesis; these chapters also show that the effect occurs in the opposite direction as well – scientific research is clearly affected by economic contexts and social influence. Alongside a government-encouraged admiration for modern science and technical breakthrough however was fear and insecurity; Wilson remarked that significant numbers of films produced in the early 1930s presented science as a route to catastrophe. Knight has suggested that there was a 'loss of scientific innocence' after World War I, with science being viewed as concerned with vested interests of

[27] Knight, 2006.
[28] *ibid*.

investors, not the inoffensive, disinterested investigation previously considered. As the motives of scientists were questioned, some public distrust followed[29]. Again, this is shown to have had an effect later in the twentieth century; consideration of the social context for mESC and hESC research (Chapters 4 and 5) clearly show that public distrust was able to influence political policy, and affect the availability of funding for particular research projects. For example, legislation would dictate that Thomson could not use federal funding or facilities for his hESC isolation research (instead depending on the private sector) (Chapter 5).

Both *Culturing life* and *Tissue culture in science and society* consider the advances made in tissue culture techniques during inter-war and post-war Britain, and their commercialisation. In the ongoing fight against disease, tissue culture could now play a significant role and would be portrayed in a more heroic light by the media. World Wars I and II shifted the focus of scientific research towards more practical uses. As highlighted in Chapter 4 of this thesis, the economic downturn of the early 1980s had a similar affect, particularly in Britain. Arguably, such commercialisation and focus on the application of techniques resulted in useful standardisation of tissue culture in the mid-twentieth century. Standardisation of cell lines was part of the motivation of Gail Martin and Martin Evans' work in the 1970s and 1980s, demonstrating that this factor was an ongoing concern – as again shown when John Gearhart and James Thomson began working on the isolation and culture of hESCs in the 1990s (Chapters 3, 4 and 5). Alongside this was the realisation that tissue culture, no longer simply an interesting phenomenon, could be clinically relevant. Again, this mirrors the realisation of something similar by Martin Evans in the 1980s, as he made his isolated mESCs clinically relevant by producing animal models of disease (Chapter 4). John Gearhart was also drawn into isolation and culture of hESCs through the need for a more clinically relevant tool for his research (Chapter 5).

Furthermore, usefulness of cell culture for genetics and heredity research became evident in the 1950s and 1960s, including the fusion of cells from different species. That these hybrid cells could survive demonstrated that, at least at the cellular level, there was some unity between species that must (biologically) allow such blending. Just as Maienschein reminded us that there is a relevance to

[29] Raina, 1999; Knight, 2006.

theoretical biology (preceding practical, or experimental, biology), Landecker noted that cell culture resulted in an entire shift of meaning of 'life' and 'organism'; these themes appear throughout Landecker's history, focusing on cell culture as a tool linking nineteenth-century physiology to twentieth-century genetics. In this thesis, I aim to provide a complementary overview, such as discussing development of disciplines in Chapter 2. The second chapter of this thesis clearly shows that there were many links between embryology, cell culture, and genetics that, arguably, have lasted into the twenty-first century. In fact, this thesis has shown that whilst earlier studies may have been influenced by the (then unknown) mechanism of heredity and inheritance, later twentieth-century and twenty-first-century studies continue to be carried out under a genetics paradigm. Biological sciences held a different position in the public mind in the early decades of the twentieth century compared to, for example, the physical sciences. Different research traditions were emerging, and there was, in some cases, little communication between the proponents of these different fields.

In the laboratory, tissue culture was becoming essential to several disciplines, including pharmacological sciences and genetics. By the 1960s, cells had become "easily accessible, available, and manipulable"[30]. Landecker has suggested that the malleability cell biology demonstrated was not primarily about the creation of 'artificial monsters', but about demonstrating the plasticity of life[31]. The parallels between both the biology and the study of the pathological and non-pathological are highlighted throughout this thesis, most notably in Chapter 3. Although Landecker suggested that such parallels were recognised in the mid-twentieth century, as it became paired with the possibilities by experimental research, Chapter 3 shows that such parallels were certainly considered in the nineteenth century, and possibly before.

Notwithstanding the apparent lack of consideration given to the ethical and legal issues connected with tissue sample collection through most of the twentieth century, Wilson's sixth chapter engaged with this important matter. Wilson reported that there was a generally positive attitude towards human tissue culture during the 1960s and 1970s, due to the positive spin created by anti-vivisectionists, who saw tissue culture as a method that could replace experiments on living animals. The 1967

[30] Landecker, 2007 p 201.
[31] *ibid* p 232.

Abortion Act however caused concern in some sections of society, with Catholic claims that perhaps abortions would be encouraged in order to provide foetal tissue for research. Such attitudes became part of the discussion of IVF research, which itself was historically important for the later isolation and culture of hESCs. Wilson helpfully described the contemporary legalities of human tissue use: surgical procedures required consent, which, when given, implied abandonment of removed tissue (including foetal tissue). Researchers then felt that once they cultured this, it became their property. Professionals from a variety of specialisms commented on the collection and use of foetal tissue, which became entangled with the pro-life movement of the mid-1970s. Developments in the storage and culture of human tissue greatly expanded the opportunities for its use, and therefore increased demand. As the market increased, so did the commodification of human tissue in this context[32]. The demand for foetal tissue would have been increased since no human embryonic cell lines existed in the 1970s and 1980s. Although a donor may afford different cultural values and individual rights to bodily materials, there was a temptation to suggest that this was somewhat negated by a sort of clinical detachment[33]. This would not only affect a physician treating an individual or a scientist receiving a donation, but a patient: the patient is more likely to feel detached from removed diseased tissue than removed healthy tissue. The MRC and National Institutes of Health (NIH), in the UK and USA respectively, responded to changes in public feeling by funding attempts to create foetal cell lines. Following several legal cases concerning tissue and cell line ownership in the USA, queries regarding ownership began appearing in 1980s Britain.

The Thatcher government of the late 1980s encouraged commercial incentives in biological sciences, and a 1988 *Lancet* article claiming that collection of and testing on removed tissues was not covered by the 1961 Human Tissue Act, led to an empowering of patients; patients now had the option of whether to donate tissue or

[32] Lesley Sharp (2000) has highlighted that commodification of the body is not a new phenomenon, or one that is particular to medicalisation of the body in the modern era. The body, "either in its entirety or fragmented form has long been an object of economic, social, and symbolic use in a host of societies" (p 292). Political and military frameworks have also commodified the body, and have separated bodies based on class, age, race or gender, for example. In a biological and medical research context, bodies have been used as objects by anatomists and collectors, and in history the pauper's body was frequently worth more dead than alive.

[33] Andrews and Nelkin, 1998 p 53.

not. Patients were no longer considered to have 'abandoned' any tissues following surgical procedures, and more attention began to be paid to personal data learned from genetic analysis. The Thatcher government's policies on biological science research becomes an important focal point in Chapter 4 of this thesis, particularly considering how policy and funding availability affected research directions.

Stem cell history and policy

By the end of the twentieth century, stem cells had become a beacon of biological and biomedical science, glowing with mysterious scientific and medical potential, whilst blushing with moral and ethical issues. From this, some non-scientists have become interested in the history, philosophy, sociology, and legality of stem cell research and regenerative medicine, publishing various papers on the topic. One of the first books to begin compiling such studies on stem cell research was put together by Renato G Mazzolini and Hans-Jörg Rheinberger, in *Differing routes to stem cell research: Germany and Italy* (2012). The volume is based on a conference held at the Italo-German Historical Institute in Trento, in 2010, which addressed two questions in particular: where did stem cell research come from, and why have international (in this case, German and Italian) manifestations varied so greatly?

Historical accounts led the discussion, by considering conceptual changes through the history of stem cell research. This included discourses on terminology (examining Ernst Haeckel's *Stammzelle*, for example), to the influence of American teratoma research in the mid-twentieth century. There are also comparisons drawn between various branches of biological study in the nineteenth and twentieth centuries, such as between pathology (in particular cancer) and embryology, again considering the influence of embryonal carcinoma cell lines derived in the 1970s. These are all discussions examined at length and in greater detail in this thesis. What also becomes clear from the historical discussion is that the stem cell as an object is multifaceted[34], and that there is no single history of stem cell research, but several, depending on which facet of the 'stem cell' one wishes to explore. Again, this is highlighted throughout this thesis, as different historical approaches demonstrate various ESC discourses; other stem cell histories have also shown that several

[34] Capocci, 2014.

histories are needed to explore the variety of stem cell types, such as the work carried out by Cooper and Kraft, for example, on haematopoietic stem cell history.

After the historical perspectives, there followed consideration of the sociology, legal, and biological aspects of (embryonic) stem cell research, especially focusing on the differences between German and Italian research (which was not apparent in the first historical chapters). Usually, it is the approach taken to ESC research in the UK that is written about in this way, since UK legislation is relatively permissive. Both Italian and German laws however are, in contrast, relatively restrictive, although for different reasons. This section of the volume is highly influenced by Sheila Jasanoff's "civic epistemology", which is used to frame how legislation in Italy and Germany has developed[35].

Increasingly in the twenty-first century, discourses on stem cell research are beginning to enlarge from the initial science/ethics/faith discussion, to include debate regarding economic, legal, social, and political factors. In particular, Herbert Gottweis, Brian Salter, and Catherine Waldby initiate discussion between the more familiar biology and ethics, and less familiar political dimensions, in *The global politics of human embryonic stem cell science* (2009). The political analysis provided by Gottweis, Salter, and Waldby is based on large national and international research projects, providing a useful transnational approach (and making the 'global' of the title worthy of its name). The authors also make use of interdisciplinary methods, utilising sociology, anthropology, politics, and economics in their work; although occasionally this makes the book a slightly fragmented read, overall, the book holds together. The "transnational influence" of factors such as investment in biotechnology, alongside regional influence of individual laws (by country, or state) for example, are highlighted by Gottweis, Salter, and Waldby, who argue that such economics, politics and "power" aid in explaining country or state attitudes and regulations for stem cell research[36]. Gottweis, Salter, and Waldby call this the "post-welfare state", in which the welfare of citizens is given a lower priority than 'big business', such as biotechnology firms, and regulations are formed based on this arrangement of political interest, as countries compete with each other for industrial finance[37], demonstrated by the reduction in national healthcare and welfare budgets[38].

[35] For example, see Jasanoff, 2007 p 247-271.
[36] Blaser, 2010 p 100.
[37] Gottweis, Salter, and Waldby, 2009 p 29.

For example, following the Bush administration's limit on stem cell research in the USA, activities in other countries were driven by the potential ability to fill this perceived gap in the market. Despite this, almost every country has a different set of regulations for stem cell research, which is explored in this book.

Gottweis, Salter, and Waldby introduce the concept of biomedicalisation[39] (as an extension of medicalisation[40]), with particular reference to stem cells and marketisation, that has affected the technological developments and economic investments in ESC research in the latter years of the twentieth century, creating "a global techno-managerial paradigm in health policy"[41]. This is highlighted further by discussion referring to the marketisation of human tissue (especially, for example, human oocytes and "reproductive tourism"[42]), intellectual property rights, commercial interests, and the effects such factors have on policy decisions in various countries. Human ESCs and related tissues become known for their "biovalue"[43]: a global, economic yield generated by biotechnology companies in their biomedicalisation of cell biology. Although Chapter 5 focuses on the lead up to the isolation and culture of hESCs, there is evidence in this history that supports the claims made by Gottweis, Salter, and Waldby. In particular, the potential commercial benefits of the isolation and culture of hESCs, and the development of standardised cell lines, was thought to be potentially fruitful enough for biotechnology company Geron to invest in both Thomson and Gearhart's work. If federal policy and economics had enabled public funding of Thomson or Gearhart's work, there is a distinct possibility that private funding and the almost immediate commercialisation of their work would not have been required. It is also possible that hESCs may have been isolated and cultured sooner.

The middle section of *The global politics of embryonic stem cell research* followed this line of enquiry by beginning with discussion of global regulations, ethical questions, and local policy development post-Dolly (1996)[44], and more detailed consideration of regulations in regions that one review called an "ethical

[38] McCall, 2010.
[39] Clarke *et al*., 2003.
[40] Zola, 1972.
[41] Gottweis, Salter, and Waldby, 2009 p 13.
[42] Caplan and Bürkli, 2009 p 15.
[43] Gottweis, Salter, and Waldby, 2009 p 8.
[44] Blaser, 2010; Caplan and Bürkli, 2009.

potpourri"[45]; regulation in Britain, USA, Japan, Italy, South Korea, and Germany have previously been discussed[46]. This volume neatly brings together interviews and policy histories for these countries, but generally adds little original research to the discussion. For example, policy shifts under different administrations in the USA (Clinton to Bush to Obama), contrasting Buddhism and science policy in Japan and South Korea, and restrictive legislation in Germany, have all been examined elsewhere[47]. *The global politics of embryonic stem cell science* would have been completed and in press prior to several important and influential stem cell policy decisions of 2008, such as the reduced restrictions for German stem cell research, changes initiated in the USA as Barak Obama became president, or the 2008 amendments to the UK's Human Embryology and Fertilisation Act, which could have added to work previously published.

Stem cell philosophy

There have, very recently, been two books published concerning the philosophy of stem cell research: Melinda Bonnie Fagan's *Philosophy of stem cell biology: Knowledge in flesh and blood* (2014), and Lucie Laplane's *Cancer stem cells: Philosophy and therapies* (2016). The publication of such texts demonstrates that the early twenty-first century is not just becoming known for its advances in stem cell biology, but also for its reflections on stem cell research. There is in addition perhaps a more coherent field of 'stem cell studies' emerging, which, according to the statements made in several published works, aims to engage with and inform global stem cell research and policies. The book by Fagan, and especially the one by Laplane, certainly appear to be in this category.

Fagan's book has been referred to as "a superb discussion of this exciting field of contemporary science"[48]; being the first book-length philosophical consideration of the topic, Fagan's book will no doubt become a 'yard-stick' by which other attempts will be measured. Fagan appears to acknowledge the responsibility of writing the first such book, carefully explaining the relevant scientific details of stem cell biology, including the relevant scientific concepts, techniques, and, to an extent,

[45] Caplan and Bürkli, 2009 p 15.
[46] For example, see Robertson, 1999; Ayer, 2002; Tauer, 2004.
[47] For example, see Walters, 2004; Wolfrum and Zeller, 1999.
[48] Ioannidis, 2015 p 285.

history. The book is organised to begin relatively generally, deepening arguments and detailing complications as one reads through.

The book is separated into three parts, the first focusing on the conceptualisation of stem cells for research. The second, middle part is concerned with examining general debates in the philosophy of biology, and showing how these are relevant to stem cell research. The final part links stem cell research with clinical medicine and systems biology. What appears to be missing is a focus at some point in the book on the importance of the cell's environment; although mentioned several times, there is no single section where Fagan draws together all of these notes to historically and biologically contextualise them. For example, somatic cell nuclear transfer has demonstrated that, theoretically, every cell (and not only stem cells) has the capacity for self-renewal and differentiation (in the appropriate environment). There is no discussion of what this sort of data means for the concept of pluripotency. Steps towards providing this discourse have been taken in Chapter 6 of this thesis.

In particular, Fagan noted that, like in several other experimental biological areas, there is a lack of 'general theories', such as those found in physics, for example. Instead, the foundation of experimental biology are models, which makes stem cell science appear rather disunified, presenting a significant methodological problem for philosophers of science[49]. Fagan's approach to this is to make use of (abstract) models and develop models herself. Making use of a much-used stem cell definition stating that stem cells are cells capable of both differentiation and self-renewal, Fagan generates the "abstract stem cell model", where self-renewal and differentiation potential or capability are the "minimal unifying framework" for stem cell research[50]. This becomes a way of demonstrating that stem cell research is not necessarily disunified, and, once she has established this, Fagan can then continue her book based on this model. For example, the model enables us to identify whether a cell is or is not a stem cell. This is an important distinction for research, Fagan claimed, as it demonstrates that there are two branches of stem cell research (based on a methodological difference): tissue-specific research, concentrating on those stem cells restricted to particular tissues, and pluripotent cell research, which focuses on cells with pluripotent properties. Unity can even be found after accepting this divide, Fagan stressed, stating that ultimately stem cell research, despite the lack of single

[49] *ibid* p 286.
[50] Fagan, 2013b p 45.

methodology or approach, has the same therapeutic goal[51]. This is not something that has been universally accepted however; for example, the important role of stem cell research historically in development studies, and how stem cells are increasingly being used in a variety of disciplines as models themselves are also relevant.

In addition to generating a first comprehensive philosophy of stem cells, Fagan considers general theories and ideas in the philosophy of science, and how they can apply specifically to stem cell research. For example, Fagan queries how experimental results from large populations of cells can tell us about the ways individual cells behave, and how we can accurately measure the capacities or potentials of single cells; these problems are referred to as "the stem cell uncertainty principle"[52]. In addition to these issues concerning individual stem cells or stem cell populations, Fagan also observed that there remains some discontinuity in the term stem cell, and whether the term identifies a single cell, a cell population, or various cell types; this reminds us of the discussions by Holger Maehle and Ariane Dröscher in particular, and the explorations of the early uses of the term *Stammzelle* or stem cell[53] (as further explored in Chapter 1 of this thesis). Experimental results are interpreted in light of such varied theories, and would therefore benefit from some unity[54]. The 'stemness alternative' has previously been suggested as a way to overcome the aforementioned uncertainty, suggesting that the stem cell may be able to move along a variety of "differentiation states"[55], rather than every cell having the same signature (which has been the favoured model up until now). This model is carefully examined in detail through Chapter 6 of this thesis. Although Fagan agreed that there are some merits in this idea (such as the action of a stem cell being influenced by its environmental context), she is largely critical.

In contrast, Laplane neatly demonstrated that her version of the stemness model is practically applicable and useful to stem cell research. In a book published in mid-2016, Laplane considered how viewing cancer stem cells (CSCs) differently might affect experimental design, and lead to new cancer treatments. Central to Laplane's theory is that all tumour cells are not equal, generally consisting of small numbers of highly proliferative cells, and larger numbers of

[51] Ioannidis, 2015.
[52] Fagan, 2013b p 64.
[53] Maehle, 2011; Dröscher, 2014.
[54] Germain, 2014.
[55] Fagan, 2013b p 71.

differentiating/differentiated cells. At least some of these cells appear to be stem-cell like – i.e., they have the ability to both self-renew and differentiate, which is what maintains the growth of the tumour. These are CSCs. The parallels between CSCs and ESCs, and how this has been exploited in research, is discussed in Chapter 3 of this thesis. The existence of CSCs would explain how cancers could reoccur, even after chemo- or radiotherapies, and can apparently 'strike' at any time, and almost anywhere in the body. Furthermore, the development of secondary tumours, some far away from the primary tumour site, could also occur by the movement of CSCs through the body. In short, CSC theory explains important facets of our current understanding of cancer, including why patients are never considered 'cured'. On the other hand, understanding CSCs may give us another weapon in our arsenal for treating cancer.

Laplane also included a useful historical overview, demonstrating that the CSC concept emerged from teratoma research (as discussed in greater depth in Chapter 3 of this thesis), and haematopoietic cancers. More recently, cell-sorting technology introduced in the latter years of the twentieth century has resulted in something of a boom in CSC research. Laplane noted in particular the work of John Dick, who transplanted purported leukaemic stem cells from sick mice into healthy mice. Dick found that these transplanted cells acted like stem cells (i.e. with the ability to both self-renew and differentiate), but would generate leukaemic cells instead of healthy cells. Laplane therefore suggested that the study of CSCs should not be separated from the study of 'normal' (i.e. non-pathological) stem cells; again, this is explored further in this thesis, in Chapter 3, although the parallels between the abnormal and normal can be observed at several points through ESC history.

With this in mind, Laplane argued that for successful cancer treatments to be developed, we must begin with stem cell biology. Laplane carefully unpacked current semantics and concepts in stem cell research, but identified issues with the way researchers think about stem cells (and their properties), and the experimental results. Stem cell and cancer biologist Hans Clevers has suggested that there has been "fuzziness" in stem cell definitions, which has affected experimental design, interpretation of results, and communication of findings[56]. This is where Laplane

[56] Clevers, 2016.

delivers a new concept of stem cells that may affect experimental design in the future, with four possible stem cell 'types', based on philosophical concepts:

1) That the properties of a stem cell (i.e. self-renewal and differentiation ability) are intrinsic, and therefore independent of its environment ("categorical")
2) That these intrinsic properties of a stem cell only emerge under the appropriate environmental conditions ("dispositional")
3) That these properties of stem cells are extrinsic, and can be induced by environmental factors ("relational")
4) That the properties of stem cells are actually extrinsic properties of tissues, rather than individual cells ("systemic").

Laplane's four potential stem cell types require further laboratory investigation, since no experiments have been designed under this new stem cell concept, nor have any results been interpreted in light of Laplane's ideas. It is not immediately clear how any currently studied stem cells fit into Laplane's framework, yet it could still be valuable for future research, since it is applicable to experimental work. It is clear from Clevers' enthusiasm for Laplane's alternative way of conceiving stem cells that the research community may be looking for new approaches to cell biology, and are open to suggestions from philosophy[57]. Although this thesis approaches this in a slightly different way (primarily from interpreting the historical results of experimentation), Chapter 6 demonstrates that a new way of conceiving stem cells may be fruitful for experimental research in the future.

3. Thesis overview

This thesis focuses on the history of embryonic stem cell research in Europe and the USA, during the nineteenth and twentieth centuries. Specifically, it will ask what the role of political and social context has on stem cell research is, what the significance of cell fate and cell fate research is, and under which paradigms ESC research developed, including whether these paradigms are still useful in the twenty-first century. The thesis will investigate several aspects of ESC history to help answer these questions; this includes exploration of the stem cell concept through history, the role of heredity and genetics research and paradigms, the parallels between cancer and

[57] *ibid*.

stem cell research, the social, political, and economic factors that governed the isolation and culture of mESCs and hESCs, including their use, and lastly, whether we should consider stem cells as entities, or 'stemness' as a state.

The first chapter of this thesis therefore explores the paradigms under which the term '*Stammzelle*' developed; since German natural philosophy was so influential in developing the concept of the stem cell, there will be a particular focus on this region and research in the earlier sections of this chapter. An overview of German nineteenth-century research into the life sciences provides important context, enabling us to appreciate how the concept of the cell developed, and how this came to be the proposed fundamental unit of life. Linking studies in embryology and evolution, Ernst Haeckel (1834-1919) developed the notion of a stem cell in two different contexts: initially as the first unicellular organism, from which other life evolved, then the fertilised egg, from which the entire embryo could develop. These ideas were popularised in English, particularly at the turn of the twentieth century. Of particular significance was the use of the term 'stem cell' in Edmund Beecher Wilson's (1856-1939) textbook *The Cell*, which was published in several editions. This appeared to solidify the use of the term in English, alongside use of 'stem cell' by researchers from Eastern Europe, where the term seemed to be in general scientific use. Wilson was also part of a small group in the USA undertaking cell lineage studies. The role of cell fate (as examined through lineage studies) became increasingly important for stem cell research through the twentieth century – possibly due to the influence of the early American approach.

Chapter 2 begins similarly to the previous chapter in that it examines the history of the conceptualisation of the cell nucleus. This becomes relevant as the paradigm of genetics is a theme emerging through this thesis, as a way in which stem cells have been explored, particularly in the twentieth (and into the twenty-first) century. In order to examine the manner in which genetics becomes so influential in ESC research, Garland Allen's claim that genetics initially developed under the embryology paradigm is tested. Using historical examples from the field of experimental biology (and in particular experimental embryology), this chapter argues that there is a difference between the conceptual and the chronological development of heredity, genetics, and embryology. Through examination of specific experiments from nineteenth- and twentieth-century life science, the chapter demonstrates that the

disciplines of genetics and embryology remain closely linked into the late twentieth century.

In order to throw light on another discipline linked to ESC research, the third chapter of this thesis considers the parallels between cancer and ESC knowledge and understanding. The chapter begins with a brief history of cancer, focusing on the nineteenth-century suggestion that cancer could arise from cells remaining in the adult from embryonic development. Later experimental work using mouse teratomas (tumours of germ cell origin) in the mid-twentieth century highlighted similarities between development and growth of these cancers, and embryogenesis. This chapter identifies several ways in which cancer and development were studied either in parallel, or were directly compared, in the mid- to late twentieth century. This includes biochemical studies, the study of cell fate (perhaps a link to the earlier cell lineage studies highlighted in Chapter 1), and, briefly, the role of the niche. Lastly, this chapter considers the cancer stem cell (CSC) concept; this idea appears to be fashionable at various times throughout the twentieth century, however is having another moment in the early twenty-first century, particularly as it may be applicable to cancer treatment. The last part of Chapter 3 is devoted to comparing the CSC and ESC concept, in light of the experiments and observations previously noted in this chapter, and Chapter 1.

Continuing to examine the links between pathological and non-pathological development, Chapter 4 continues where Chapter 3 concluded, with the research on teratomas in the mid-twentieth century. Since ESCs were not available for experimental research, teratoma cells were considered a useful alternative. After developing methods to culture these murine embryonal carcinoma cells (ECCs), similar techniques were applied to mouse embryos, in order to isolate and culture mouse ESCs; this is explored in Chapter 4. Also of significance is what the researchers who isolated mESCs, Gail Martin (USA) and Martin Evans (UK), decided to do with their new tool. This chapter argues that science policy and economics affected the research directions taken in the USA and the UK by Martin and Evans respectively. This therefore requires some description of the social, political, and economic situation of the 1980s in the UK and USA, followed by an investigation of how this affected research. The chapter concludes that, despite the capitalist ideologies of the USA, there was more funding to carry out fundamental, or 'pure' research available to Martin. In contrast, under Thatcher, Evans needed to

demonstrate applicable outcomes for his research, driving him to develop murine models of disease in the UK.

The fifth chapter of this thesis provides an in-depth consideration of a claim made by philosopher Michel Morange. In 2006, Morange wrote a paper commemorating the 25th anniversary of the isolation and culture of mESCs in 1981; in his paper, Morange compared this event with the isolation and culture of hESCs in 1998. Morange claimed that mESCs and hESCs were not equivalent because the scientific contexts they were isolated under were different. This included the motivations and goals involved in their isolation and culture. Chapter 5 therefore examines the events which led up to the isolation and culture of hESCs (mESCs were described in Chapter 4). The role of the American biotechnology company Geron is considered, since Geron funded the 1990s work of both Thomson and Gearhart. The chapter concludes that Morange was correct in his assertion that the scientific context was different between 1981 and 1998, although there were also some parallels between the projects of Martin and Evans, and Gearhart and Thomson. This chapter offers a further, complementary addition to Morange's argument: the political, social, legal, and ethical context of the work Thomson and Gearhart carried out were different to those same contexts in the late 1970s and 1980s (which was partly referred to in Chapter 4). The chapter concludes that context is important for assessing motivations and results of research.

The last research chapter of this thesis queries the paradigm under which stem cells have been studied over the past two centuries, and proposes an alternative paradigm for future stem cell research. To support this claim, some work is required to better understand the philosophy of stem cell research. The first half of Chapter 6 makes use primarily of the research from philosophers Melinda Fagan and Lucie Laplane, who have been the first to carry out any in-depth consideration of stem cell philosophy. Taking the work of Laplane and Fagan as a starting point, Chapter 6 considers the importance of the definition of stem cells (i.e. the ability to both self-renew and differentiate), and whether stem cells may be natural kinds. The chapter also discusses the importance of cell fate on our understanding of stem cells. Cell fate, as previously highlighted throughout the thesis as an important factor in the concept of the stem cell, and stem cell research, is discussed from the position of the niche, embryonic cells, molecular markers, and plasticity. This last approach demonstrates that the current stem cell paradigm is still being used in the laboratory in

the twenty-first century: the stem cell entity paradigm. The examples used in this chapter suggest that it may be time for a different approach to understanding stem cell biology: 'stemness' (i.e. the properties of self-renewal and differentiation) as a 'state', or phase, that cells can pass through at different times in their life. This means that potentially, any cell has the potential to become a stem cell. As a way of testing the potential of this suggested new paradigm, results of previous stem cell research beliefs and experiments are examined. It is argued in this chapter that results of stem cell research in the twentieth and twenty-first centuries support the state view, and that perhaps a change in the stem cell concept is required for development of the science in the twenty-first century.

Through these chapters, the three overarching research questions of this thesis can be answered. Although specific to stem cell research, some of these responses could be extrapolated to cell biology, biomedical science, and even biological science in general.

The first query considers the importance of political and social influence in science, evidenced in this thesis as occurring from the nineteenth to the twenty-first centuries. For example, in Chapter 1, the political and social situation in Eastern Europe affected the language that ultimately became used in cell biology, which affected conceptualisations of various cell types. The switch in most-used language in biological science, from German to English, also occurred due to the political situation in Germany and surrounding countries at the beginning of the twentieth century; the threat of war led many researchers to move westwards to Britain and the USA. This theme is particularly evident in Chapter 4, which explicitly demonstrates that political, social, and economic contexts significantly affect the research that is carried out. Chapter 4 shows that the importance of application of research led to Evans' creation of genetically engineered mice, whereas Martin was able to continue with 'pure' research. In Chapter 5, Morange's claim that motivations and goals between mESC and hESC isolation were different, because of the different scientific contexts the four researchers - Martin, Evans, Gearhart, and Thomson - were working in. In addition to this, Chapter 5 also demonstrates that the political and social context also significantly influenced the work of the four researchers. The specific examples provided in this thesis have not been documented in this manner previously. It is useful to expore these particular examples since the isolation and culture of

mESCs and hESCs are arguably pivotal moments in stem cell research, and an understanding of how social and political factors influence such key research may help to influence policy in the future.

Another question queries the importance of cell fate in stem cell studies. As mentioned in Chapter 1, Haeckel's *Stammzelle* concept emerged out of the importance of potential: in the fertilised egg was the potential to develop into an entire being, including all of the specialised cells, tissues, and organ systems required. Cell lineage, or cell fate studies also became a focal point of research in early twentieth-century USA, as previously highlighted by Maienschein. Utilising cell fate research to learn more about stem cells also appears through Chapter 2, particularly via the discipline of heredity and genetics (see also below). Chapter 3 in particular demonstrates the importance of cell fate studies, not only for ESC studies, but in CSC studies as well. Throughout this chapter, comparisons between normal and abnormal cell fate appear in much of the research carried out, ultimately demonstrating the influence of identifying parallels between non-pathological and pathological growth and development. This is followed in Chapters 4 and 5, as Martin and Evans utilise the ability of ESCs to vary their cell fates in their different research projects through the 1980s, and Gearhart's and Thomson's knowledge of cell fate directs their approach to isolating hESCs in the 1990s. Lastly, in Chapter 6, the importance of differentiation ability, or the ability to change cell fate, becomes particularly important for developing our understanding of stem cell properties. In the late twentieth century and early twenty-first century, it has become apparent that cell fate is not a one-way street (as proposed, for example, by Conrad Hal Waddington [1905-1975]). Instead, it has been shown that cell fate is significantly more flexible than previously thought, leading to the distinct possibility that cells can move into and out of a stem cell state throughout their lives. Cell fate and cell fate research have not previously been considered in the context of ESC research in this detail. This thesis especially focuses not only on cell fate itself, nor only on cell fate research, but a combination of both. This is important since it demonstrates how theory impacted on practice (i.e. how ideas about cell fate impacted on research into cell fate). The lack of current research into the link between cell fate research and ESC research is unacceptable, since this thesis shows that each affects the other. Therefore, a history of one is incomplete without a history of the other. Cell fate is also an important

concept in the development of the stem cell concept, and the impact this has on paradigms such research is carried out under (see below).

Lastly, the thesis asks about the importance of paradigms in ESC research, in particular the genetics paradigm and the stem cell entity paradigm. As the idea of the stem cell was evolving in the nineteenth century, it became entwined with the notion of potentiality; this was perhaps why Haeckel believed that an additional term was required, rather than 'fertilised egg'. Haeckel's *Stammzelle* was initially the first unicellular organism, from which all other life could evolve. Shortly after, Haeckel also referred to the fertilised egg as the *Stammzelle*, since it had this potential for creating an entire organism. Part of this potential however was to make use of those traits inherited from both the mother and father in the creation of an entirely unique individual. Chapter 2 focuses on the genetics paradigm in stem cell research, and how both the study of embryology and heredity were developing through the late nineteenth and early twentieth centuries, becoming the discipline of genetics in the later twentieth century. As this was occurring, so was the expanding science of stem cell research; just as the structure of DNA was becoming understood in the mid-twentieth century, so were stem cells being isolated and available for experimentation. This led to the two disciplines developing together, arguably with the application of genetics influencing much of stem cell research through the late-twentieth and into the twenty-first centuries. For example, as in Chapter 4, Evans made use of the understanding and application of genetics to his mESCs, resulting in production of genetically modified mice; this was considered such an achievement that Evans and his colleagues were awarded a Nobel Prize. Chapter 6 also demonstrates how important genetics has become to biological science; a particular section of this chapter explores the efforts made by some researchers to find genetic markers of stem cells. Genetics, by the late twentieth century, had become so crucial to biology that cells were becoming defined based on their genetic profiles. It was a natural development from this to find such a genetic profile for stem cells then. Despite the efforts of several groups, obtaining a particular genetic profile for stem cells has not been achieved. This failure to obtain what has become so fundamental to our concept of cell type has been a contributing factor to our doubting whether stem cells are entities at all. Although the genetics paradigm of the twentieth century has been written about several times previously, it has not often been considered specifically in the context of embryonic stem cell research (although there are of course links to

embryology and development). It is important to investigate the specific effect of the genetics paradigm on stem cell research as arguably, stem cell research is a significant discipline of the late twentieth and early twenty-first centuries. Since so much of the experimental design is carried out under the genetics paradigm, its effect on stem cell research is essential to explore. Moreover, the additional exploration of the stem cell entity and stem cell state views is also important for future experiment design. This thesis argues that most (if not almost all) stem cell research has been carried out under the assumption that stem cells are entities; if, as this thesis argues, stemness is a state, it will require scientific researchers to change their appriach to stem cell research, and may allow us to learn more, and better understand the properties that can both cause us harm (as in cancer), or provide hope for treatment (as in bone marrow transplants).

As noted above, this thesis begins with an overview of the conceptualisation of the stem cell, required to appreciate exactly what a stem cell is and how these ideas emerged. This follows in Chapter 1.

CHAPTER 1:

THE CONCEPTUALISATION OF CELLS, AND THE EMBRYONIC STEM CELL

1. Introduction

The overarching aim of this chapter is to demonstrate how the term 'stem cell' came about in German (*Stammzelle*), then English; and in particular, how did the concept of an 'embryonic stem cell' come to light? This includes examination of the earliest definitions, and contexts of the term's use. To achieve this, four smaller goals are sought. Firstly, how biologists came to understand what a 'cell' was, and how they are created. Secondly, how biologists connected the cells observed in the early embryo to those in the adult. Once the idea of a 'stem cell' was in place, how did this concept fit into existing ideas, particularly regarding development? Lastly, what were the different research directions in early-twentieth century USA and Europe, and how were these affected or influenced by the concept of an embryonic stem cell?

In order to achieve this aim and answer those questions set out above, this chapter will begin with a consideration of nineteenth-century European biology, with a particular focus on Germany; this includes some examination of the political environment of the era, especially how international politics affected the reception of work and ideas from various research groups. Heavily influencing cell biology of the nineteenth century, Theodor Schwann's (1810-1892) Cell Theory will be considered, including Schwann's influences, and the role of Cell Theory in understanding multicellular organisms. Since Schwann's Cell Theory was refuted relatively soon after publication, it is also important to consider why and how the supposed 'unifying theory' of biology was debunked. Since this thesis has a focus on embryonic stem cells, it is also useful to examine the state of embryology during the nineteenth century, and what was understood about early development. This includes the theory that the egg was a cell, and identifying that cells of the early embryo were analogous to the cells of the adult.

In addition to an examination of nineteenth-century biology, it is also useful to consider the use of language and its connotations when querying the origins of 'stem cell'. The first individual to publish the term *Stammzelle* was Ernst Haeckel. Understanding how Haeckel came up with the term, and how it became the dominant term from a pool of other phrases, is also useful in developing an understanding of the concept of the stem cell.

Lastly, the chapter will consider what was occurring in early twentieth century stem cell research, since there appears to be a difference in research direction regarding cell biology of this period, with USA researchers more interested in developing an understanding of cell lineages. In addition, the movement of several researchers from Germany and Russia to the USA had a general effect on publication language, as well as research interests in different regions. Examination of this will aid in further understanding of the embryonic stem cell concept.

2. Early studies in embryology and development

2.1 Earliest theories

Through the seventeenth century, observations into embryology were made by anatomists Hieronymus Fabricius ab Aquapendente (1537-1619) (*De formatione foetus*, 1604), William Harvey (1578-1657) (*Exercitationes de generatione animalium*, 1651), and physician Marcello Malpighi (1628-1694) (*De formatione pulli in ovo*, 1673) for example.

Italian physician Marcello Malpighi demonstrated that the microscope was useful for embryology, anatomy, and physiological studies, greatly extending knowledge regarding structure and function of the human body. Malpighi's work was known not only in Italy, but was popular with the Royal Society in England[58]. Another member of the Royal Society, Robert Hooke (1635-1703), developed his microscope in the 1660s, writing a book, *Micrographia* (1665), based on his observations. Amongst items described in *Micrographia* is cork, which Hooke described as comprising "cells" or "little Boxes"[59], comparing them to the 'cells' of monasteries, where monks would sleep.

A significant discussion of the seventeenth century was between the ovists and spermists. In 1678, Dutch textile merchant-turned microscopist, Anthoni van Leeuwenhoek (1632-1723) identified spermatazoa. Leeuwenhoek, and those who followed his work, believed that the sperm was the germ, considering the egg a nest required for 'hatching'. The opposing belief was that the egg was the true germ. The ovists (or ovulists) believed that the sperm were relatively insignificant[60].

[58] Lancaster, 2014 p 29-30
[59] Hooke, 1665.
[60] Harris, 2000.

A second discussion point of the period concerned the epigenesists and the preformationists. The epigenesist argument was that the development of an egg or embryo was an entirely new construction or creation. The preformationists argued however that embryonic development was akin to a flower bud unfurling: the embryo was preformed. As Harvey remarked in *Exercitationes de generatione animalium*, "the vegetal primordium whence the fœtus is produced...pre-exists"[61]. This latter approach was favoured by noted microscopists Malpighi and Jan Swammerdam (1637-1680), as well as Albrecht von Haller (1708-1777) and Charles Minot (1852-1914)[62].

2.2 Experiments in development (to 1800)

Neither the epigenesis/preformation nor ovist/spermist debates were resolved in the seventeenth century; little was achieved in the eighteenth either. It is likely that this is due to the lack of experimental progress that was made in embryology, which in turn is a demonstration of slow technological progress during this period. This said, some researchers were still concerned with developmental biology, however no works really surpassed the excellent studies carried out by Malpighi a century prior. One individual who did concern himself with embryology was Haller, who in the mid-1700s, carried out dissections on animals post-mating. Focusing on larger mammals, such as sheep, Haller would claim that he could see nothing for the first fortnight post-mating, and that only a fluid substance could be observed in the uterus. This fluid, Haller claimed, 'curdled' (*gerinnen*)[63], forming the embryo. Haller's conclusions were considered to be plausible and the most reliable explanation, leading his theory to be taught in universities across Europe through the late eighteenth century[64]. There was an opposing theory available however. In 1797, Scottish anatomist William Cumberland Cruikshank (1745-1800) published observations he had been making since the 1770s. Cruikshank dissected rabbits after mating. He reported seeing ova three days post-mating in the oviducts, and four days post-mating in the uterus. Cruikshank made significant numbers of observations which were eventually published in the *Philosophical Transactions of the Royal Society*; Sarton

[61] Harvey, 1847 p 465.
[62] Harris, 2000.
[63] Translation by Sarton, 1931.
[64] Harris, 2000.

suggested however that there was such faith in Haller's theory that Cruikshank's work was given little consideration[65].

2.3 Fertilisation to embryo (1800-1840)

Formation of the chick embryo had been studied for centuries; one of the earliest works describing such observations is *De generatione animalium* by Aristotle. Despite this, no connection had been made between early embryonic development and (what we now understand to be) cells. So far, there was also no evidence to show that there was any correlation between the embryonic development of mammals and the development of birds, for example.

Jean Louis Prévost (1790-1850) and Jean Baptiste Dumas (1800-1884) described the furrowing of frog eggs after treating them with fluid expressed from frog testicles[66]. For them, the segmentation occurred only after fertilisation. Although initially they only appear to describe the changes at the surface, their comparison of the dividing egg to a raspberry suggests that Prévost and Dumas understood that this was not only a surface phenomenon.

Mauro Rusconi (1776-1849), whilst at the University of Pavia, built on the work of Prévost and Dumas, describing in detail the 'segmentation' of the egg post-fertilisation. Rusconi carefully described 'furrows' on the surface, which he observed to eventually result in division and subdivision, creating ever-smaller units. Rusconi's comprehensive descriptions (and significant experimental detail) indicate that he clearly understood the process that was occurring[67]. For instance, after these first subdivisions, Rusconi described the development of *une masse granuleuse*. Rusconi published his observations in 1834, although he had started his studies in 1826. Like Prévost and Dumas, Rusconi also made it clear that he believed segmentation occurred post-fertilisation; in Karl Ernst von Baer's (1792-1876) work (which mostly ignored Rusconi's findings), he theorised that the segmentation previously described by Prévost and Dumas occurred before fertilisation. von Baer believed this process occurred in order to give all parts of the egg access to the sperm.

[65] Sarton, 1931.
[66] Prévost and Dumas, 1824.
[67] Harris, 2000.

2.3.1 Identification of 'embryonic cells'

English physician Martin Barry (1802-1855) was the first to identify the individual cells of the early embryo, comparing them to cells of the adult. In three papers titled *Researches in Embryology* published in 1838, 1839, and 1840 in the Royal Society's *Philosophical Transactions*, Barry described the mature egg, fertilisation, and the early development of the mammalian embryo in a fashion that makes it clear he believed that the subdivision of the fertilised egg was equivalent to cells observed and described in adults.

In *Researches in Embryology: First Series* (1838), Barry began with a brief history, considering Regnier de Graaf's (1641-1673) theory that the ovum existed pre-formed in the ovary, and Haller's opposition[68]. Later, Cruikshank would support de Graaf's theory, but lacked the evidence to be taken particularly seriously. Evidence was collected by Prévost and Dumas, and later von Baer (as noted above). Barry himself went to Germany to work with Johannes Müller (1801-1858) and his students to learn about animal development and microscopy (see section 3). The skills Barry learned enabled him to dissect and section mammalian ovaries; the *First Series* described his observations regarding ova development, maturation, structure and size. Barry believed that the germinal vesicle was formed first.

The *Second Series* (1839) focused on development of the ovum, tracing the early stages of development. Barry noted that there was still a "dark period" (between mating and appearance of vertebrae) in mammalian development - little was understood regarding this time, and Barry aimed to shed some light[69]. To help him, Barry used rabbits (although one of the carefully drawn figures also includes the ovum of a tiger!). Barry claimed to have examined hundreds of ova, both through dissections and preserved samples, carefully measuring and drawing what he saw. The figures in Barry's paper clearly reflect this attention to detail.

[68] Here, it is likely that Barry is referring to Haller's earlier views. As a student, Haller followed the teachings of Herman Boerhaave (1668-1738), whose lectures he attended in Leiden (1725-1727); Haller therefore supported preformationism, with a bias towards the male. In the 1740s, Haller switched his allegiance, believing that the epigenesis theory was the more likely explanation. Haller changed his mind for a third time however, having carried out work on chicken eggs. For the latter years of his life, Haller became an ovist preformationist. This suggests that Haller would have supported de Graaf's theory that the ovum exists pre-formed in the ovary. (For a more detailed discussion of Haller's thoughts on embryology, see Roe, 1975.)

[69] Barry, 1839 p 307.

Barry described his stages of development in intricate detail alongside his figures. For example, in the sixth stage of development, Barry described the second set of cell divisions:

"The centre of this fluid was occupied by four large vesicles. These vesicles were spherical, but somewhat flattened…Some of these vesicles presented in their interior a minute pellucid space, which may possibly have been a nucleus"[70].

A note with the figure suggested that Barry observed a nucleus in all similar vesicles he observed. The seventh and eighth stage resulted in more vesicles, although smaller. By the ninth stage, Barry stated that the small vesicles hung together like a "mulberry"[71]. At the tenth stage, there were even more vesicles, and within each was "an object resembling the 'germinal vesicle-like nucleus' observed by Valentin in 'globules' from various parts of the nervous system"[72]. Here then, Barry is clearly indicating that what he is seeing in the developing embryo can be directly compared with the adult cells observed[73]. By doing this, Barry is establishing that what he sees at the embryonic level is analogous to the 'subunits' of adult animals[74]. Although Barry refered to 'vesicles' as opposed to cells, I do not think this is particularly significant; the term *Zellen* only came into common use following publication of Theodor Schwann's (1810-1882) 1839 monograph popularising Cell Theory (see below). Prior to this, 'vesicle' was a term used to describe what we would now consider to be 'cells' (in both plants and animals).

Carl Bergmann (1814-1865), whilst working as Rudolf Wagner's *Assistent* in Göttingen, would also describe furrows in a similar fashion to Prévost and Dumas. Bergmann's work studying the eggs and embryos of newts in the late 1830s and early

[70] *ibid* p 323.
[71] *ibid* p 324.
[72] *ibid* p 324-5.
[73] Barry is referring to Gabriel Gustav Valentin (1810-1883), a German physiologist, known particularly for his descriptions of cells in nervous tissue.
[74] A further indication that Barry understood the 'vesicles' of the early embryo as those which would become the 'vesicles' of adulthood is in the discussion of methods. Barry utilised the most modern techniques available for his observations, which were primarily histological methods. For example, in the *Second Series* paper, Barry described using 'kreosote water' for preserving ova. This is a solution Müller had shown to Barry, which he used to preserve tissues of the nervous system. Barry must have believed that the 'substance' of the ovum must be similar to the 'substance' of nervous tissue to believe that Müller's kreosote water would be as useful for preserving ova as it was nervous tissue.

1840s made him conclude that the 'furrows' observed were cell divisions, and that these cell divisions gave rise to the cells of the embryo.

Shortly after Bergmann's observations were published, H. Bagge published his dissertation[75]. In his work, Bagge claimed that the successive segmentation of the egg eventually gave rise to the cells of the embryo in nematode worms. Furthermore, Bagge described the division of the nucleus prior to division of the cell in *Ascaris*. Professor of zoology and anatomy at the University of Königsberg, Heinrich Rathke (1793-1860), came to similar conclusions as Bagge, although Rathke studied other invertebrates. Rathke's work was published just a few months later in 1842. As opposed to 'furrows', Rathke preferred the term *Durchfurchung* ('cleavage'), when describing the segregation of the egg. Such a term suggests that Rathke wanted to make clear that this was not only a surface phenomenon. Likewise, German embryologist Adolf Grube (1812-1886) also used the tern *Durchfurchung* in his description of division of the fertilised leech egg[76]; this has been translated to "fissures", leading to clear division of the fertilised egg into four blastomeres[77].

3. Nineteenth century biology: a review

3.1 The cell as the basic unit of life

"Give me an organic vesicle endowed with life and I will give you back the whole of the organised world."[78]

3.1.1 'Vital phenomena'

Henri Dutrochet (1776-1847) is known for highlighting two fundamental ideas in the life sciences: the search for the identity of the vital phenomena in plants and animals, and his particularly materialistic view of the phenomena of life. In achieving these aims, Dutrochet, following German physiologist Kasper Friedrich Wolff (1735-1794), considered cells as physiological entities, where the most basic biochemistry of life occurred. Dutrochet introduced the ideas of endosmosis and exosmosis, and experimental work followed; these were the first steps towards contemporary cell

[75] Müller, 1842; Harris, 2000.
[76] Grube, 1844.
[77] Blyakher, 1955 p 520-527.
[78] Raspail, 1833 p 547.

physiology[79]. It is possible that through his work, Dutrochet was one of the first to observe an animal cell, likely to have been a ganglion cell[80].

In the mid-nineteenth century, many German anatomists were using new microscopy techniques to establish the structure and function of the cell. François-Vincent Raspail's (1794-1878) approach however concerned cell chemistry, much as Dutrochet's had done, considering cells as little laboratories. Raspail was also a pioneer in microscopy, developing methods to freeze tissue samples and slice thin sections for microscopy; this helped Raspail in his cytochemistry work, only really appreciated long after it was carried out. Harris suggested that this may have been the case for three reasons (none of which are related to the quality of his research): firstly, Raspail was a French republican, who was imprisoned on more than one occasion. For a ten-year period (1853-1863), Raspail lived in exile in Brussels. In addition to his political beliefs being an obstacle to the acceptance of Raspail's work, Harris suggested his work was ignored since Raspail eventually became interested in medicine and therapeutics, leaving 'pure biology' behind. Lastly, Harris refered to the conflict between the Germans and French of the era - influential German scholars failed to appreciate Raspail's contributions to cell biology and microscopy[81]. This is an early example of politics affecting scientific belief - a theme that will be returned to.

3.1.2 Cell Theory (1839-1855)

The Breslau School

Johann Evangelista Purkyně (1787-1869) was a Czech-born scholar working in Germany. The war between Germanic and Bohemian peoples in the seventeenth century had resulted in increasing Germanisation of the Bohemian peoples, including the Czechs. The Bohemian peoples were considered second-class citizens, regardless of the continued Germanisation of the region through the eighteenth century. Such Germanisation included academia - the University of Prague, founded in 1348, although open to Czechs, Poles, and Germans, was an entirely German-speaking establishment by the mid-eighteenth century. The *Kulturkampf* of the mid-nineteenth century saw the rise of an educated middle-class who identified as Czech - Purkyně

[79] Harris, 2000 p 28-9.
[80] *ibid*.
[81] *ibid*.

was one such individual. Purkyně attended the University of Prague and became chair of pathology and physiology at the University of Breslau (Wroclaw) from 1823 (before returning to Prague in 1859, as professor of anatomy and physiology). Purkyně, the Czech, however was given less consideration than his German colleagues, due to his ethnic background; Purkyně also occasionally published in Czech, greatly reducing the readership for his work. This said, Purkyně established a following - a 'school' - at Breslau.

Breslau, founded in 1811, was known for its rivalry with the University of Berlin, founded a year prior[82]. Purkyně's counterpart at Berlin, Johannes Müller, established his own school. Purkyně was in the midst of nineteenth-century German biology, at a university with high-standing, and able to participate in ongoing debates with Berlin scientists. Müller occupied a more dominant position in German biology at the time however (given Purkyně's Bohemian roots), and it was predominately Müller's theories that became popular[83].

More recently, closer study has revealed the importance of Purkyně's work, even if it was only selectively noted by Müller and his students. Harris suggested that in fact it was Purkyně who was the pioneer of Cell Theory[84]; evidently, Harris was not alone. In his 1828 publication *Über Entwickelungsgeschichte der Thiere*, von Baer concluded that Purkyně had already exhausted the topic[85].

Unlike the prolific publication of Müller and his students, much of Purkyně's school's works were recorded in theses, reports, and lectures. Theses of Purkyně's students reveal that Breslau scholars were interested in animal tissue and cell structure, made comparisons between animal and plant tissue, and identified the cell nucleus. Purkyně's term for the cell at the time was *Körnchen* (i.e., a granule), a term that was supposed to draw focus onto the interior of the cell[86]. The Breslau school were known for their significant contributions to microscopy, leading some to dub Purkyně's laboratory as the 'cradle of histology'[87].

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[82] Although Berlin was known for being an innovative university, it is likely that this is due to state pressure, rather than enlightened faculty. Weindling, 1981.
[83] Harris, 2000.
[84] *ibid*.
[85] von Baer, 1828.
[86] The slightly later term *Zellen*, preferred by Schwann and popularised by Schwann's Cell Theory, suggested an empty bag, focusing on the outer membrane and wall of cells. Schwann however identified his *Zellen* and Purkyně's *Körnchen* to mean the same object.
[87] Heidenhain - in Harris, 2000 p 91.

Gabriel Gustav Valentin (1810-1883) was a student of Purkyně, whose work focused on tissue and cell structure, as much of the work at Breslau did. Valentin was a significant individual, whose work on comparing animal and plant cells pre-dated Schwann's by a few years; the Insitiut de France published Valentin's first paper on the topic in 1834. Valentin wrote about his observations in greater detail in work published in 1836; clearly prior to Schwann's published monograph of 1839. Whilst Schwann acknowledged Valentin's work, the comparisons made between plant and animals cells were dismissed as brief observations of morphology[88] (see below).

The Berlin School

Müller, finding himself "somewhat limited" at the university in Bonn, took the unusual step of 'applying' for a vacant role at the University of Berlin in 1832. Carl Asmund Rudolphi, the professor of anatomy and physiology at Berlin (and, incidentally, Purkyně's father-in-law), had died in November 1832. Seeing an opportunity, Müller wrote to Moritz Seebeck (1805-1884), the minister responsible for finding Rudolphi's replacement[89]; despite the unusual approach (it was not typical for individuals to put themselves forward for such jobs), Müller was deemed a suitable candidate and was offered the professorship. Müller's situation at Berlin was unusual for another reason: he had no physical laboratory. Instead, he and his students would work in small rooms, niches, and guesthouses, either privately owned or around the university[90]. Despite this hindrance, Müller was fanatical about his work, sometimes spending up to ten hours at a time at the microscope, fuelled by coffee. He would have trouble sleeping, and is known to have had at least five depressive episodes during his lifetime, and possibly committed suicide[91]; perhaps the hallmarks of a man with bipolar ('manic') depression.

Müller's text *Handbuch der Physiologie des Menschen* (1833) was considered to be the 'go to' source for human physiology throughout the nineteenth century. Müller, as well as being an excellent scholar in his own right, trained a school of

[88] Harris, 2000.
[89] Since German universities were state-funded, the state made appointments. Faculties were encouraged to make recommendations, but the state had ultimate authority. Weindling, 1981.
[90] Weindling, 1981; Otis, 2007. Although the state had a very reasonable budget for universities, the ongoing development of institutes and rising student numbers put some pressure on university funding. Many professors believed that their facilities were inadequate; Műller perhaps was an individual for whom this gripe was justified!
[91] Otis, 2007.

microscopists and experimentalists whose aim appeared to be the mapping of the entire human body at the cellular level[92]. Jakob Henle (1809-1885) was Müller's first (and possibly favourite) student. Theodor Schwann was another of Müller's Berlin students, completing his doctoral dissertation in 1834, and becoming assistant to Müller until 1839 - the period he was developing his Cell Theory[93]. Henle's work under Müller referred occasionally to the work of Purkyně, however would highlight Purkyně's archaic terminology[94].

Körnchen *or* Zellen*?*

Around the time Schwann was developing his Cell Theory, the Purkyně school were also developing their theories in cell biology. Schwann was not the only individual at the time who was working towards identification of similarities between plant and animal cells. Publishing *Ueber die Analogien in den Struckturelementen des Thierischen und Pflanzlichen Organismus* in 1840, Purkyně described several analogies between plant and animal cells. There was a significant difference between the two however, according to Purkyně. When referring to animal cells, Purkyně used the term *Körnchen*. Plant cells however were *Zellen*. This implied that Purkyně did not see plant and animal cells as analogous at all[95]. The use of *Zellen* for plant cells suggests Purkyně saw them as mostly fluid, although he did describe solid parts attached to a more solid cell wall. This refers to a focus botanists had with the plant cell wall at the time. Conversely, *Körnchen* were considered to be solid throughout, or rather, the term would draw attention to the interior - in animal cells this was considered to be more important that than the outer cell membrane. Furthermore, Purkyně suggested that in his attempt to find a unifying theory, Schwann relied too heavily on Matthias Schleiden's (1804-1881) plant observations, stating that Schwann should not have applied so much of Schleiden's plant work to animal cells.

The most likely reason for *Zellen* becoming the most popular term (as opposed to Purkyně's *Körnchen*) is the popularity of Schwann's text. The popularity of Schwann's monograph meant that it became particularly influential; Purkyně's work

[92] *ibid*.
[93] Parnes, 2003.
[94] Harris, 2000.
[95] This difference between animal and plant cells had been noted significantly earlier; Leeuwenhoek for example referred to 'free cells' (those without cell walls), and 'animacules' in the 1670s. Magner, 2002; Lancaster, 2014.

was never likely to be as successful as a German's work during the nineteenth century. Harris also claimed that Purkyně's work was occasionally misinterpreted. For example, Karl Bogislaus Reichert (1811-1883) (a student of Müller), considering Purkyně's *Körnchentheorie*, mistook *Körnchen* for 'nuclei', leading him to dismiss Purkyně's account of cell development[96]. Instead, Reichert promoted Schwann's *Zellentheorie*. Reichert referred to this again in other papers written during his career, particularly in the influential journal Müller's *Archiv*, propagating the error and encouraging others to follow suit. As previously noted, Schwann's work became the basis of Cell Theory, whilst Purkyně's theories were considered incorrect and insignificant. There seems to have been little resistance to the use of *Zellen* to describe all cells, since even Purkyně's students are known to have used the term[97].

The botanist's contribution to Cell Theory

Matthias Schleiden (1804-1881) is particularly noted for his work that contributed to Cell Theory; he is also known to have made some significant observations regarding the nucleus and nucleolus. The attitude Schleiden had, which would be unacceptable today, was to not refer to those who had previously made similar observations. For example, in his description of the nucleus, Schleiden noted that he is not the first to observe it, but never mentions who influenced his work. In a further example, when Schleiden wrote about the nucleolus of plant cells, he neglected to say that Rudolf Wagner (1805-1864) had actually described it three years previously[98]. Occasionally this has the effect of leaving the reader wondering where previous work concluded and where Schleiden's began.

Schleiden's significant contribution to Cell Theory was regarding the nature of the nucleus. Schleiden, renaming the nucleus the 'cytoblast', believed that the role of the nucleus was to generate the rest of the cell, and that once this had been achieved, the nucleus was dispensable[99]. Schlieden described the process of cell generation beginning in the *Kern*, and the membrane of the new cell emerging from the

[96] Harris, 2000.
[97] *ibid*.
[98] *ibid*.
[99] *ibid*.

cytoblast; in his Cell Theory, Schwann would also decribe this process in animal cells[100].

The zoologist's contribution to Cell Theory

Schwann, a loyal Müller-trained scholar of the Berlin school, is well-known for his contribution to Cell Theory. In order to better understand Schwann's contribution to Cell Theory, Ohad Parnes has closely examined the available notebooks of Schwann, written between 1836 and 1838. Although these are not complete[101], Parnes shows how these notebooks can offer some insight into Schwann's approach. Regarding Cell Theory, Parnes stated that despite the time period over which these notebooks were written, they offer no clear declaration that Schwann was embarking on a quest to find any elementary 'building block' of both animals and plants, despite a perception that this is what Schwann was setting out to do[102]. Instead, Parnes added, Schwann's notebooks appear to be "documentation of a persistent and consistent attempt to make sense of life in terms of causal agencies"[103]. Instead of using observational techniques (such as microscopy) to simply look for similarities between plant and animal life, it should perhaps be the case that Schwann should be equally well-known for his contribution to scientific methodology; Parnes claimed that Schwann's late 1830s work was aimed at introducing new experimental design into physiological studies. Through these new methods, the causal relationship between the specific agents required to produce specific physiological phenomena could be found[104]. This prevented 'vital forces' being resorted to as an explanation for physiological occurrences. The question we should actually be asking then, Parnes highlighted, is not where Schwann conceived of the idea that a cell was a fundamental building block, but where did he get his motivation for changing the way physiological research was carried out[105]?

[100] Parnes, 2003.
[101] Parnes suggested that this may have been because Schwann simply removed those pages of his notebooks in which he had written work for publication. This in turn suggests that Schwann perhaps did not spend a significant amount of time agonising over details of the papers he sent for publication, preferring to send them as initially drafted, alongside his practical notes and experimental results and observations. Parnes, 2003.
[102] For example, as in Harris, 2000.
[103] Parnes, 2003 p 120.
[104] Parnes, 2003 p 121.
[105] *ibid*.

Parnes began his investigation into Schwann's motivations by claiming that Schwann was preoccupied with the "foundations of physiology"[106]; perhaps this is where previous errors have been made, assuming that such a 'foundation' was the search for unity. Schwann's first step appears to have been "banishing the question of the seat of the mind from physiology"[107]; this enabled one to resist any temptation to opt for 'vital phenomena' as an explanation for physiological phenomena, and required instead an account based on causal relationships. Schwann began this work, according to his notebooks, by studying muscle contraction, then stomach digestion; each of these projects should, Schwann reasoned, enable him to identify the causal agents involved, if he could get the experimental design correct. For his digestion experiments (carried out between 1835 and 1836), Schwann was keen to understand the *Wesen* (essence) of the digestive process; prior to Schwann's work, whilst the process was understood as a chemical reaction, it was belived that a 'vital environment' was needed for this reaction to occur[108]. A few months of experiment demonstrated to Schwann that this was not the case; instead, an enzyme was the responsible causal agent, which he named 'pepsin'.

These experiments that Schwann carried out in the mid-1830s, are, Parnes argued, vastly important for modern experimental research in the life sciences; the epistemological step that Schwann took enabled researchers to characterise specific physiological causative agents (which could not have been found through previous methods, that relied on observation or chemical precipitation, for example)[109]. It was this approach to work in the life sciences that would elevate biology (to be considered alongside other sciences), since it took its methodology from chemistry and physics. Again, this "epistemological step"[110] is Schwann's 'unifying' achievement in physiology, not Cell Theory *per se*.

To really test his new approach to physiology then, Schwann needed to move beyond experiments on single systems (such as muscle contraction or stomach digestion); Schwann had to show that this methodology could be applied to all physiological processes in all creatures. To this end, he moved onto the study of

[106] *ibid*.
[107] This quotation is taken from a letter written by Schwann to his brother, in March 1835, which Schwann included in his notebook (translated by Parnes); Parnes, 2003 p 121.
[108] Parnes, 2003 p 124.
[109] Parnes, 2003 p 125.
[110] *ibid*.

respiration, and designed an experiment through which he could demonstrate that *infusoria* (mirco-organisms) required a specific agent in air to be able to respire. Schwann heated the air to destroy the agent, and found that the *infusoria* could not live under these conditions[111]. Between 1835 and 1837 then, Schwann had carried out several experiments that demonstrated the usefulness of his new methodology.

In February 1837, Schwann's notebooks reveal that he was carrying out fermentation experiments (showing that yeast was the agent of alcohol fermentation, for example). Schwann identified yeast as a microscopic plant, arguing that the actual place where fermentation took place to be within the 'globules' (cells); Parnes argued that the construction of plants appeared to be made-up of these 'globules' was a generally accepted view, however Schwann was the first to identify that a physiological process was occurring inside such a structure[112].

In 1879, Schwann claimed that it was Schleiden who inspired his intellectual curiosity into cells. Schlieden, Schwann claimed, was telling him about the role of the nucleus in development of plant cells[113]; it struck him that he had observed similar structures in the notochord. "I grasped the extreme importance that my discovery would have if I succeeded in showing that this nucleus plays the same role in the cells of the notochord as does the nucleus of plants in the development of plant cells"[114]. Parnes' reviews of Schwann's notebooks from the time however suggest that Schwann's recollection of his inspiration may have been written through the rose-tinted glasses of hindsight. Instead, Parnes argued that Schwann's conception of cells (as in Cell Theory) was not based solely on simple observation. Instead, Schwann had realised from his conversation with Schleiden that the nucleus might be the causative agent of cell generation[115]. This would explain why Schwann rejected Valentin's prize-winning work of 1834, suggesting that it was simply observational and descriptive; Schwann was looking for something more than similarity of morphology. According to Harris, Schwann argued that such was not good enough to aid in the quest for a general law[116].

[111] For further detail on these experiments, see Parnes, 2003.
[112] Parnes, 2003.
[113] This was later published as Schlieden, 1838.
[114] Schwann, 1879 p 38 (transl. Hughes, 1959).
[115] Parnes, 2003 p 130.
[116] Harris, 2000.

Schwann proceeded to examine how the nucleus was able to generate new cells in animals; his tissue of choice was the branchial cartilage of frog larvae. This work was carried out in the first half of 1838 and, Parnes claimed, became the first part of Schwann's volume *Mikroskopische Untersuchungen über die Übereinstimmung in der Struktur in dem Wachstum der Thiere und Pflanzen*, published in 1839. Schwann concluded in his notebook from early 1838 however that the observation of cells with nuclei in the frog larvae was not enough; "If rigorous proof could be delivered, that in the animal body there exist corpuscles analogous to the plant cell, then we could parallel these two and then we could consider the generation of animal organisation as nothing other than a modification of plant organisation"[117]. Schwann continued with this thought: in order for the parallel to be confirmed, then he needed to show firstly that the structures he observed in the frog larvae were cells, and secondly that the functions, such as "nutrition and growth", also occurred in a similar manner[118].

Following this, Schwann constructed what Parnes referred to as the second part of *Mikroskopische Untersuchungen*, which was drafted in mid-1838. Here, Parnes argued, Schwann begins to move on from observing the similarities between plant and animal cells, to describe similarities in processes[119]. Again apparently inspired from the previous conversation with Schlieden, Schwann's notebooks reveal his investigation into the processes of cell generation, where the membrane of the new cell would develop inside the old cell, emerging from the nucleus. Schwann's study of the process in animal cells provided a functional link between plant and animal cells.

The third and final part of *Mikroskopische Untersuchungen*, as Parnes interpreted Schwann's work, was carried out at the end of 1838, and in January of 1839; it also summarised the work Schwann carried out previously, such as the muscle contraction and digestion experiments from preceding years. Now that Schwann had shown that cells were the fundamental units of animals (just as they were in plants), and that processes were carried out inside these cells (such as the yeast metabolism observation), Schwann could now re-interpret the results of his previous work in light of his new concept.

[117] Parnes, 2003 p 130-131; translation from Schwann's notebook of 1838.
[118] Parnes, 2003 p 131; translation from Schwann's notebook of 1838.
[119] Parnes, 2003 p 132-133.

"We have seen that all organized bodies are composed of essentially similar parts, namely cells; that these cells are formed and grow in accordance with essentially similar laws; and, therefore, that these processes must, in every instance, be brought about by the same powers."[120]

Schwann continued to highlight the ability of cells to change – that is, to differentiate and specialise, through 'chemical alteration'; Parnes interpreted this as making "the specific out of the non-specific". Through these processes, cells, Schwann argued, demonstrated themselves to be the "fundamental, force-exerting principles of life" – a series of processes caused by specific agents[121].

Response to Cell Theory

Following publication of his monograph, Schwann was offered a chair at Louvaine, which coincided with a change in his research focus. There are two prominent hypotheses for why Schwann may have stopped working on Cell Theory. The first is that it jarred against his Catholic beliefs too much for him to be able to reconcile his faith with his research. God was supposed to have created all life (from nothing), yet Schwann's work had shown that spontaneous generation was not an adequate theory for explaining cell formation. How could Schwann believe in both the Catholic doctrine that God created all life, and that cells could not be created through spontaneous generation? Schwann's uneasiness can be observed in his letters to the Archbishop of Malines (the primate of Belgium). The archbishop replied to Schwann's letter, stating that he had no concerns with his work; we have no record of how much this eased Schwann's conscience however. The second theory offered by Harris suggested that Schwann gave up Cell Theory simply because he knew it was incorrect and chose to remain quiet on the topic[122]! This would explain how swiftly Schwann gave-up his work in cytology and development once he moved to Louvaine. It may also explain why Schwann's Cell Theory was disregarded within a decade of Schwann's *Mikroskopische Untersuchungen* being published[123].

[120] Parnes, 2003 p 133; translation from Schwann's notebook of 1839; also in Schwann's *Mikroskopische Untersuchungen*, p 227.
[121] Parnes, 2003 p 134.
[122] Harris, 2000.
[123] *ibid*.

Following the criticisms of Cell Theory after publication of Schwann's *Mikroskopische Untersuchungen*, Schleiden also became less dogmatic in his own works regarding Cell Theory[124]. This is not to say that Cell Theory was discounted entirely however. Cell Theory provided Berlin in particular (but cytology in general) the opportunity to unite anatomy, physiology, botany, and zoology into the latter decades of the nineteenth century, and into the twentieth. This also maintained the organicist view, held particularly in German universities, through the nineteenth century. Professors of this era were concerned with 'the totality of man's nature', and believed that the state's attempts to hire chairs in many specialties undermined the organicist approach[125].

The end of Schwann's Cell Theory

Harris suggested that Schwann's Cell Theory caused significant confusion for embryologists in the ten years following its publication; embryology became split into two schools: those who agreed with Schwann (and who tried to make their observations fit with Schwann's Cell Theory), and those who did not (whose work was interpreted to go against Schwann's doctrine wherever possible)[126]. Despite its theoretical errors, Schwann's monograph was still a useful piece of work for its histology and admirable aim. Since at its core the book held the Cell Theory however, Schwann's monograph was eventually considered to be wholly misleading[127].

In 1855, Polish German embryologist Robert Remak (1815-1865) published his book on embryology: *Untersuchungen über die Entwicklung der Wirbeltiere*. Remak's observations clearly showed the egg membrane and what was occurring during the first few cell divisions – available through Remak's pioneering work on histological techniques. Remak identified that all animals consisted of nucleated cells, and that each nucleus was descended from the first nucleus (created when the egg was fertilised), by cell division[128]. Technical advances finally allowed significant investigative work to begin tracing the transmission of hereditary characteristics[129].

[124] *ibid*.
[125] Ringer, 1969; Weindling, 1981
[126] Harris, 2000.
[127] *ibid*.
[128] Remak, 1855.
[129] Harris, 2000.

3.2 The egg as a cell

Although loyal to Schwann, many of the Berlin school were not entirely convinced by his Cell Theory. For example, in *Allgemeine Anatomie* (1841), Henle devoted a significant part of the second section (*Die Entstehung der Zellen*) to a critique of Schleiden and Schwann's work. Henle argued that although Schwann had attempted to unify plant and animal cells, his model was based on an analogy which was, as yet, unestablished. Henle also disagreed with Schwann's opinion that the egg was a cell (and the germinal vesicle was the nucleus) - Henle instead was of the opinion that the germinal vesicle itself was a cell[130]; such differences of opinion at the time made the mode of generation of the egg uncertain.

3.2.1 Carl Gegenbaur

Carl Gegenbaur's (1826-1903) parents had expected him to become a physician, and sent him to Würzburg University to study medicine. However, Gegenbaur found himself far more interested in the natural sciences. Gegenbaur became the founder of the 'Gegenbaur school' of comparative morphology, initially at the University of Jena, and later continuing the tradition at the University of Heidelberg[131]. Building on current theories of evolution and comparative anatomy (previously developed by Müller), Gegenbaur insisted on a systematic approach to comparative anatomy: a deviation from the traditional method at the time, which was generally based on publishing isolated snippets of observations[132].

In 1861, Gegenbaur produced an influential work in anatomy: the cellular nature of the egg (*Ueber den Bau und die Entwickelung der Wirbelthier-Eier mit partielleer Dotterbildung*) in *Archiv für Anatomie, Physiologie und wissenschaftliche Medicin*. Gegenbaur was the first to identify the egg (of mammals, reptiles, fish and birds) as an individual cell. The cell as the fundamental structural unit, and the basis for life science, was emerging as a new approach; one that was closely followed by Gegenbaur and Haeckel through their careers[133].

[130] Henle, 1841.
[131] Fröber, 2003.
[132] Hoßfeld, Olsson & Breidbach, 2003.
[133] Weindling, 1989.

4. *Stammzelle*

4.1 Ernst Haeckel

Ernst Haeckel was born in 1834 in Prussia, the second son to Charlotte and Carl Haeckel. Carl and Charlotte encouraged their son's interest in nature, as did Haeckel's tutor, Karl Gude. Early in his life, Haeckel read naturalist Lorenz Oken's (1779-1851) *Allgemeine Naturgeschichte für alle Stände*[134]. Oken's search for a system to exemplify the unity of nature ultimately became a concern of Haeckel's, and Oken hinted at a form of Cell Theory: another influence on Haeckel's later work[135]. Haeckel also read Matthias Schleiden's 1848 *Die Pflanze und ihr Leben*, and from this he learned about development (*Entwicklung* [or as Haeckel elected to spell it, *Entwickelung*]), the origins of life, and the cellular composition of living things[136].

After completing his secondary education in 1852, Haeckel found himself at Berlin studying medicine and natural science. After a few months at Berlin, Haeckel moved to Würzburg University, where he had several fortuitous meetings that would influence much of his later life and work. Firstly, the lecturer Albert Kölliker (1817-1905), a morphologist and histologist. Di Gregorio suggested that for Haeckel, Kölliker became a mentor, and they stayed in touch for many years, despite their differences concerning the correct approach to the natural sciences[137]. Secondly, Haeckel met another of Kölliker's students, Carl Gegenbaur (eight years Haeckel's senior, and from a similar social background), a significant influence in Haeckel's early professional life. Whilst at Würzburg, Haeckel also met Rudolf Virchow (1821-1902)[138]. Virchow's political convictions were reflected in his work on cellular pathology, in which he suggested tissues were formed from many individual cells all of equal standing[139]. Virchow provided the hints of an answer to Oken's and Haeckel's concerns regarding the unity of nature, since he argued that Cell Theory, demonstrating that cells controlled the actions of all living things, provided unification for the life sciences. The cells, in turn, were subject to the laws of

[134] These were a series of books on natural science topics, such as botany, and geology. The series were published between the late 1820s and early 1840s.
[135] Di Gregorio, 2005; Richards, 2008.
[136] Wedekind, 1976; Di Gregorio, 2005.
[137] Di Gregorio, 2005.
[138] Pagel, 1931; Pagel, 1945.
[139] Ackerknecht, 1953.

chemistry and physics, representing a further unification[140]. Despite Virchow having such a significant influence on the life sciences as a whole and on Haeckel's work specifically, Virchow and Haeckel never became friends; Ackerknecht suggested that this was simply down to a difference in personality. Haeckel was insecure yet warm-hearted, whilst Virchow was over-confident and cold[141].

In 1854, Haeckel returned to Berlin, where he could attend lectures by Johannes Müller, another significant influence. Di Gregorio observed that Haeckel's earlier works in particular appear based in Müller's physiology and morphology teachings; Haeckel also considered himself a "philosophical scientist", after his lecturer Müller[142].

Despite taking up a minor academic position at Würzburg in 1856, Haeckel returned to Berlin in 1857 to submit his dissertation on crayfish, and was declared Doctor of Medicine shortly afterwards. In 1858, Haeckel set-up a small medical practice (which he loathed) in his father's house, and became engaged to his cousin, Anna Sethe[143].

By the late 1850s, Gegenbaur was a well-respected member of the Jena faculty, and was able to put into practice his new approach to natural science: separation of anatomy and zoology. Gegenbaur became the chair of anatomy, but needed an associate for the work in zoology. Gegenbaur selected Haeckel for this role. In May 1858, Haeckel first met with Gegenbaur and the university *Kurator* Seebeck to discuss a research trip to Italy. Gegenbaur fell ill, leaving Haeckel to go on his own. Haeckel's trip, including the Gulf of Naples and the small island of Ischia, formed the basis of his work for many years[144]. It also enabled Gegenbaur to officially offer Haeckel a post at Jena in 1860[145]. Once in his new job, Haeckel was able to write-up his experiences in Italy in his first monograph: *Radiolaren* (1862). Another name that appears several times in *Radiolaren* is that of Thomas Henry Huxley (1825-1895), the English naturalist. Huxley had taken his scientific approach

[140] *ibid*; Bynum, 1994.
[141] Ackerknecht, 1953.
[142] Di Gregorio, 2005 p 46.
[143] Di Gregorio, 2005.
[144] Haeckel's student, Anton Dohrn, would later (1872) found a zoological station on the island of Ischia, a particularly prestigious research centre amongst several new marine labortories being set-up in Europe and America. Hopwood, 2009.
[145] Gegenbaur, learning that Haeckel was also seriously considering a position at the Berlin Academy of Art, promised him swift promotion to Extraordinary Professor in December 1860. Di Gregorio, 2005.

from the German tradition, and was also concerned with finding natural systems. Like his German contemporaries, Huxley was looking for a unifying theory of life, approaching the problem using Cell Theory[146].

Together at Jena, Gegenbaur and Haeckel strongly influenced the field of developmental biology, encouraging biologists to recognise its relevance to evolution (and vice versa); to them it was obvious that understanding ontogeny (*Entwicklungsgeschichte*) was essential to understanding phylogeny (*Stammesgeschichte*)[147]. (This became the basis of biogenetic law, which Haeckel expressed as 'ontogeny recapitulates phylogeny'; the hypothesis was that during development, the embryo would go through phases that resembled the creature's previous evolutionary phases[148].) Moreover, the concept of homology in anatomy and morphology had arisen in the late eighteenth and early nineteenth century, identifying similarities ('sameness') between structures in various organisms.

4.2 Darwin's influence: Trees

As well as sending a copy to Huxley, Haeckel sent *Radiolaren* to Charles Darwin (1809-1882) in late 1863. Despite his apparent gratitude, it is likely that Darwin never read the book carefully[149]. Haeckel however had clearly been influenced by Darwin's work, and explicitly referred to it in *Radiolaren*, suggesting that Darwin's theory, as outlined in *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life* (1859), was an opportunity for naturalists to understand "the great law of development"[150]. For Haeckel, there was one area of deficiency in Darwin's theory however: the origin of the primordial organisms (*Urorganismen*).

According to Haeckel's own translated copy of Darwin's *Origin*, he began reading it in the summer of 1860; his annotations however suggest that Haeckel did

[146] Di Gregorio, 1982; 2005.
[147] Olsson, 2003. In this paper, Olsson suggested that evolutionary and developmental biology progressed along separate paths through most of the twentieth century. However into the twenty-first, Haeckel and Gegenbaur's belief that the two disciples are relevant to each other has become realised again. Olsson gave examples of more recent studies in the fields, particularly concerning lungfish and amphibians, that show evolutionary and developmental biology are becoming intertwined once more.
[148] Hopwood, 2009.
[149] Di Gregorio, 2005; Richards, 2008.
[150] Di Gregorio, 2005 p 75.

not read the book in detail until 1864[151]. Haeckel's notes suggest he was particularly interested in heredity, external influences on reproduction, and variation; these notes indicate that Haeckel believed Darwin's natural selection explained how heredity and adaptation created new life forms. Haeckel and Darwin's ideas could co-exist[152]:

"the natural system is founded on embryology which distinctly reveals the common descent…many embryos resemble the common root-form[,] the embryo [is] the whole class"[153].

Haeckel's contribution to Gegenbaur's anatomy reforms at Jena was embryology, concluding that the success of these reforms was due to this contribution[154].

4.2.1 *Stamm*

As highlighted above, Haeckel was strongly influenced by Müller and Virchow, and the developing Cell Theory of Schwann and Schleiden. A further important influence was Gegenbaur's suggestion that eggs are cells. From this interpretation, Dröscher argued, Haeckel developed his stem trees; he used terms such as *Stammeltern* (progenitors), and *Stammorganismen* (stem organisms). Dröscher suggested that the German *Stamm* conjures images of genealogies and origin, as well as strength and community[155]. Haeckel was not alone in his use of *Stamm*; the philosopher Immanuel Kant (1724-1804), and other biologists such as von Baer and Gottfried Treviranus (1776-1837) also used the prefix to conceptualise organic organisation. Dröscher suggested that Haeckel's *Stamm* however had a more explicit role both linguistically and pictorially, with terms such as *Stammform* (stem form) appearing frequently[156].

The biological *Stamm* (phylum) and the concept of the *Stammbaum* (literally 'stem tree', but used in the context of a genealogical or evolutionary 'tree') became the linguistic origin of Haeckel's *Stammzelle*. In this context, the stem cell was considered as the cell that first gave rise to unicellular and multicellular organisms of

[151] *ibid*.
[152] *ibid*.
[153] Di Gregorio, 2005 p 82; translation from Haeckel's notes in Bronn's 1860 translation of Darwin's *Origin*.
[154] Di Gregorio, 2005 p 82.
[155] Dröscher, 2012; 2014.
[156] Dröscher, 2014.

the animal and plant kingdoms. Stem cells, Haeckel argued, derived from the *Moneren*; clumps of protein that were thought to be the first forms of life. Monera were considered to be the most undifferentiated beings, originated by spontaneous generation. This is different to the way the term is used in Haeckel's later work, *Anthropogenie* (1877), in which the *Stammzelle* (or *Cytula*) is the cell that gives rise to all other cells of a multicellular organism: the fertilised egg. This appears to be an idea evolved from Gegenbaur and Haeckel's 'biogenetic law': that ontogeny was a recapitulation of phylogeny.

"The name "stem cell" seems to me the most simple and appropriate one, because all other cells stem from it and because it is in its most literal sense the stem father as well as the stem mother of all the countless generations of cells of which later on the multicellular organism is composed".[157]

Since for Haeckel, each organism moved through each of its previous phylogenic states during development, his ontogenetic stem cell (i.e. the fertilised egg) was the equivalent of the single-cell organism: the phylogenetic stem cell.

4.3 *Stammzelle*

Why though did Haeckel believe that a new term was required? What was wrong with the 'fertilised egg' - a concept all biologists would have understood? Haeckel did not define *Stammzellen* in *Natürliche Schöpfungsgeschichte*; Dröscher claimed that he did not need to - that the term was "easily understood as the starting point of evolution and as the basis of his stem trees"[158]. Maehle furthermore suggested that this was Haeckel's effort to distinguish the fertilised egg from the egg cell: the fertilised egg was a combination of mother and father, and represented the future offspring[159]. This is a more likely explanation; *Stammzelle* captures the essence of great potential far more than 'fertilised egg' does.

4.4 *Keim* and *Ur*

Beginning with Haeckel's biogenetic law, and the results of Wilhelm Roux's (1850-1924) experiments suggesting that chromosomes were composed of regions

[157] Haeckel, 1877 (transl. Maehle, 2011).
[158] Dröscher, 2014.
[159] Haeckel, 1877; Maehle, 2011.

distributed amongst daughter cells, August Weismann (1834-1914) developed his model of heredity and development (see also Chapter 2). Following Haeckel, Weismann linked embryology with evolution and heredity; Dröscher suggested that this approach provided a plausible account of the material basis, and conceptual explanation of development[160]. This included theory relevant to stem cells: Weismann suggested that germ cells developed separately to somatic cells, and it was only these germ cells that would pass on hereditary material. Just as Haeckel had utilised the prefix *Stamm*, Weismann introduced *Keim* ('germ'). The germ cells, as carriers of inheritance, possessed all material needed to create the next generation in the *Keimplasma* (germ plasm), and these germ cells developed along the *Keimbahn* (germ track). The somatic cells did not contain all of the *Keimplasma*; instead each cell division resulted in division of the *Keimplasma*, resulting in irreversible differentiation and specialisation. It was the nucleoplasm, Weismann argued, that contained the "hereditary tendencies" of each cell type; the first ectoderm cell would divide unequally, creating a cell containing the nucleoplasm required for the nervous system, and another containing the nucleoplasm needed for the skin[161]. The germ cells however needed the entire nucleoplasm. Weismann believed this was the route of inheritance and development until the 1890s, when he saw that Roux's frog embryo experiments offered a different explanation (see section 5.1). Weismann re-imagined the transmission of information from one cell to the next in the early 1890s: "…the hereditary substance of the egg-cell, which contains all the hereditary tendencies of the species, does not transmit them *in toto* to the segregation cells, but separate them into various combinations, and transmits these groups to the cells"[162]. Accordingly, Weismann placed the *Urzelle* at the start of development; it was the *Urzelle* that contained the potential to become an entire organism. Weismann's cell trees then provided a causal explanation of development as well as a description of it.

Weismann never used Haeckel's term *Stammzelle*, preferring *Urzelle*; the meaning appears similar: a cell that has great potential, a cell that is the ancestral remnant and the beginning of ontogenesis. Weismann's *Urzelle* however seems to be a more specific idea than Haeckel's *Stammzelle*. Weismann described the method of increasing differentiation (either as decreasing complexity or composition) via the

[160] Dröscher, 2014.
[161] Weismann, 1891 (vol. I) p 189
[162] Weismann, 1893 p 205.

nucleoplasm. Haeckel also suggested that there were more external forces at work during development, whereas Weismann placed more emphasis on intracellular events. On this basis then, it is interesting that *Stammzelle* is the phrase that has remained, when *Urzelle* appears to more accurately describe our current understanding of stem cell biology.

4.5 Stem Cell

Jan Sapp suggested three tenets of Cell Theory: 1) that all plants and animals are made of cells; 2) cells possess all of the attributes of life; 3) all cells arise from division of pre-existing cells[163]. Staffan Müller-Wille suggested a fourth: that the cell can be regarded as a 'unit of life'[164]. These observations are crucial, Müller-Wille argued, in allowing biologists to conceive cell populations as a succession of generations. This was crucial for emerging nineteenth-century ideas regarding heredity. In the early twentieth century, the requirement for a unifying approach to biology was falling away again. Cytology, useful to a large array of disciplines, was becoming too large for anyone to comprehend it in its entirety. For this reason, although American biologist Edmund Beecher Wilson's *The Cell in development and inheritance* (1896) covered three decades of changes in the field (the third edition being published in 1926), it was the last work in cytology to have been all-inclusive whilst written by a single author[165].

As well as becoming a known phrase in English (see below), *Stammzelle* was establishing its context in German. For example, Berlin anatomist Richard Weissenberg (1882-1974), previous assistant of Oscar Hertwig (1849-1922), used the term to refer specifically to precursors of egg cells and sperm cells[166].

In 1892, Valentin Haecker (1864-1927), then an assistant of Weismann at the University of Freiburg, used *Stammzelle* with reference to a cell that becomes internalised during embryonic cell migration in crustacean development - the daughter cells of this progenitor give rise to the mesoderm and germ cells[167]. (It has been suggested that as Haecker did not use the term more widely in his publications,

[163] Sapp, 2003 p 75.
[164] Müller-Wille, 2010 p 225. Here Müller-Wille is not repeating the second tenet suggested by Sapp, but is instead referring to the single cell stage of life that all organisms pass through.
[165] Maienschein, 1991.
[166] Weissenberg, 1926; Maehle, 2011.
[167] Haecker, 1892; Maehle, 2011.

it is possible that he 'borrowed' the term from elsewhere[168].) Theodor Boveri (1862-1915) (then working under Richard Hertwig (1850-1937)[169] at the University of Munich) also used the term in a similar way to Haecker[170]. In a lecture given to the Munich Society for Morphology and Physiology on the horse roundworm, Boveri described the cells derived from the fertilised egg that would eventually become the primordial germ cells as *Stammzellen*.

Despite the growing popularity of Haeckel's *Stammzellen*, Dröscher suggested that the term was "too speculative in its evolutionary part, and too generic in its embryological part, to find followers among the experimental biologists"[171] (although this did not take into consideration the relationship of Boveri and Wilson[172]). This is not to say that Haeckel's concept had no influence at all however; *Stammzelle* enhanced the previously rebuffed Schleiden and Schwann Cell Theory. The initial focus on cell walls, membranes, and the nucleus was diverted somewhat to the protoplasm and the behaviour of chromosomes. Just after Haeckel's *Natürliche Schöpfungsgeschichte* was published, Oscar and Richard Hertwig described in detail the merging of the maternal and paternal nuclei during fertilisation, and began investigations into the actions of chromosomes.

The term 'stem cell' was not popularised in English until the publication of Wilson's *The Cell*, in which Wilson reviewed the work of Haecker and Boveri; in fact, Boveri's cell lineage diagrams and drawings were featured in Wilson's text. Boveri and Wilson had previously worked together in the early 1890s at the Zoological Institute in Munich, and had remained friends[173]. Wilson began writing the 1896 edition of *The Cell* between 1892 and 1893, based on lectures given at Columbia University in New York. Initially, Wilson claimed that the book was simply going to be a book of his lectures for a general university student audience; this aim shifted slightly following the publication of Oscar Hertwig's book (*Zelle und Gewebe*, 1893) and his research project investigating the history of centrosome function in fertilisation[174]. This appears to have delayed the publication of *The Cell*

[168] Ramalho-Santos and Willenbring, 2007.
[169] Richard Hertwig had himself been a student of Haeckel.
[170] Boveri, 1892. In the lecture, Boveri explicitly states that he had adopted the term 'stem cell' from Haeckel. Maehle, 2011.
[171] Dröscher, 2014.
[172] For example, see Baltzer, 1967; Maehle, 2011.
[173] Baltzer, 1967.
[174] Wilson, 1896 p vii.

slightly, as Wilson was clearly keen to ensure that the information contained within was as up-to-date as possible. There are only a couple of references to the stem cell in the 1896 version of *The Cell*. These appear in the chapter on *Origin and growth of the germ-cells*. On pages 110 and 111, Wilson wrote "Haecker has recently traced very carefully the origin of the primordial germ-cells in *Cyclops* from a "stem-cell" (Fig. 56) clearly distinguishable from surrounding cells by the early blastula stage, not only by its size, but also by its large nuclei rich in chromatin, and by its peculiar mode of mitosis, as described beyond". Figure 56 is from Haecker's work; the figure legend reads: "*A*. Young embryo, showing stem-cell (*st*). *B*. The stem-cell has divided into two, giving rise to the primordial germ-cell (*g*). *C*. Later stage, in section; the primordial germ-cell has migrated into the interior and divided into two; two groups of chromosomes in each"[175]. In *The Cell* then, 'stem cell' clearly referred to the progenitor of germ cells, as in Boveri's work. This is diagrammatically explained in Figure 55 (from Boveri), where Wilson's legend reads: "*A*. Two-cell stage dividing; *s.* stem-cell, from which arise the germ-cells"[176]. Wilson continued his explanation by stating that at the 4-cell stage, there are two stem cells and two somatic cells; the stem cells are larger, and are richer in chromatin. These cells continue to divide eventually resulting in the primordial germ cells, which then only give rise to germ cells. "Through this remarkable process it comes to pass that in this animal [*Ascaris*] *only the germ-cells receive the sum total of the egg-chromatin handed down from the parent. All of the somatic cells contain only a portion of the original germ substance*" [original emphasis][177]. Citing Boveri, Wilson went on to explain the function of the stem cell further: "The original nuclear constitution of the fertilized egg is transmitted, as if by a law of primogeniture, only to one daughter-cell, and by this again to one, and so on; while the other daughter-cells, the chromatin in part degenerates, in part is transformed, so that all of the descendants of these side-branches receive small reduced nuclei" (p 437 in Boveri, 1891)[178].

There is very little change to Wilson's use of 'stem cell' in the 1900 edition of *The Cell*, where the term still appears in reference to Boveri's and Haecker's work,

[175] Wilson, 1896 p 112.
[176] Wilson, 1896 p 110.
[177] *ibid*.
[178] Wilson, 1896 p 111-112.

and assumes that the stem cell is a primordial germ cell[179]. This second edition was reprinted several times until at least 1911. After a relatively significant gap, Wilson published the third edition ('with corrections'), in 1925, which also included a subtle title change to *The cell in development and heredity*. In this version, the original account of stem cells changes very little; the term is still used alongside images and text from Haecker and Boveri. In addition however, Wilson included his own diagram, referring specifically to the cell lineage of the early *Ascaris* embryo. The zygote itself is referred to as a stem cell, as are three further cells from the first three cell divisions. These divisions are asymmetrical, so the first cell divisions result in the embryo comprising three somatic cells and one stem cell. The stem cell then no longer produces somatic cells when it divides, but only germ cell progenitors (and eventually germ cells). The somatic cells continue to divide to produce the rest of the cells required by the developing embryo. "[T]he germ-line may be followed without a break back to a stem-cell that is distinguishable as such already in the 2-cell stage of the embryo, and in each succeeding cleavage. This cell differs from the somatic cells at every stage in the fact that it alone retains the sum-total of the nuclear substance, while every somatic nucleus has cast out a portion of its chromatin"[180].

The popularity of *The Cell* could have been partly due to the distinguished positions Wilson had held at Columbia University[181]; at the time of publication of the first edition, Wilson was professor of invertebrate zoology (having previously held the position of professor of biology). Following publication of his monograph, Wilson became praised as the world's leading cytologist; such was the influence of *The Cell*, many have argued that it paved the way for acceptance of Mendelian ideas of heredity, and that TH Morgan's opinions on chromosomes in inheritance were revised according to work following on from Wilson's textbook[182].

Wilson considered the cell as the fundamental unit of development, and therefore organisation (since, for Wilson, these two problems were inseparable)[183]. As previously mentioned, Cell Theory was to establish biology as a scientific discipline, and united several fields of research (including, for example, physiology, microscopy, and natural history). The influence of *The Cell* can be seen in other texts

[179] Wilson, 1900 p 147, 149, 300.
[180] Wilson, 1925 p 323.
[181] Ramalho-Santos and Willenbring, 2007.
[182] Gilbert, 1978; Maienschein, 1991; Dröscher, 2002.
[183] Dröscher, 2002.

that were produced in the years that followed; for example, then University of Michigan assistant professor of zoology, Robert William Hegner (1880-1942), also used Wilson's phrasing and Boveri's diagrams in *The germ-cell cycle in animals* (1914).

Wilson's use of 'stem cell' (to mean a progenitor of germ cells) appears elsewhere in the early twentieth century, following publication of *The Cell*. For example, SJ Holmes' paper *Early cleavage and formation of the mesoderm of Serpulorbis squamigerus*, describing research carried out in the 1890s under Charles Otis Whitman (1842-1910) at Chicago, was clearly written in the style of cell lineage studies (see section 5.2). The use of the term 'stem cell' in this paper is not defined in any way, and makes a single appearance. This makes it somewhat difficult to know exactly which cell Holmes is referring to as the stem cell, and why. Holmes appears to be referring to a cell from which the mesoderm layer originates (from which the germ cells will eventually emerge), at around the 24-cell stage[184].

In 1907, at the Seventh Zoological Congress, JP Munson included the term 'stem cell' in his paper *Generation and degeneration of sex cells*, which desribed the development of the butterfly *Papilio rutulus.* Again Munson's 'stem cell' appears to be similar to that of Boveri and Wilson; the cell from which germ cells develop. The origin of sperm cells, Munson claimed, was the "grandmother stem cell", or "the original germ cell"[185]. This stem cell had a large nucleus, and long "protoplasmic processes or strands", at the end of which were cells also rich in chromatin; Munson refered to these cells as "mother branch cells", which divided asymmetrically, one of which remained near the stem cell, and the other eventually becoming a sperm cell[186]. A similar set-up may be seen in the female, and had also been observed in the tortoise.

A paper published in 1918, *Oogenesis and early embryology of Ascaris*, still described the stem cell as the cell responsible for the eventual creation of germ cells. AC Walton referred to asymmetric division soon after fertilisation, resulting in a particular cell lineage moving towards germ cells. "These observations, while scanty,

[184] "The next cleavage occurs in the macromere *D*, and results in the formation of a yolk-laden cell, lying obliquely above the larger stem cell in such a way as the indicate that the division was laeotropic. This cell corresponds exactly as regards its time and mode of origin with the primary mesoblast cell of other molluscks". Holmes, 1900 p 118.
[185] Munson, 1907 p 327.
[186] *ibid*.

go to prove that Ascaris canis agrees with Ascaris megalocephala (Boveri, '99), in that there are five stem cells which give rise to a soma cell and a stem cell, the sixth stem cell giving rise only to pure propagation cells. Each of these five soma cells, or its immediate daughter cells, undergoes the process of 'chromatin diminution'"[187]. It appears that Walton also believed that the amount of material in the cells that would not become germ cells reduced following each division (hence the term 'diminution').

This use of the term 'stem cell' is narrower than the term as its used by Vera Danchakoff (1879-1950) in her work. In her research, published in English after 1910, Danchakoff instead referred to a cell that is not only the progenitor of germ cells, but of other tissues as well; in particular, Danchakoff's research focused on haematopoiesis. Thus far, Danchakoff's working model of the stem cell appears far more similar to the definition we would use today, than the narrower definition popular in the work of Wilson (and others).

The economic climate (particularly in Germany) following the end of the First World War saw a reduction in stem cell research in Europe, and an increase in America (which also led to a change in the publication language from German to English). The Russian Vera Danchakoff, for example, began her career at Moscow University, before continuing her research in New York (at Columbia University, The Rockefeller Institute and The Wistar Institute, where she moved to prior to 1916). Whilst in America, Danchakoff observed that most (haematopoietic) stem cell research was still being carried out in Europe, but her publications and lectures suggest that 'stem cell' was a term also familiar to an American audience[188].

The meaning of 'stem cell' appears relatively precise in Danchakoff's work, although it is in a different context to the use described above (by Wilson, for example). Although the term 'stem cell' is occasionally interspersed with 'mother cell' (a term also used elsewhere), it is Danchakoff who provides an explicit description of a stem cell that would be recognisable today[189]:

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[187] Walton, 1918 p 567.
[188] Danchakoff, 1916.
[189] Boveri previously mentioned similar similar properties regarding *Ascaris* prior to Danchakoff, particularly through the 1890s, partially in response to Weismann's germ plasm theory (see Baltzar, 1967 p 114-119; also Maehle, 2011 p 361-362), however Danchakoff's succinct summary is more similar to the definitions used in the late twentieth and early twenty-first centuries (see Chapter 6). Boveri's, Haecker's, and Wilson's previous work are focused more specifically on stem cells as the precursors of germ cells however, whereas Danchakoff's definition (and use) applies to other tissues and systems as well.

"In the early development of the blood cells …many similar features may be noticed during the simultaneous differentiation of the granuloblasts and erythroblasts from a morphologically and genetically identical mother-cell. This stem-cell maintains its own existence by uninterrupted multiplication, on the one hand, and on the other it differentiates…"[190].

Other terms were also used for the 'progenitor cells' of the haematopoietic system, such as 'haemoblast'. Despite this variation, Danchakoff described some features of stem cells that are still considered essential characteristics today: that a stem cell may last for the entire life of the organism, retaining its potential for self-renewal and differentiation[191]. Danchakoff appears to have been the first researcher to use the term 'stem cell' in English to refer to precursor cells of tissues other than gametes. This may have been because she had recently moved from Eastern Europe however, since the term '*Stammzelle*' had been used to refer to progenitor cells of the haematopoietic system prior. For example, Artur Papenheim (1870-1916) would state that the precursor of blood cells was the *Stammzelle*[192]. Pappenheim also referenced works that refered to stem cells as precursors of gametes (as above), as well as sensory cells, ganglion cells, neuroglia cells, and connective tissue[193]. For Pappenheim then, the *Stammzelle* was an embryonic cell that was the ancestral cell of any tissue type. Pappenheim was not the only researcher to use '*Stammzelle*' in this fashion; for example Alexander Maximow (1874-1928) would also tell the Berlin Haematological Society in 1909 "the 'lymphocyte' was 'the common stem cell' of all tyhpes of blood cells, both during embryonic devlopment and in the adult life of mammals"[194]. These views then were all commited to the concept of a single ancestor cell of the entire haematopoietic system[195].

[190] Danchakoff, 1916 p 401.
[191] *ibid*.
[192] Pappenheim, 1896.
[193] *ibid* p 600-601, 635-636, 640.
[194] Maehle, 2011 p 364.
[195] This 'unitarian' view, as it was described by pathology professor Ernst Neumann (1834-1918), contrasted with the 'dualist' view of Paul Ehrlich (1854-1915), who believed that different white blood cells had different precursor cells. Neumann suggested that if such cells could be grown in culture, like Robert Koch (1843-1910) had done with bacteria, then this may settle the debate. See Maehle, 2011 for further discussion.

5. Embryonic stem cells

5.1 Experimental embryology of late nineteenth century Europe

In the late 1880s, Wilhelm Roux began 'pricking experiments' using two-cell frog (*Rana esculenta*) embryos. Using a fine, hot needle, Roux would puncture one of the cells (with the aim of killing it, and rendering it no longer capable of contributing to development), whilst leaving the second cell to develop normally. Roux observed that the usual result of the experiment was that half an embryo would develop from the cell left intact. Therefore, Roux argued, the material for development of one half of the embryo was contained in one of the cells at the two-cell stage[204].

A few years later, Hans Driesch (1867-1941) experimented with early sea urchin (*Echinus microtuberculatus*[205]) embryos, separating cells by shaking them in sea water. This experiment was important, since it demonstrated that Roux's findings were not as a result of any effect from the pricked cell[206] (i.e. the death of one cell at the two-cell stage). Instead of observing half-embryos as Roux had, Driesch's sea urchins were notably smaller, but nevertheless formed fully developed larvae. The same occurred after separating cells at the four-cell stage, and, occasionally, the eight-cell stage (also refered to as "¼ blastomeres"[207])[208].

"The isolated half-cells did in fact cleave as if they were still connected with their sisters, and formed half-cleavage stages resembling half of a hollow ball. However, this then closed to a small whole ball, and I obtained on occasion, quite contrary to my expectations, a dwarf pluteus"[209].

Driesch also considered whether the same results would be found using amphibian embryos, however claimed that he was not skilful enough to make this experiment a success[210].

Such new observations required new experimental protocols to examine the changing ideas about developmental biology (see above). Swiss anatomist Wilhelm

[204] Spemann, 1938 p 19-20; Baltzer, 1967.
[205] Baltzer provides the alternative nomenclature *Psammechinus microtuberculatus* (1967, p 107).
[206] Spemann, 1938 p 21.
[207] Baltzer, 1967 p 109.
[208] Driesch, 1893.
[209] Driesch, 1951 p 74.
[210] McKinnell, 1978 p 8.

His (1831-1904) demanded that developmental biology needed to describe the structure and function of the fertilised egg as it developed, and that these descriptions should be derived from mechanical explanations and direct causal relationships, allowing each step to be understood as a consequence of the proceeding one[211]. For emerging developmental biology, it was considered important to track the fates of the early embryonic cells, still believed to relive their phylogenies. August Rauber (1841-1917) also took His' approach, and set to work carefully observing cleavage events in order to better understand the importance of geometrics in morphogenesis. Weismann was part of another investigative strand of His' developmental biology, concerning himself with the carriers and passage of hereditary material.

5.2 American cell lineage studies

Development of evolutionary theory after the publication of Darwin's *Origin* (1859) meant that the development of individual organisms could no longer be adequately explained by referring back to conformity of species type – these 'types' no longer existed (if species were changing all of the time). The emerging ideas about evolution suggested that individuals could inherit from distant as well as recent ancestors[212]; this was captured in Haeckel's biogenetic law, however it was attracting criticism. Gegenbaur's ideas (basically rejection of Haeckel's 'phylogenies beget ontogenies' beliefs) were particularly influential in American embryology of the era, especially influencing a new school of study: cell lineage. This was the study of exactly what happened to each cell of the developing fertilised egg over the course of the first few cell divisions of the early embryo[213]. In particular, Mainenschein has highlighted that there were six members of this school (in addition to students or the occasional visitor); in her account, the school began and ended with these researchers. The six researchers included EB Wilson (see above), CO Whitman, Edwin Grant Conklin (1863-1952), Aaron L Treadwell (1866-1947), AD Mead (1869-?), and Frank Rattray Lillie (1870-1947)[214]. For Wilson, Whitman, Conklin, Treadwell, Mead, and Lillie, examination of the earliest stages of development would shed light on fundamental biological processes, taking a more modern approach to interpreting

[211] His, 1874 p 2
[212] Maienschein, 1978.
[213] Maienschein, 1978; Guralnick, 2002.
[214] Maienschein, 1978.

Haeckel's biogenetic law. They accepted that ontogeny and phylogeny were likely related; however, they saw that although phylogenies of adult forms may have similarities to ancestral ontogeny, there was no causal relationship[215]. The section below will briefly highlight the contributions of Conklin, Lillie, and Wilson, since Conklin's and Wilson's interpretations of results were so different to the extent of almost opposing each other, whereas Lillie's, although more like Conklin's, were more moderate[216].

Two centres became crucial for cell lineage study in the US: the University of Chicago, and the Marine Biological Laboratory (MBL) at Woods Hole, Massachusetts (where Conklin was the first director, from 1888). In 1894, at an MBL Friday Evening Lecture, Wilson noted that "no-one believes that ontogeny is actually a true and complete record of phylogeny"[217]. Wilson and Gegenbaur appeared to have similar views: that homology could only be accurately assessed using comparative anatomy and morphology[218]. (The search for homologies also became a key concept for cell lineagists, who used this approach to help understand body plans and variation, for example[219].) The aim of the cell lineage researchers was to first, learn the extent to which an individual embryo is a product of its distant ancestors, and secondly, to learn how the individual embryo is affected by external pressures (perhaps resulting in adaptation and change). This fit well with the proposed Darwinian theory of natural selection; it should also shed light on whether changes due to selective pressures occurred in the past, or whether they were still occurring[220]. Maienschein claimed that although not the priority of cell lineage studies, it was the clarification provided on the relationship between ontogeny and phylogeny that enabled later study of development[221]. Gross goes as far as to say that the cell lineage studies were successful as they could be carried out "largely free of preoccupation with phylogeny", enabling the researchers to demonstrate the somewhat superficial nature of Haeckel's style of embryology; without such shackles, a new way of studying the developing form of the embryo could emerge[222].

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[215] *ibid* p 134.
[216] Guralnick, 2002.
[217] Wilson, 1894 p 102.
[218] Laubichler and Maienschein, 2003.
[219] Guralnick, 2002.
[220] Maienschein, 1978 p 135.
[221] *ibid* p 157.
[222] Gross, 1985.

Wilson's contemporary and, Maienachein claimed, "inspirational leader" of the cell lineage group, Conklin, concluded that the germ layer was not the best place to start looking for homologies[223]. Instead, Conklin preferred to study the cleavage patterns of of blastomeres. This would also help Conklin learn more about the various factors which he believed had an influence on evolution, including growth, differentiation, variation, metabolism, and inheritance, for example[224]. From his studies on blastomeres, Conklin could examine any phylogenic significance of early cleavages without the many extra factors at play that affected cleavage in later development. Conklin saw development as an expression of internal or intrinsic factors alone, believing from his results that the cleavage and cell divisions were all precisely inherited functions (i.e. unaffected by any external influence). For Conklin then, understanding early ontogeny was the key to understanding evolution[225]. Conklin therefore rejected Haeckel's biogenetic law. Conklin's approach was historical; he explained that whilst cleavages were morphogenetic, this did not fit with recapitulation theory[226]. Laublichler and Maienschein highlight the difficulties with such studies however; although the practicalities of such experiments were difficult enough, there were also issues with disseminating results. It was expensive to publish the results of such observational studies, since so many figures and plates were required to adequately describe the processes occurring[227].

Lillie's cell lineage work began around a decade after Conklin's, after attending an 1892 session at the MBL, then working with Whitman there. Lillie completed his PhD under Whitman in 1894. Lillie worked with *Unio* (a fresh-water mussel), receiving acclaim for providing impressive insight in his 1895 paper *The embryology of Unionadae*[229]. Lillie believed that the cleavage options of the fertilised egg were limited by information inherited from the parents, and the orientation of its cytoplasm. External pressures would not affect any adaptation at this stage, however could come into play on the organism as a whole; like Conklin then, Lillie believed that ontogeny was influenced only by internal factors.

[223] Maienschein, 1978 p 134.
[224] *ibid* p 146.
[225] *ibid* p 147, 149.
[226] Guralnick, 2002 p 549.
[227] Laubichler and Maienschein, 2003.
[229] Maienschein, 1978 p 151.

Wilson began his cell lineage work in the 1870s, whilst still studying under William Keith Brooks at Johns Hopkins University. Wilson's early papers, published in the late 1870s and early 1880s, trace the early developmental stages of various invertebrae[230]. Wilson interpreted his observations as showing that each cell of the 4-cell embryo developed into a different part of the body. He also learned that cleavage for cell division occurred in a specific way, so that new cells always developed at a slight angle, resulting in what became known as 'spiral cleavage'[231]. As his research continued, he found that he became less and less convinced by Haeckel's biogenetic law, having rejected most of it by the early 1890s. Guralnick claimed that Wilson's *Nereis* (a polychate worm) work from 1892 in particular demonstrated that a different approach from Haeckel's version of recapitulation was needed, as Wilson launched a "vitriolic" attack on Haeckelian methods in his MBL lecture of 1894[232]. Instead, Wilson believed that ontogeny was actually a series of organogenies – each organ appeared to develop from a single cell (a blastomere). To achieve this, the egg divides depending on the role the new cell will later have, so the morphology and location or pattern of cleavage and division was regulated to achieve this. This regulation may have been affected by both internal and external (such as environmental) factors. The impact of external factors meant that although early embryos may have the same cleavage events and cell divisions, and the resulting new cells all be arranged in the same way, they may eventually have different morphologies. Each cell was influenced by its inherited factors, whilst the whole embryo was also influenced by its immediate environment[233], a belief that most cell lineagists would agree with[234]. This appeared to set Wilson's next goal: to elucidate how external factors could affect internal functions, resulting in differences to adult homologies; yet again, cell lineage studies were useful here, but were not the only way to learn more about embryology[235]. Eventually, by the beginning of the twentieth century, Wilson had also accepted cell homology, and summed up the work of cell lineagists by noting the similarities revealed by studies on molluscs and

[230] Maienschein, 1978.
[231] Guralnick, 2002 p 541.
[232] *ibid* p 544; Wilson, 1894 p 104.
[233] Wilson, 1892; Maienschein, 1978.
[234] Guralnick, 2002.
[235] Maienschein, 1978.

annelids, and other such creatures as all basically following the same general plan of development[236].

The cell lineage studies of the late nineteenth and early twentieth centuries resulted, claimed Gross, in the modern approach to studying embryonic development. Gross argued that embryology had been viewed as an especially useful discipline through which evolution could be studied; by the end of the nineteenth century however, embryology had begun to be seen as a discipline in its own right (see also Chapter 2)[237]. The question of inheritance *versus* adaptation was important in the latter half of the nineteenth century, and cell lineage studies were able to begin investigating this, eventually showing that both internal and external factors were influential[238]. In the 1890s, cell lineage work was mainly based on observation. This is a different methodological approach to the emerging experimental embryology of researchers such as Roux in Europe (see Chapter 2). Whilst observation was of course initially useful, the experimental embryologists argued that observation alone could not provide explanation for developmental phenomenon (Grualnick has argued however that Wilson's *Nereis* work supported mechanistic explanations of cleavage patterns[239]). The experimental embryologists, making use of similar creatures for their work as the cell lineagists, demonstrated that the early cleavage events could be disrupted without affecting the embryo's capacity to continue normal development[240]; observation alone could not have achieved such understanding. Arguably, cell lineage studies had helped to elevate the field of embryology from its study under the paradigm of Haeckel's biogenetic law. The study of the embryo and embryogenesis could potentially reveal much more about multicellular life than the study of recapitulation theory[241]. Potentially, this may have also been a significant factor in the end of cell lineage studies in the early twentieth century; as highlighted by Guralnick, published accounts of new cell lineage studies had stopped by 1907[242]. Guralnick offered a potential explanation for the decline of cell lineage studies: that researchers stopped carrying them out because the general patterns of cleavage

[236] Guralnick, 2002 p 559.
[237] Gross, 1985 p 76.
[238] Guralnick, 2002.
[239] *ibid* p 537.
[240] Gross, 1985 p 71.
[241] *ibid* p 76.
[242] Those papers published after 1900, also focused more on comparative homologies than causation. Guralnick, 2002.

observed were relatively similar, whilst simultaneously, specific cell divisions of embryos showed too much variation[243]. Although significant amounts of data had been produced by this time, no-one really knew how to process it all into a theory of embryonic development. It was only through experimental embryology, particularly as it developed in the early twentieth century, that biologists such as Hans Spemann (1869-1941) became capable of assimilating and evaluating the observations produced regarding cell lineage studies, utilising the data alongside experimental results. In 1915 for instance, Spemann noted the significant influence of the homology theme of cell lineage work, claiming that it was useful to link the causal-analytical and historical approaches of previous embryological studies[244].

6. Conclusions

There was significant growth in cell biology studies through nineteenth-century Germany, for which several factors were responsible. Firstly, there were significant funds available through the state for university appointments and research. Secondly, the academics employed, and trained in new techniques of systematic observation and experiment, also had access to dyes and microscopes (for example, Paul Ehrlich's [1853-1915] influence on the use of dyes in microscopy in the late nineteenth century[249]). Organicism drove the search for the cell, and then its structure and function. In Germany in particular, this was aligned with idealistic views of the state; Haeckel, for example, compared cells to good citizens of the *Kulturstaat*, which could only flourish by the division of labour. Different political views would be manifested in different theories on 'the cell state'. Eventually, social concepts such as 'colonies', 'migration', and 'culture' would remain in the cytology that developed[250].

Henri Dutrochet's idea of the vital phenomena that allowed all life to flourish, and the translation of his thoughts to experimentation, took the first steps towards modernising cell physiology. So great were Dutrochet's microscopic skills, that he was one of the first to observe a somatic cell. Experimentation was also taken up by

[243] These variations, Guralnick argues, are not addressed in the cell lineage papers published. In part, this may have been because there was no easy way (at the time) of analysing the mass of data produced on such variation in any meaningful or quantitative manner. Guralnick, 2002 p 537; 561.
[244] Spemann, 1915; Guralnick, 2002.
[249] For example, see Ehrlich, 1877.
[250] Weindling, 1981.

Raspail, now considered important for the development of the discipline of cytochemistry. Franco-German politics however diminished the reception of Raspail's work, and the initial impact it had in Europe.

A further example of such political influences on scientific endeavour, that of Purkyně's contemporary at Berlin, Müller, being far more influential, since he was German as opposed to Czech. Although Purkyně's student Valentin was one of the first to publish comparisons between animal and plant cells in the 1830s, his efforts were dismissed by the popular Müller student Schwann, when he published *Mikroskopische Untersuchungen* in 1839.

Although the details of Schwann's Cell Theory were repudiated within a relatively short amount of time, the basis of Cell Theory (i.e. that all organisms were made-up of cells) was influential in biology for much longer; the ideal of unifying the disciplines of zoology, botany, anatomy, and physiology, strengthened by German organicism, would influence the type and structure of research in the biological sciences well into the twentieth century.

This had an effect on the developing discipline of embryology. Although there had been an interest in development throughout history, it was the development of the microscope and staining that gave scientists the first opportunities to take a closer look at early mammalian development. Fuelled by debates concerning epigenesis, evolution, and preformation, pre-nineteenth century studies focused on dissection and microscopy. Following the initial boost of information provided by microscopy in the late 1600s, little technological development in microscopy through the 1700s stymied progress. Late eighteenth-century dissections were of some use, but the popular conclusions drawn by Haller distracted some research avenues as other theories were dismissed.

It was not until 1861 that Gegenbaur clearly argued that the egg itself was a cell. I would argue that this is an important point in embryonic stem cell history - without identifying the unfertilised egg as a cell, it would be difficult to consider the first 'products' of the fertilised egg as cells too. The first influential observations on fertilised eggs were made in the early nineteenth century. Prévost and Dumas described how 'furrows' would appear in the hours following fertilisation in the rabbit egg. Rusconi followed suit, going further than Prévost and Dumas by declaring that the furrowing was not only a surface phenomenon - in fact there was segmentation

occurring throughout the fertilised egg, resulting in division and subdivision of the egg.

Soon after Rusconi published his observations, Barry published the first of three papers that would describe his studies on egg development and maturity, fertilisation, and early embryonic development in detail. In the second paper (1839), Barry described segmentation like Rusconi, referring to division of vesicles with nuclei. Barry compared the vesicles he observed with globules identified in the nervous system by Valentin. This, I argue, is the first time cells of the newly-forming embryo were compared with the cells observed in an adult.

Barry's and Rusconi's observations were confirmed by others working with invertebrates. The theory was strengthened by the publication of Remak's book on embryology. Remak claimed that the nuclei in cells of the adult were all produced from the first nucleus created at fertilisation. This again shows that the preformation / epigenesis and ovist / spermist debates of previous centuries still required conclusions; Remak's work seems likely to have been influenced by this, given the conclusions provided.

It is important to consider how the works of Remak, Barry, and others influenced stem cell biology. Since Haeckel initially coined the term *Stammzelle*, it is prudent to understand how and why, and in what context. Haeckel was influenced early on in life and education by botany and botanists. Haeckel's time at Würzburg and Berlin where he met Müller, Kölliker, and Gegenbaur (amongst others) was important, since this influenced Haeckel's interests and career path. Dröscher argued similarly - that Haeckel's exposure to Cell Theory, Müller, Virchow, Darwin, and Gegenbaur allowed him to conceive his 'stem tree' idea, from which influences Haeckel would develop *Stammeltern* and *Stammorganismen*. The use of *Stamm* appears to follow from the works of others in similar disciplines of study. *Stammzelle* seems to alter its meaning in Haeckel's works, dependent on context. *Stammzellen* were derived from *Moneren*, giving rise to the first unicellular and multicellular organisms. In *Anthropogenie*, *Stammzelle* referred to a fertilised egg. This concept of the stem cell intergrated well with Haeckel's idea that ontogeny begets phylogeny - the ontogenic stem cell being both the fertilised egg of multicellular organisms, and the equivalent of a unicellular organism. *Stammzelle* also appeared to capture the essence of the great potential the cell had for life.

Across the Atlantic, American biology was taking a slightly different research direction. Wilson's *The Cell* has been referred to as the last comprehensive text in cytology; during the early decades of the twentieth century the discipline was becoming too complex for a single textbook to explain all of its nuances. Its popularity was significant for stem cell history - it included the first time 'stem cell' was used in English. The term was becoming popular in the two most influential languages of biology at the beginning of the twentieth century - German and English.

Experimental techniques had been improving to the point that early embryos of some species could be cultured in the laboratory. In particular, frogs, sea urchins, and similar creatures proved useful as experimental animals for observing and testing embryonic development. The term *Stammzelle* or stem cell would be used in various contexts in the first three decades of the twentieth century, both in America and Europe.

Cell lineage studies were the most popular use of new experimentation techniques in the USA, particularly as Haeckel's biogenetic law was reducing in popularity[251]. Cell lineage studies were considered a useful way of learning about fundamental biological processes; Conklin was particularly influential in this area of study. Meanwhile in Europe, a reduction of stem cell research in Europe (due to political disruption and, eventually, the outbreak of war) resulted in a small exodus of researchers to the USA[252]. Vera Danchakoff was one such emigrant. Danchakoff's focus was haematopoiesis, however still produced works important for general stem cell biology.

Having explored the history of the stem cell concept, another concept now needs to be surveyed to aid further understanding of the development of genetics, and its role in ESC research. The following chapter considers the role of the nucleus, and the relationship between heredity, genetics, development, and embryology.

[251] Maienschein suggested that this was, in part, due to the lack of uniformity observed where ontogeny was supposed to repeat phylogeny. Maienschein, 1978.
[252] For further detail on this movement of researchers, see Medawar and Pike, 1999.

CHAPTER 2:

GENETICS UNDER THE EMBRYOLOGY PARADIGM: FANTASTICAL EXPERIMENTS

1. Introduction

In 1985, Garland Allen wrote a paper proposing that since embryology and heredity were so closely conceptually and historically related, that heredity research initially developed under the paradigm of embryology. Allen's paper begins with the early relationship between genetics and embryology (from the end of the nineteenth century and into the first years of the twentieth). The paper then discusses the developing divergence between genetics and embryology up to 1940, and Thomas Hunt Morgan's (1866-1945) role in this. Initially, Allen argued, the study of heredity emerged in the latter half of the nineteenth century, as part of studies of embryology, since these areas were conceptually linked. For example, researchers such as August Weismann and Ernst Haeckel proposed that information was 'transmitted' to offspring from parents, then 'translated' into traits seen in the offspring. Towards the end of the nineteenth century however, newer generations of researchers wanted a shift in scientific methodology, preferring experiment and testable hypotheses – Morgan was one such researcher. These researchers made use of embryology (and in particular experimental embryology) to test their hypotheses about heredity, however discovered that this was difficult. Whilst theoretically it made sense to consider development and heredity together, practically it was more useful to separate them. Allen argued in his 1985 paper that there was therefore a separation of the study of 'transmission' (genetics) and 'translation' (embryology) in the early twentieth century; therefore, it was after developing into the field of genetics, Allen suggested that heredity was able to distance itself from embryology. Morgan's work in particular is used by Allen to illustrate and support this claim. This chapter aims to develop Allen's ideas, which were focused on the early decades of the twentieth century, and apply them to research into heredity, genetics, embryology, and development later in the twentieth century; in particular, this will be explored by considering the experiments carried out which would yield chimeras, hybrids, and clones.

This chapter will demonstrate that there is a difference between the chronological development and conceptual development of research fields. Initially, the conceptualisation of the cell nucleus needs to be considered, including recognition of its structure and its function in heredity. This includes discussion concerning several individuals who were influential in the field, such as van Leeuwenhoek,

Scottish botanist Robert Brown (1773-1858), Purkyně, and Weismann (see also Chapter 1). Each of these men were individually important in identifying and naming the cell nucleus, and suggesting a function for it.

In addition, this chapter will also give a brief history of experimental embryology, selecting examples from the early, mid-, and late twentieth century. The work in the early twentieth century is exemplified by the research of Nobel Prize winner Hans Spemann in particular. It was Spemann who proposed the 'fantastical experiments' referred to in the title of this chapter: these would be experiments that examined the role of the nucleus by transferring it between cells at early stages of development. Decades after Spemann's experiments, Robert Briggs (1911-1983) and Thomas King (1921-2000) worked towards achieving the fantastical experiments, swapping nuclei between fertilised frog eggs, and achieving continued development of the recipient eggs. Following the successes of Briggs and King, John Gurdon (1933-) was able to achieve live births following his nuclear transfer work, again using frogs. In 1962, Gurdon published his initial findings, including a photograph of the first mature adult vertebrate created using nuclear transfer. Moving on from frogs and into the latter decades of the twentieth century, this chapter will consider the work of Karl Illmensee (1939-), who began attempting to clone *Drosophila* in the late 1970s. Although this feat was not achieved until much later (and not by Illmensee), it was another hint that nuclear transfer was a technique being utilised throughout Europe and North America to learn more about development and heredity. In the 1980s, Ilmensee claimed that he had used nuclear transfer to create mice, however later accusations of fraud impacted the trust individuals had concerning his earlier work cloning mammals.

The chapter will then move on to consider the history of theories concerning genetics and embryology. As mentioned above, this will focus on the interpretation made by Allen: that the field of genetics developed initially under the paradigm of embryology, before Thomas Hunt Morgan initiated a split of the disciplines. In order to more closely examine Allen's claims, a brief review of Morgan's contribution will be given, including a critique of Allen's claims by philosopher of biology Robert Meunier, who suggested that Allen's claims are too Morgan-centric. In an effort to look at Allen's claims from another angle, the chapter considers historian Gregory Radick's account of Raphael Weldon's (1860-1906) explanation of heredity.

Lastly, this chapter will conclude that Allen was correct to observe the development of genetics under the paradigm of embryology. Using Meunier's critique, Radick's Weldonian genetics, and examples of nuclear transfer research through the twentieth century, this chapter will also demonstrate that there was no definitive split of disciplines, but that the fields of genetics and embryology are still closely linked with each other late into the twentieth century.

2. Conceptualisation of the cell nucleus

It is valuable to consider the conceptualisation of the nucleus, since elucidation of its structure and function are relevant to the way the nucleus' role was understood. This section will distinguish between the initial identification of the cell nucleus in plants and animals, including the language used to distinguish it from other organelles. The section will then move on chronologically and conceptually to consider how function of the nucleus was established. This links back to theories expressed as part of Cell Theory (see Chapter 1), and the first observations and experimental procedures that were carried out in order to identify a function for the nucleus. Again, this is relevant since the framing of nucleus' function would affect the experimental design of later work carried out in the field.

2.1 Identification of the cell nucleus

It has been suggested that the first to see the cell nucleus was the Dutch merchant Antoni van Leeuwenhoek; in a letter to English microscopist Robert Hooke dated 3 March 1682, van Leeuwenhoek described the blood cells of fish: "…I came to observe the blood of a cod and of a salmon, which I also found to consist of hardly anything but oval figures…it seemed to me that some of them enclosed in a small space a little round body or globule…"[253]. The editors of Leeuwenhoek's correspondence note that this is the 'Discovery of cellular nucleus'; others are more tentative about what Leeuwenhoek is describing. For example, in *The birth of the cell*, biologist Henry Harris (1925-2014) concluded that "Leeuwenhoek saw globules

[253] Hooke, 1682 p 158. The shape of red blood cells, or erythrocytes, are slightly different to most other cells, having a central 'dip'; this is to increase surface area for gaseous exchange. To further increase opportunity for gaseous exchange, erythrocytes have no nucleus or other organelles; these cells therefore do not circulate for longer than a few months, before being replaced by new erythrocytes.

everywhere"[254]; a particular criticism is that Leeuwenhoek is describing small fish blood cells (erythrocytes). When Leeuwenhoek described the equivalent, larger erythrocytes of frogs, he did not refer to any globules, but instead to a central shadowing, which is typical of mature erythrocytes.

It was Italian biologist Felice Fontana (1730-1805) who was probably the first to describe the nucleus. Fontana observed the skin of eels, and his illustrations suggest that the epithelial cells he saw contained nuclei, placed at different points in each cell - these were referred to as globules. Inside each of these globules was a central body[255]. Unbeknownst to Fontana at the time, epithelial cells would have been a much more appropriate choice for observing cell structures than erythrocytes. In comparison to the erythrocyte, the epithelial cell is naturally flattened, making the nucleus more prominent. This would have been especially useful for early microscopy. Thin, single-cell thick layers of epithelium can be easily extracted from the skin of many animals for viewing under the microscope.

Unsurprisingly, the nucleus of the plant cell was also observed following introduction of the microscope. Franz Andreas (later Francis) Bauer (1758-1840) was an Austrian botanical draughtsman who had links with other botanists in England; his drawings of plants were known to the Fellows of the Royal Society of the late eighteenth and early nineteenth century. In 1802, Bauer made a sketch of the stigma and stigmatic surface of *Bletia tankervilleae* (an orchid), in which he described seeing "one, two or three granular, more opaque greenish yellow specks, looking like young seeds of an Orchis"[256]; the rest of the sketch showed these same structures in almost every cell, suggesting Bauer regarded the greenish yellow specks as a regular feature. Despite Bauer's illustrations not being published until the late 1830s, they were well-known by Robert Brown.

Scottish botanist Robert Brown is considered to be the individual who introduced the term 'nucleus' into common parlance (Brown is also known for his observation of Brownian motion). In a paper read to the Linnean Society on 1 and 15 November 1831, Brown described the nucleus of the cell in plants, which included a detailed discussion of Bauer's drawings and notes[257]. Brown suggested that he had

[254] Harris, 2000 p 77.
[255] Fontana, 1781.
[256] Bauer and Lindley, 1838 table VI.
[257] Brown, 1831.

seen this nucleus in a variety of plant types, describing it as "distinctly granular", "slightly convex", and having "no regularity as to its place in the cell"[258], describing many tissues where nuclei could be observed, and its presence in other plant species. Brown did not claim to have discovered the structure, referring to previous work not only by Bauer, but also by Franz Meyen (1804-1840), Purkyně, and Adolphe-Theodore Brogniart (1801-1876) (although, Brown claimed, his predecessors appear to have attached little importance to the structure, sometimes not even referring to it in figure descriptions[259]); Brown's work is still considered important for his naming of the structure however. Through his paper, Brown actually uses two terms interchangeably - nucleus, and areola. Harris suggested that there is little reason why nucleus came to be the preferred term for the structure, except perhaps that the Latin translation conceived a nucleus as a solid structure, as opposed to the open space implied by 'areola'[260]. The problem with this explanation however is that, in the 1830s, it had not yet been demonstrated whether the nucleus was solid or not. A further explanation may be the influence of the German nomenclature, where the nucleus was referred to as the *Kern* (kernel)[261].

Whist the influence of Bauer and Brown was relevant for plant biology, we need to return to Purkyně for early nineteenth-century influence in animal biology. In 1825, Purkyně observed the *vescula germinativa* in the hen's egg. The role of the nucleus was initially considered based on its observation in eggs, which is further linked to identification of the nucleolus. Although it is possible that the early nineteenth-century microscopists may have observed the nucleolus as a darker region of the nucleus, it is generally accepted that the nucleoli were first described by Rudolf Wagner in 1835[262]. Wagner's work examining Graafian follicles of sheep allowed him to observe Purkyně's *vescula germinativa*, inside which Wagner described seeing a dark spot. Wagner claimed that he was drawn to this spot, since he had also noticed it in other animals. He named it the *macula germanitiva* (germinative spot), and

[258] *ibid* p 710.
[259] *ibid* p 713.
[260] Harris, 2000 p 80.
[261] As highlighted in Chapter 1, Purkyně's term for animal cells was *Körnchen*, implying either that animal cells were more solid than plant cells (*Zellen*), or to draw attention to the cell interior. Furthermore, a student of Müller, Karl Reichert, misunderstood Purkyně's use of the term *Körnchen*, believing that the term was referring to the nucleus, not the entire cell.
[262] The nucleolus was also identified independently by physiologist Gabriel Valentin the following year.

suggested that it was a structure important in development. The context in which the nucleus and nucleolus is described then is particularly influential - Wagner's study of the germinative spot had evolved from his observations of the Graafian follicle; with these studies in mind, Wagner surmised that the germinative spot had a role in development, concluding that the presence of the spot was the first sign of the embryo, having seen the first germinal layer arising from this spot.

Purkyně, despite the general use of microscopy during the nineteenth century, elected to carry out his studies of the hen's egg with a hand lens. Purkyně's studies on the germinal vesicle were published in 1825, in a volume prepared by the Breslau medical school to mark the fiftieth anniversary of the graduation of the German naturalist Johann Friedrich Blumenbach (1752-1840). Purkyně's contribution, *De evolutione vesiculae germinativae* (*Keimbläschen*), described Purkyně's attempts to isolate the delicate germinal vesicle from the hen egg. Purkyně's work on the germinal vesicle was influential and highly regarded; von Baer for example announced that Purkyně had virtually exhausted the topic, and had little to add on the subject of the germinal vesicle in 1828[263].

Purkyně however did not consider the germinal vesicle as a cell nucleus; instead, Purkyně believed that this vesicle may be another whole cell. Purkyně may have been persuaded otherwise later on - a Polish student of Purkyně's, Adolph Bernhardt (1801-1870), submitted a doctoral thesis considering the existence of a structure not dissimilar to the vesicle Purkyně described, within the mammalian ovum. Harris believed that Bernhardt's thesis of 1834 then suggested that it was no longer feasible to consider that the structure described by Purkyně was not the cell nucleus of the egg[264]. Through examination of the later work of Purkyně's students, it becomes apparent that the delivery of a Plossl (Vienna) compound microscope in 1832, and another new microscope constructed by Pistor and Schiek (Berlin) in 1836, heavily influenced the theses of Purkyně's students[265]. Czech histologist František Karel Studnička (1870-1955), in his work considering the doctoral theses produced by Purkyně's students[266], noted several theses that refer to a cell nucleus. These include Alphons Wendt (1833), whose thesis described the structure of human skin. Wendt's

[263] von Baer, 1828.
[264] Harris, 2000 p 85.
[265] *ibid*.
[266] Studnička, 1927.

illustrations showed the presence of granules (*textura granulosa*) in the various skin layers, which themselves contained smaller granules. Later, Wendt described these granules as existing in all tissues. Another student, Carolus Deutsch, described corpuscles (*Knochen-Körperchen*) in bone (in a similar manner to Purkyně's work describing corpuscles in cartilage)[267]. With hints of Cell Theory to come, Purkyně's student Isacus Raschkow made a specific comparison between plant and animal cells in his thesis (1835). Raschkow's doctoral research was focused on mammalian tooth development, and, describing the *Körnchen* he saw in the epithelium of the dental papilla, suggested that these were similar to the cells of the parenchyma of plants ("*paranchyma plant arum cellulis simillimum*")[268].

2.2 Function of the cell nucleus

The work of van Leeuwenhoek, Fontana, Bauer, Purkyně and his students may have described a structure we now recognise to be the nucleus, however they did not suggest any possible role or significance to it. This said, it was the first structure identified as an organelle, with other cellular components eventually being described over the following century; these are reviewed in Edmund Beecher Wilson's 1926 edition of *The Cell*.

Conceiving a function for the nucleus was a somewhat different endeavour, however developed from the structure's identification in many cells in both animals and plants. In his Cell Theory, Schleiden proposed that that the plant cell nucleus had a role in generating new cells, referring to it as the cytoblast. Schwann, focusing on animal cells, had also observed the nucleus; after conversing with Schleiden about these structures, Schwann wanted to demonstrate that the nucleus in the animal cell was also responsible for the creation of new cells[269]. Schwann's research led him to argue that both plant and animal cells contained cell membranes, cytoplasm, nuclei, and nucleoli; it was the presence of the nucleus that characterised true cells in plant and animal tissues[270]. Remak's following work demonstrated the problems with several of the incorrect assumptions incorporated into Schwann's Cell Theory,

[267] Deutsch's work on bone was referenced by one of Johannes Müller's students, Jacob Henle. The references to Deutsch and Purkyně's work in Henle's 1841 textbook *Allgemeine Anatomie* demonstrate that the Berlin school were aware of the Breslau school's useful contributions to the field. Harris, 2000.
[268] Translation by Harris, 2000 p 86.
[269] Magner, 2002.
[270] *ibid* p 183.

including the role of the nucleus in cell division. Remak studied the developing frog embryo, and observed that the nucleus would divide prior to the rest of the cell[271]. Further clarification that Remak was observing cell division came from his study of the haematopoetic system of the developing chick. From his research, Remak concluded that cell number would increase by cell division. Virchow accepted Remak's version of events, and popularised this with the declaration of *omnis cellula e cellula*. This idea was supported by the findings of Prévost and Dumas, Rusconi, and Barry in the 1820s and 1830s - fertilised eggs would undergo cleavage events that led to an increasing number of cells in the early embryo (see Chapter 1).

So it became understood then that the nucleus was present in almost all cells, and would divide prior to the rest of the cell during cell division. What then would this information lead researchers to believe the function of the structure was? It was later in the nineteenth century that the function of the nucleus with regards to heredity was considered. Lois N Magner suggested that this way of thinking developed through the work of cytologists attempting to link their work to theories of heredity and evolution[272]. Microscopy techniques had developed through the nineteenth century which now allowed some staining of tissues prior to observation; such staining suggested to cytologists that the nucleus was different in both form and function to the cytoplasm[273]. Some stains were even capable of highlighting chromosomes.

August Weismann carried out influential work at the end of the nineteenth century that resulted in a new framework for thinking about development and heredity, in which the nucleus had a central role[274]. Weismann had, up until the mid-nineteenth century, made use of the microscope and the techniques that went with it for his research; in 1864 however, he developed a disorder which made microscopy and experimental work painful for him. Weismann then moved into developing his theoretical work - Magner argued that Weismann made his most valuable contributions as a theoretician[275]. Weismann agreed with Charles Darwin's idea that adaptions are generally made through small, subtle changes; Darwin had described a mechanism through which evolution could occur, and biologists needed to describe

[271] For Remak's overview of his own work in this area, see Remak, 1862.
[272] Magner, 2002 p 395.
[273] *ibid* p 395.
[274] See Churchill, 2015 for further detail on the life and works of Weismann.
[275] Magner, 2002 p 395.

the mechanism through which inheritance could occur. Weismann concluded that although looking at evolving populations was useful, it was important to understand how cells and individuals were involved in inheritance between generations first[276]. Weismann therefore developed his germ plasm (*Das Keimplasma*) theory, which dictated that there is a continuous line of descent from one generation of cells to the next, beginning with the germ cells.

The germ plasm theory of 1892 stated that differentiation was controlled by 'determinants', which controlled 'elementary vital functions'[277]. Therefore, each cell type's structure and function would be controlled by its determinants. Determinants were derived during mitosis (i.e. a process which does not occur in germ cells) and separation of the 'ids' (roughly translated to the genome[278]).

"In the first cell division every id divides into two halves, each of which contains only half of the entire number of determinants, and this process of disintegration is repeated at every subsequent cell division, so that the ids of the following ontogenetic stages gradually become poorer as regards the diversity of their determinants, until they finally contain only a single kind".[279]

Germ cells retained the entire 'idioplasm'. Wihelm Roux's late 1880s work apparently supported this theory (see below).

3. History of experimental biology

As previously noted at the beginning of the previous section, the understanding of nucleus function was relevant to the way experiments were designed. In this section, those experiments will be considered in more detail. This section will move chronologically, beginning in the late nineteenth century with the work of Wilhelm Roux and Yves Delage, moving into the twentieth, particularly looking at the contribution of Hans Spemann. Spemann's work in experimental embryology was

[276] *ibid* p 395.
[277] Spemann, 1938 p 16.
[278] McKinnell, 1978.
[279] Weismann, 1893b (transl. Parker and Rönnfeldt). In the original German: "Jedes Id spaltet sich schon bei der ersten Zelltheilung in zwei Hälften, von denen jede nur noch die Hälfte der Gesammtzahl der Determinanten enthält, und bei jeder folgenden Zelltheilung wiederholt sich dieser Zerlegungsprocess der Ide, so dass die Ide der ontogenetischen Stadien von Stufe zu Stufe ärmer an Verschiedenartigkeit ihrer Determinanten werden, bis sie zuletzt nur noch eine einzige Art derselben enthalten." Weismann, 1892 p 596.

highly influential, and won him a Nobel Prize in 1962. What Spemann was interested in particularly was how the early developing embryo would organise itself, leading to many useful experiments being carried out to this end. It also led Spemann to claim that much could be learned from a 'fantastical experiment' - the transfer of nuclei between cells in order to observe their influence. In the mid-twentieth century, Robert Briggs and Thomas King were able to continue Spemann's work by carrying out nuclear transfer in frogs, and were ultimately able to do this successfully, with eggs developing, though not to the embryo stage. It would be another decade after Briggs and King began their work that an adult frog would result from nuclear transfer; this would be achieved by Cambridge researcher, John Gurdon.

After Gurdon's success, attempts were made to clone other animals in the latter decades of the twentieth century. To examine this, this chapter will consider the work carried out by Karl Illmensee; this is not to suggest that Illmensee's work was carried out in isolation. On the contrary, Illmensee's network included several other influential biologists from the fields of cell biology and development, including Leroy Stevens and Beatrice Mintz, for example. Focusing on Illmensee's contributions highlight where late twentieth century researchers were using embryological methods to learn about genetics and where genetics was being used as data support for developmental biology theories.

3.1 Fantastical experiments I: Late nineteenth and early twentieth century

In his 1938 text, Spemann suggested that it was Weismann's *Das Keimplasma* and anatomist His' *Über unsere Körperform* (1874) that kick-started the work in experimental development. During the latter half of the nineteenth century, Spemann reported that the science of morphology was thriving in Germany[280]. His made the study of chick embryos his work, in an attempt to elucidate the formation of layers and their folding in the developing embryo. Roux made advances in developmental embryology using experimentation to determine the direction of development (as proposed by the germ plasm theory), and what caused these. Whereas Roux felt that he could demonstrate that 'internal forces' were the only controlling mechanisms of development (as in the germ plasm theory), another physiologist, Eduard Friedrich Wilhelm Pflüger (1829-1910), believed that external forces could also have an effect

[280] Spemann, 1938 p 14.

(in a similar way that gravity appeared to have an effect on the growth of plants)[281]. Roux discounted Pflüger's theory by continually changing the position of the developing fertilised egg, and demonstrating no deviances from normal development[282]. Spemann went on to recall Roux's following 'pricking experiments', "which exercised an enormously stimulating influence on future research"[283] (see also Chapter 1). In 1907, Michael Guyer (1874-1959) (whilst at the University of Cincinnati) used a very similar technique (pricking one cell with a fine capillary containing blood cells). Many eggs developed to the blastulae and gastrulae stage, and two became swimming tadpoles[284]. Guyer accredited this to the injected leukocytes, stating his belief that the female pronucleus took no part in proliferation[285]. Cell biologist Robert McKinnell suggested this experiment (and it's results) were particularly important for two reasons: firstly, it suggested that at least some cells from adult organisms retained developmental potential; secondly, this was the type of 'fantastical experiment' Spemann referred to[286].

In the late 1880s, Roux began his pricking experiments using two-cell frog (*Rana esculenta*) embryos. Using a fine, hot needle, Roux would puncture one of the cells (with the aim of killing it, rendering it unable to contribute to development), whilst leaving the second cell to develop normally. Roux observed that the usual result of the experiment was that half an embryo would develop from the cell left intact. Therefore, Roux argued, the material for development of one half of the embryo was contained in one of the cells at the two-cell stage. Later (during the 1890s), Roux decided that since the remaining half appeared to be developing normally, there is no need for the second half of the egg. The 'causal topographical conception' as Roux called it, stated that as long as the living half of the fertilised egg

[281] *ibid*; Pflüger, 1883.
[282] Spemann, 1938; Roux, 1884; Roux, 1887.
[283] Spemann, 1938 p 18.
[284] McKinnell, 1978 p 16.
[285] Guyer, 1907. Repeats of these experiments later in the twentieth century demonstrated that Guyer had actually observed parthenogenesis; i.e the 'female pronucleus' was the only contributor to proliferation (as opposed to taking no part whatsoever). It is also not necessarily true that Guyer's opinions on contributions to heredity are shared; for example, Edwin Grant Conklin, then at Princeton University, stated in a presentation given in 1916 that "Practically all students of heredity are agreed that there is a general equivalence of inheritance from father and mother, and O. Hertwig (1892) cites this as one of the evidences that the chromosomes only contain inheritance material…" (Conklin, 1917 p 101).
[286] McKinnell, 1978 p 17.

had all of the conditions required to develop (e.g. oxygen, heat, etc.), the remaining half could continue its development normally[287].

Similar work (with similar results) was carried out by Laurent Chabry (1855-1893) (using a glass needle) at the same time as Roux, using early *Ascida* (sea squirt) embryos. A few years later, Hans Driesch experimented with early sea urchin (*Echinus microtuberculatus*) embryos, separating cells by shaking them in sea water. This experiment was important (according to Spemann), since it demonstrated that Roux's findings were not as a result of any effect from the pricked cell[288]. Instead of observing half-embryos as Chabry and Roux initially had, Driesch's sea urchins were notably smaller, but nevertheless fully developed larvae.

Hermann Endres (1895) and Spemann (1901) were both able to repeat Driesch's experiments in urodele embryos, whilst Albert Brachet (1904) and later GA Schmidt (1933) repeated the work using anuran eggs. It was noted that if the "gray crescent material" was distributed evenly between separated blastomeres (using hair-loop constriction[289]), two normal (but small) embryos would develop[290]. Boveri would comment on these experiments in the early twentieth century, interpreting the results as demonstrating that if the nucleus was in-tact when "fragments" were taken from the early embryo, then such a fragment could "give rise to normal dwarf [smaller than average] embryos"[291]. Much later, similar work was carried out in mammals (with single blastomeres implanted into the oviducts of pseudopregnant females), specifically rabbits[292] and mice[293].

It has been argued that prior to the published work of Spemann in the late 1930s, the French biologist Yves Delage (1854-1920) proposed nuclear transfer[294]. In 1895, Delage published *La Structure du Protoplasma, les théories sur l'Hérédité et les grands problèmes de la Biologie géneralé*, in which he suggested that differentiation occurred due to differences in the cytoplasm between daughter cells.

[287] Spemann, 1938 p 19-20.
[288] *ibid* p 21.
[289] Hair loop constriction was a method described by Spemann, whereby a fine hair would be used to loop around two cells, which, when constricted, would result in separation of the cells.
[290] McKinnell, 1978 p 8.
[291] Boveri, 1914 (transl. Harris, 2008) p 11.
[292] Moore, Adams and Rowson, 1968.
[293] Tardowski, 1971.
[294] Beetschen and Fischer, 2004.

This then caused changes in the nucleus. Delage proposed an experiment that would demonstrate this:

"…if, without any deterioration, the egg nucleus could be replaced by the nucleus of an ordinary embryonic cell, we should probably see this egg developing without changes."[295]

It can convincingly be argued then that the original aim of nuclear transfer was to test Weismann's germ plasm theory (part of which stated that differentiation occurred as a consequence of unequal nuclear division throughout embryonic development)[296]. The theory suggested that the differences in the nuclei that resulted during cell division would determine (differentiated) cell type. The first attempts to experimentally test this theory were probably made by August Rauber (1841-1917) in the late 1880s. During this period, Rauber was the Head of the Institute of Anatomy, Histology and Embryology at the University of Tartu (Dorpat) in Estonia[297]. Amongst many other experiments utilising early embryos and fertilised eggs, Rauber exchanged the nuclei of frog and toad eggs one hour after fertilisation; neither egg developed, possibly as a result of 'rough treatment'[298]. It appeared however that nuclear transfer did not demonstrate clearly Rauber's hypothesis (that the nucleus and cytoplasm together transmit hereditary traits to the offspring)[299]. Roux was apparently well aware of Rauber's work, although Driesch probably did not earlier

[295] Delage, 1895, transl. Beetschen and Fischer, 2004 p 610.

[296] Not, as some have suggested, that Delage and/or Spemann equated this technique with cloning (Beetschen and Fischer, 2004). Spemann has also been (wrongly) associated with Nazism; for example: "The first practical model [to create identical organisms] was suggested in 1938 by German embryologist Hans Spemann (1869-1941), who is often called the "father of modern embryology." Working in Nazi Germany, with its own fantasies of breeding a "master race," he proposed what he called a "fantastical experiment": to remove the nucleus from an egg cell and put into its stead a nucleus from another cell" (Gilman, 2001 p 307). [This said, Gilman's text demonstrates itself to be a far from reliable source regarding cloning; on the same page Gilman suggests that Dolly (the cloned sheep) was given her name by scientists to state their intent to demonstrate cloning was 'the real thing' (as opposed to a silicone breast implant). Ian Wilmut, the researcher at the head of the team who created Dolly, has stated that firstly, 'scientists' did not come up with the name (rather it was the agricultural workers who looked after her on the Roslin Institute farm who decided on her name), and secondly, she was so called because the somatic cell the nucleus was obtained from was a mammary cell, and therefore 'Dolly' seemed appropriate (Wilmut, 2012).]

[297] Rauber had only recently been appointed to this post, having been essentially a recluse for the decade prior to this. Brauckmann, 2006.

[298] Beetschen and Fischer, 2004 p 607.

[299] Brauckmann, 2006 p 446. Since the experiment did not work, it is likely that this was also another reason why Rauber decided not to persevere.

on[300]. Later, Driesch referred to Rauber's work, but only briefly, since Rauber "failed to get any results at all"[301].

Oscar Emile Schotté (1895-1941) obtained his doctorate under Emile Guyénot (1885-1963) at the University of Geneva in 1925, before moving to Freiburg and Spemann's group with a Rockefeller Foundation Fellowship in 1928. At the time, Spemann and his group were concerned with the interaction of tissues in embryonic development; Schotté's contribution would highlight the role of genetic transmission in embryonic development[302]. In Freiburg, Schotté carried out xenotransplantations between frog and salamander embryos. Transferring the ventral ectoderm of a frog gastrula to a salamander gastrula mouth region would result in developing salamander larvae with jaws and suckers of a frog. The reciprocal transplant would result in tadpoles with balancing rods and dentine teeth of salamanders[303].

Hans Spemann was the first embryologist to win a Nobel Prize for work in developmental biology[304]. Spemann believed that the nucleus of the morula provided the genetic information required to produce an entire embryo. To demonstrate this, over approximately 15 years, Spemann devised a method to introduce a morula nucleus into an egg which had had its nucleus removed. Spemann also believed that it would have been a useful experiment to inject the nucleus of a more differentiated cell into an enucleated egg, whilst conceding that a failure to develop would not necessarily be as a result of transplantation of an incomplete genome; in his 1938 text, Spemann stated that "genes may be lost or become ineffective in other ways besides that of elimination out of the cell"[305].

When talking about this work in the Silliman Lectures (delivered at Yale University in 1936), Spemann suggested that the main problem in furthering this work was obtaining a denucleated egg. If this could be achieved, he argued, it might

[300] Oppenheimer, 1965.
[301] Driesch, 1908 p 235.
[302] Liversage, 1999.
[303] Spemann and Schotté, 1932.
[304] Spemann was awarded the Nobel Prize in 1935, "for his discovery of the organizer effect in embryonic development" (Nobel Foundation, 2014). Much of the practical work on this topic was carried out by one of his students, Hilde Mangold (née Proescholdt) (1898-1924), who died in a fire a year after her dissertation on the topic was written in 1923 (*Ueber die Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren*). Mangold's doctoral dissertation was published in early 1924 (prior to her death), although Spemann insisted on being named as first author (Mangold had objected to this) (Hamburger, 1984; Doty, 2011).
[305] Spemann, 1938 p 210.

demonstrate that the nuclei of differentiated cells could initiate normal development in the correct environment (i.e. the oocyte cytoplasm)[306]. This suggests that Spemann could see the importance of the argument that the oocyte cytoplasm was essential to development, and also his anticipation into somatic cell nuclear transfer or cloning. Maienschein has suggested that Spemann's foresight into the possibilities of nuclear transfer was due to Spemann's interest in transplanting "just about anything experimentally"[307].

3.2 Fantastical experiments II: Mid-twentieth century

This chapter has already briefly considered the work in heredity that was occurring under the embryology paradigm at the beginning of the twentieth century, which is probably most well-known for Spemann's conceiving of a 'fantastical experiment', where genetic transmission could be studied as part of embryological development (by nuclear transfer). In the mid-twentieth century, such fantastical experiments became possible, again perhaps highlighting a role for the study of heredity under the embryological paradigm.

Robert William ("Bob") Briggs was born in Massachusetts in 1911, moving to be raised by his grandparents at the age of 2 (after his mother and brother died of tuberculosis). Briggs stated that he had a happy, stable childhood, surrounded by aunts and uncles. Although his lifelong love of music was encouraged by his family, his passion for biology was stirred by a high school teacher; Briggs spent his time collecting small animals and plants and examining them under magnifying glasses or the school's microscope. After completing his schooling, Briggs went to Boston University, working nights to fund himself. After completing his first degree, Briggs was convinced that his future was in biology, and went to graduate school at Harvard, receiving his Ph.D. in 1938 for a project investigating metabolic changes during frog development[308]. Continuing with amphibian research, Briggs became a fellow at McGill University before moving to the Lakenau Hospital Research Institute in Philadelphia[309], where he continued research using frog embryos[310].

[306] Maienschein, 2003 p 116.
[307] *ibid* p 116.
[308] DiBerardino, n.d.
[309] The Institute later became the Institute for Cancer Research, and is now known as the Fox Chase Cancer Center.
[310] DiBerardino, n.d.

Thomas Joseph King Jr was born in New York in 1921. King's mother died in childbirth, and so King was raised by his aunt. King earned his B.S. from Fordham University in 1943, after which he served as an army instructor for the Army Medical Technicians School in Georgia. Leaving the army three years later, King returned to New York to New York University, earning a Masters degree for his work on tumours in *R. pipens*. It was during this period that King trained in microsurgery; this skill encouraged Briggs to recruit King to his laboratory in Philadelphia in 1950 as a research fellow[311]. McKinnell claimed that Briggs and King carried out "the first successful experiments involving the introduction of a living, undamaged embryonic nucleus into an activated and enucleated recipient egg" in 1952[312].

It has been reported that Briggs applied for several grants before the research could begin with funding from the National Institutes for Health[313]. Briggs and King were ultimately successful however, and were able to fund their research taking the nuclei of donor cells from *R. catesbeiana* (the bullfrog) and inserting them into eggs of *R. pipens* (northern leopard frog). This resulted in development to the blastula stage[314]. Donor blastulae were produced by inseminating *R. pipens* eggs with *R. catesbeiana* sperm. The egg nucleus was removed, producing androgenic haploid hybrids. Other eggs fertilised in the same way were allowed to develop to diploid hybrids. After 18 hours, all of the fertilised eggs had developed to the mid- to late blastula stage, and their nuclei harvested for transplantation. A useful control mechanism is important here: *R. pipens* and *R. catesbeina* hybrids do not survive past the mid-blastulae stage. Approximately half of the enucleated eggs which received donor nuclei developed to the late blastulae stage before arresting. Many displayed abnormalities related to cell division, and some had chromosomal abnormalities (presumably due to earlier irregular division). This said, Briggs and King considered development to this stage a success (since the cells could not have been *R. pipens* x *R. catesbeina* hybrids if the eggs developed to this late stage). In the conclusions of their publication, Briggs and King suggested that successful development of the nuclear transfer method would be particularly important in studying nuclear differentiation. It

[311] Cohmer, 2012.
[312] McKinnell, 1978 p 21.
[313] Lewis, 2001 p161-2.
[314] Briggs and King, 1952.

may also have other uses, such as testing the impact on variously treated nuclei, or developing an optimal nuclear medium (something which should have "real importance for future studies of nuclear biochemistry")[315]. McKinnell praised the authors for their convincing argument that the enucleated egg cytoplasm was the ideal 'test site' for a variety of nuclear tests (also referring to Briggs, Green and King, 1951)[316]. Later, Briggs and King would be able to develop these nuclear-transferred eggs to the larval stage[317].

In order to contribute to the debate over the contribution of male and female pronuclei/cytoplasm, Briggs and King also carried out experiments to investigate the importance of cytoplasm. At the end of the 1950s, Briggs and King announced their findings that endoderm cytoplasm (by itself) could not elicit or alter cleavage or differentiation on enucleated or nucleated eggs[318]. By the late 1970s, further work had been carried out in this field (such as work by DiBerardino), leading McKinnell to conclude that "The elucidation of the molecular events by which the cytoplasm effects a reprogramming of inserted nuclei is perhaps the most exciting area in cell biology today"[319].

The donor nuclei used by Briggs and King were from blastula-stage cells only, not more differentiated cells (as Spemann and Delage had suggested); development after transplantation of nuclei from cells cultured to a later stage was not successful[320]. However, for their pioneering work on nuclear transplantation, Briggs and King would be awarded the Charles-Leopold Mayer Prize by the French Academy of Sciences in 1972; the first Americans to receive the award[321]. Maienschein has observed that Briggs and King considered nuclear transfer to be the starting point for research into heredity and development[322], perhaps again indicating that, despite the separation of heredity and genetics as disciplines, heredity studies were still carried out under embryological paradigms.

[315] Briggs and King, 1952 p 463.
[316] McKinnell, 1978 p 22.
[317] Briggs and King, 1960.
[318] Briggs and King, 1957.
[319] McKinnell, 1978, p 62.
[320] Also reviewed in King and Briggs, 1956.
[321] McKinnell, 1978; DiBerardino, n.d. (DiBerardino gives the date as 1973.)
[322] Maienschein, 2003 p 119.

Notwithstanding John Gurdon's apparent lack of expertise after becoming aquainted with biology for the first time as a teenager[323], he was offered a place at Oxford to study Zoology[324], and allowed an extra year to make up for the learning he had not been able to do at school. After failing at his first attempt at applying for graduate work, Gurdon was "fortunately accepted" to work under Michail Fischberg in 1956, then the embryology lecturer at Oxford[325]. This gave Gurdon a pedigree which would lead back to Hans Spemann and Theodore Boveri[326]; Fischberg had also worked under CH Waddington in Edinburgh[327].

Following the methods of Briggs and King, injecting nuclei into eggs, Fischberg encouraged Gurdon to carry out similar work, in an attempt to achieve nuclear transplantation in *Xenopus* (clawed frogs). Following the work of Weismann (1892), Spemann (1928) and later Briggs and King (1952), Fischberg was aware of the importance of genetics in studying development. In addition, Gurdon claimed that Fischberg had selected *Xenopus* as his experimental model of choice since it would grow to maturity within a few months, produced eggs all year round, and could be easily kept in the laboratory. Initially, Gurdon was frustrated by the protective jelly of *Xenopus* eggs. Fischberg's newly-obtained UV microscope was purchased in order to eradicate egg chromosomes (resulting in enucleated eggs, ready to accept a donor nucleus), however Gurdon also found that very low wave lengths were also useful in dissolving the frustrating jelly. Gurdon reported that a second piece of good luck was the discovery of an individual frog that would produce single-nucleolated diploid embryos[328], making it a very useful genetic marker of nuclear transplantation – there was no need to carry out enucleation in the donor eggs, so any embryos produced would have to have been a result of successful nuclear transfer. Gurdon's further research would eventually result in the first cloned adults (Figure 1).

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[323] Gurdon quotes part of his teacher's report after he had been studying biology for a term in the late 1940s: "I believe Gurdon has ideas about becoming a scientist; on this present showing this is quite ridiculous; if he can't learn simple biological facts he would have no chance of doing the work of a specialist, and it would be a sheer waste of time, both on his part and of those who would have to teach him". Gurdon, 2006 p 2.

[324] Gurdon modestly suggests that he gained a place at Oxford University since "at that time, the universities were short of applications". Gurdon, 2006 p 2.

[325] Gurdon, 2006 p 2.

[326] Buscaglia and Duboule, 2002.

[327] Gurdon, 2006.

[328] These were eggs with only a single set of ribosomal genes, which provided a useful marker. This is described in greater detail in Elsdale, Fischberg, and Smith, 1958; Brown and Gurdon, 1964; Wallace and Birnstiel, 1966.

Figure 1: The first mature adult vertebrate produced using nuclear transplantation. From Gurdon, 1962a.

As Briggs and King reported in 1957, Gurdon also concluded that as cells differentiate, the nuclei become less able to allow development of enucleated eggs. That said, Gurdon then observed that nuclei from differentiated cells of the tadpole intestine were also capable of producing (fertile) adult frogs[329]. This led Gurdon to conclude that even in differentiated cells, the genome was stable. This was not, Gurdon confessed, generally accepted, particularly by more senior researchers. Eventually Gurdon went on to further demonstrate his theory, by transferring nuclei from other differentiated cells, including muscle, lung, skin and kidney[330].

In addition to showing what differentiated and undifferentiated cells were capable of genetically, these experiments also demonstrated the "remarkable powers" of egg cytoplasm[331]. Gurdon went on to continue working with this in mind through the late 1950s and 1960s.

3.3 Fantastical experiments III: Late twentieth century

As a student of Briggs in the mid-1970s, Maienschein has described Briggs' surprise that the public was not more interested in this type of work and the cloning possibilities it presented[332]. It was not only the public that missed Briggs and King's work: Maienschein has suggested that genetic determinists have often ignored the role of the cytoplasm too. It has not been ignored by developmental biologists however,

[329] Gurdon, Elsdale, and Fischberg, 1958; Gurdon, 1962b.
[330] Laskey and Gurdon, 1970; Gurdon, Laskey, and Reeves, 1975; Gurdon *et al*., 1984.
[331] Gurdon, 2006 p 4.
[332] Maienschein, 2003 p 120.

who appreciated the importance of the zygote's organelles[333]. As late as the 1980s, some scientists remained sceptical of the benefits of cloning and developmental genetics (for research if not therapeutics); in their 1983 textbook *Recombinant DNA: A Short Course*, James Watson, John Tooze and David Kurtz wrote:

"In the immediate future there is little likelihood of nuclear transplantation being attempted with any other mammalian species…If the efficiency and reproducibility can be improved, the method may, however, find a place in animal breeding. In theory, it could be attempted with human eggs and embryonic cells, but for what reason? There is no practical application."[334]

* * *

The Austrian Karl Oskar Illmensee was born in Lindau in 1939. He moved to Ludwig Maximilian University in Munich to study chemistry and biology, receiving his doctorate in 1970[335]. During the period of Illmensee's education, reproductive biology studies in mammals had established an artificial insemination method, which was soon used in standard practice (and was a huge boost to, for example, dairy farms of the period)[336]. In 1965, George Pincus had published his book *The Control of Fertility*, which was intended for a wider audience, despite the scientific details and data it contained[337]. Maienschein described the era as a time when learning and talking about sex and reproduction became more socially acceptable, and a "great deal" had been learned from developmental studies carried out on animal embryos[338]. It was during the 1960s when the Cambridge physiologist Robert Edwards (1925-2013) began studying the cells of the pre-implantation embryo[339] and Richard Gardner (1943-) produced a chimaeric embryo by inserting cultured embryonic stem cells into murine blastocoels[340] (producing a mouse which had cells developed from

[333] *ibid*, p 31, 256. Karl Illmensee by this definition then would be a 'developmental biologist' as opposed to a 'geneticist', since his research included work on the cytoplasm and the effect it had on embryonic cells.
[334] Watson, Tooze and Kurtz, 1983 p 207-208.
[335] Karberg, 2007.
[336] Maienschein, 2003 p 140-1; McCarry, 1999.
[337] Maienschein, 2003 p 141-2.
[338] *ibid* p 141-2.
[339] Edwards has commented that his work on stem cells had to be placed on the 'back burner' as the demands of his work on *in vitro* fertilisation with Patrick Steptoe were greater. Edwards, 2001b.
[340] The hollow area inside a developing embryo.

both the original and the donor cells)[341]. Edwards has written that by this time "…'ES' [embryonic stem] cells became a familiar term [to scientists], reflecting their immense potential in colonizing chimaeras"[342].

llmensee's first publication, written whilst in Munich, described the transplantation of embryonic nuclei into unfertilised eggs in *Drosophila melanogaster* (fruit flies)[343]. Following his own interests in cloning, Illmensee had moved to Indiana University in the early 1970s, into the Department of Zoology. Later Illmensee would claim that he believed that since cloning had been carried out in frogs, why not try it in insects[344]? Furthermore, *Drosophila* had been used for several decades by this point[345], which included some studies on chromosomes and genetics (see below)[346]. Also using fruit flies, Illmensee eventually managed to remove the nuclei of more differentiated cells and insert them into enucleated eggs, with 1% developing to the larval stage (and one example almost to the pupae stage)[347]. Illmensee continued his work using *Drosophila*[348] alongside his colleague Anthony Mahowald[349]. It had previously been demonstrated that primordial germ cells (PGCs) of *D. melanogaster* form at the posterior tip of the developing embryo at the preblastoderm stage (the phase following fertilisation of the egg). Illmensee and Mahowald set out to determine whether the formation of PGCs could be induced from the posterior polar plasm (i.e. the cytoplasm at the posterior tip of the developing blastocyst) at the preblastoderm stage; in the introduction to the paper, Illmensee and Mahowald referred to Boveri's 1887 work in *Ascaris*, describing cytoplasmic determinants in the formation of germ cells, although not to Schotté's earlier work transplanting various sections of embryos between frogs and salamanders. Illmensee

[341] Gardner, 1968.
[342] Edwards, 2001a p 349.
[343] Illmensee, 1968.
[344] Karberg, 2007.
[345] For example, Thomas Hunt Morgan made use of fruit flies during his time at Columbia University in the first decades of the twentieth century. Morgan, in a paper referring to rat breeding, preferred using *Drosophila* as they were a wild species, and study of evolution should be carried out on non-domesticated creatures. Morgan, 1909; Kohler, 1994 p 1, 42-3.
[346] Rudkin and Schultz, 1956.
[347] Boveri, 1887; Illmensee, 1968.
[348] At the time there was no tool appropriate for handling the microscopic fly eggs. During the first year of his Ph.D., Illmensee spent much of his time designing a small glass pipette and other implements required for manipulation of the eggs and nuclei (Karberg, 2007). Earlier glass microneedles and pipettes had initially been created for amoeba work in the 1930s (Comandon and de Fonbrune, 1932; de Fonbrune, 1934).
[349] Illmensee and Mahowald, 1974.

continued working with Mahowald on manipulation of fruit fly cytoplasm during 1975-6[350]. This work demonstrated that the cytoplasm contained germ cell determinants and the effect this had on early embryo cells.

His intricate work on the manipulation of fruit fly eggs earned Illmensee the reputation of having 'blessed hands' (despite his failure to produce a cloned fly[351]). It was after his move to the Institute for Cancer Research in Philadelphia in 1974/5 that Illmensee met and worked with Beatrice Mintz (1921-) on mosaic mice produced by using malignant teratocarcinoma cells[352]. Following his work on fruit flies, Illmensee learned to manipulate the oocytes of mice. The first of the two papers published by Illmensee and Mintz described the process by which ECCs (with a normal karyotype) were taken from embryoid bodies[353] (cultured as ascites tumours for 8 years) and injected into blastocysts (see also Chapter 3). Illmensee and Mintz stated that their intention was to test the reversibility of malignancy, developmental capacity and 'genetic constitution' of the ECCs.

"The most rigorous test possible for developmental totipotency would be significant contributions of the carcinoma cells to the normal differentiation of virtually all tissues of the mouse. For this to occur, the initially malignant cells would presumably have to be brought into association with early embryo cells so that the latter could provide an organizational framework appropriate for normal development."[355]

The ascites tumour used was, like most others, obtained from Leroy Stevens' laboratory, and had been maintained in inbred strain 129 mice. This was useful as it allowed those carrying out similar work to compare their results more directly. The blastocyst injection technique was used to create the mosaic mice: blastocysts from a different inbred strain of mouse (named C57) were each injected with 5 ECCs[356] near to the inner cell mass. Following this procedure the blastocysts were incubated for four hours at 37ºC before being transferred to the uteri of pseudopregnant albino female mice. Of the fourteen foetuses and postnatal mice analysed, three were found

[350] Mahowald, Illmensee and Turner, 1976; Illmensee and Mahowald, 1976.
[351] Only in 2004, after 820 trials, were five live *Drosophila* clones produced by a group in Canada (Haigh, MacDonald and Lloyd, 2005).
[352] Mintz and Illmensee, 1975; Illmensee and Mintz, 1976.
[353] Stevens, 1960.
[355] Mintz and Illmensee, 1975 p 3585.
[356] These cells were from the 'core' of the embryoid bodies, after the removal of the yolk sac 'rinds' (Mintz and Illmensee, 1975). This is illustrated in Figure 2.

to be mosaic mice, labelled as *mosaic mouse no.1*, *no. 2* and *no. 3* (the remaining eleven had developed from C57 cells only). The published paper described the cellular composition of each mouse in detail.

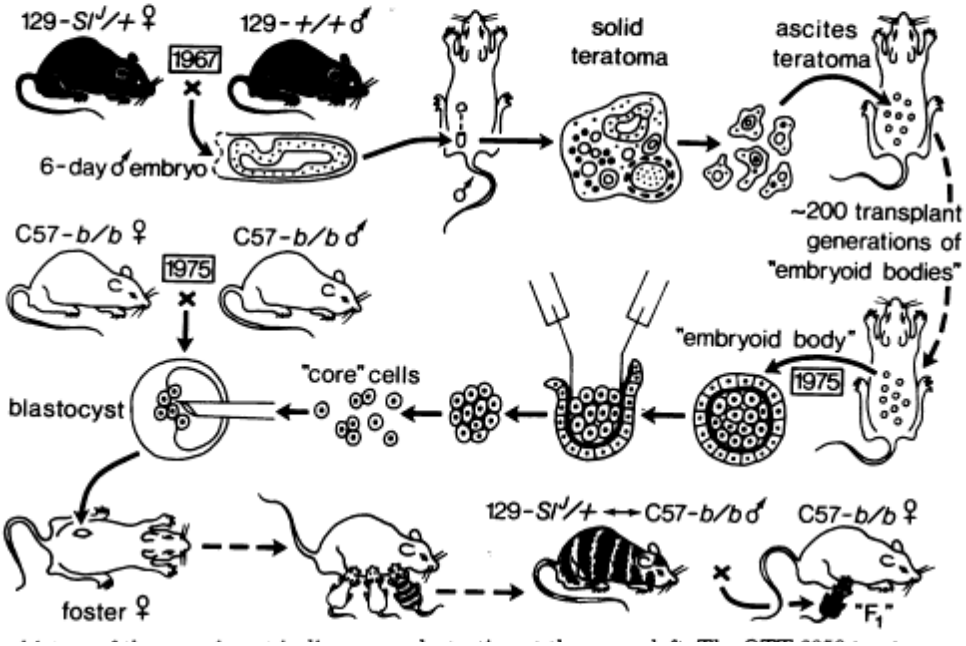

Figure 2: The eight-year history of the mosaic mice created by Mintz and Illmensee using teratocarcinoma cells. From Mintz and Illmensee, 1975.

Evidence of the contribution of the teratocarcinoma cells to the normal development of the mice could easily be observed by differences in coat colour, where, for example, *agouti* and *non-agouti* hair could be observed in the same mouse[357]. Further tests carried out on the mosaic mice demonstrated that the circulating blood cells were predominantly typical of strain 129 mice, although some C57-type blood cells were also observed in *mosaic mouse no. 1*, whilst no strain 129 blood cells were observed in *no. 2*. Gel electrophoresis was used to further establish that the liver of *no. 1* contained cells derived from the original C57 blastocyst and the strain 129 teratocarcinoma donor cells. *No. 2* contained cells of strain 129 origin in her kidneys and thymus. Morphogenically, her reproductive tract was normal,

[357] In the male *mosaic mouse no. 1*, the *agouti* coat of the strain 129 derived cells and the *non-agouti* of the C57 cells could be observed as 'stripes' across the animal. Mintz and Illmensee, 1975 p 3586.

although some XY cells from the strain 129 cells were observed[358]. Likewise, male *no. 3* also had strain 129 cell contribution to the liver (33%), spleen (20%) and kidneys (33%). This mouse was culled at 3 days of age, so was not bred from.

The initial results of this experiment were presented at a meeting by Mintz, much to the surprise of Stevens, who had provided the original ascites teratoma[359]. It was only at this meeting that Stevens became aware of these experiments, although he personally communicated with Mintz and Illmensee prior to the publication of this 1975 paper. Stevens had answered a query from Mintz and Illmensee, regarding a coat colour gene named *steel*, which was also evident in *no. 1*. Stevens had confirmed that the mother of the mouse from which the original teratoma had been taken had a *steel* allele[360]. Don Varnum, a technician in Stevens' laboratory at the time, spent a few years working with strain 129 mice. He reported that by adding the gene *steel* to the strain 129 mice, teratoma formation in males increased to 10%, increasing the number of teratomas available for research[361].

Mintz and Illmensee continued their work by mating *no. 1* with C57 females; the 61 offspring produced were all normal, and demonstrated that the sperm from *no. 1* were from the 129 strain (some progeny had inherited the *steel* gene).

Importantly, this study demonstrated that teratocarcinoma cells were capable of contributing to normal development (i.e. differentiation and proliferation in a controlled manner). Mintz and Illmensee also demonstrated that the core cells of the embryoid bodies remained tumourigenic; when tested by subcutaneous inoculation, this resulted in teratoma formation. The transplanted cells were also capable of normal differentiation. The range of cell types derived from the original 5 cells transplanted also suggested that these cells had already partially committed to a line

[358] Although female, *no. 2* was still found to have XY cells derived from the original (male) XY strain 129 cells injected into the blastocyst.

[359] Mintz had been a visitor to Stevens' laboratory, there to learn mouse embryology techniques. By this time, Stevens had been maintaining teratoma-derived cells (using serial transplants) for eight years. Stevens' daughter, Anne Wheeler, suggested that the reason her father was so surprised when Mintz presented her results was that he had mentioned the experiment to her as work he was considering carrying out. Mintz, with a larger laboratory and more research funding, had produced the chimeric mice first. Wheeler recalled her father suggesting that this was the end of an era where researchers shared their ideas and helped each other. Instead, research became more secretive and information would not be so freely shared. Lewis, 2001 p 136.

[360] This is a relatively important question that suggests Illmensee's good attention to detail – particularly with reference to possible inherited genes. This is contrasted by his apparent complacency later on, leading to accusations of fraud (see below).

[361] Lewis, 2001 p 132-5.

of differentiation and were multipotent, not pluripotent. Furthermore, this suggested that an entire embryo could not be created using only ECCs.

In addition to this paper, Mintz and Illmensee published for the second time only five months later; this paper described in further detail analysis of other mosaic mice and the contribution of the strain 129 cells to each organ[362]. It was also possible to inject only one 129 cell into a blastocyst and for mosaic mice to be created. It was shown that despite the demonstrated tumourigenicy of the cells used, only one of the 21 mice developed cancer (a pancreatic adenocarcinoma, shown to have developed from strain 129 cells). None of the animals gave any indication of teratoma development, however as a note added in proof, Illmensee and Mintz highlighted a paper published by Virginia Papaioannou *et al*. (1975) in which several mosaic mice (created by a similar method) developed tumours[363].

In late 1977, alongside Peter Hoppe at Jackson Laboratories (JAX – see Chapter 3), Illmensee published a paper describing what was essentially the cloning of mice; the title of the paper referred to these clones as "homozygous-diploid uniparental mice"[364]. Shortly after being fertilised, one of two pronuclei of the eggs was removed, leaving the haploid egg. These were first cultured in media which allowed nuclear division but not cytokinesis (this technique allowed the remaining pronucleus to become diploid) before being transferred to media which would allow normal cell growth and division. These early embryos were then transplanted into pseudopregnant females; of the 93 transplants, 7 live births resulted, all female. Five of these offspring were derived from the maternal genome (gynogenesis) whilst two mice had inherited the paternal genes (androgenesis)[365]. Homozygosity was shown across several different genetic loci in all of the mice. Six of the seven mice born had a normal diploid karyotype (including two X chromosomes) and were shown to be fertile. In this publication, Hoppe and Illmensee mentioned the previous work of Oxford University zoologist CF Graham, who had also created 'uniparental'

[362] It was also noted here that of 161 blastocysts injected with strain 129 cells and surgically transferred, 71 survived (44%). Of the 71 animals born, 30% were shown to contain strain 129 strain cells in at least one tissue. Illmensee and Mintz, 1976.
[363] Papaioannou *et al*., 1975. See also Chapter 3.
[364] Hoppe and Illmensee, 1977.
[365] This occurred as either the maternal or paternal pronucleus had been removed during the first phase of the experiment, leaving some fertilised eggs with the maternal pronucleus and some with the paternal pronucleus.

embryos[366]. In the acknowledgements, Stevens is thanked for his "generous support"[367]. As with Evans' work on developing an embryonic stem cell line (see Chapter 4), Hoppe and Illmensee's comments in this paper suggested that these mice could be used as a tool in further research (for example, "studying gene action during mammalian embryogenesis"[368]), and not simply as an end in itself. In a later review, Illmensee also saw the 'uniparental' mouse model as useful in the study of X chromosome inactivation and as a way of comparing paternal and maternal gene activity during development[369]. This perspective appears to have changed somewhat in later years, as mammalian cloning began to be recognised as useful for many applications, and Illmensee felt his early contribution to the field had not been sufficiently appreciated. This was likely due to the claims of fraud against Illmensee and this work (see below). In addition, Illmensee was prevented from carrying out work on cloning for several years due to problems in attracting funding – again as a result of the fraud accusations. More recently, Illmensee has been involved in cloning as a useful application in infertility treatment; although funded, this work is still controversial and illegal in some countries.

In 1964, Lewis J Kleinsmith and G Barry Pierce had demonstrated that teratocarcinoma cells remain pluripotent (by showing that they can clonally give rise to various tissue types typical of teratocarcinomas)[370]; when injected into mouse blastocysts, normal development had been observed[371]. In 1978, alongside Hoppe and Carlo Croce (at the Wistar Institute, Pennsylvania), Illmensee reported that he had produced chimaeric mice from mouse teratocarcinoma cells and human fibrosarcoma cells[372] (see Figure 3). The mouse-human hybrid cells initially produced were injected into mouse blastocysts. The resulting mice were shown to retain at least one human chromosome (17) in resulting mosaic organs (although human gene products were only weakly identified in two of the mosaic tissues – heart and kidney).

[366] Graham, 1974.
[367] Hoppe and Illmensee, 1977 p 5660.
[368] *ibid* p 5657.
[369] Illmensee, 1982. This type of work eventually came to fruition, with later publications by Illmensee. For example, in 1987, Illmensee published a paper describing the expression of phosphoglycerate kinase (PGK-1), an X-linked enzyme, in early mouse embryos (Fundele *et al*., 1987).
[370] Kleinsmith and Pierce, 1964.
[371] Brinster, 1974; Mintz and Illmensee, 1975; Illmensee and Mintz, 1976; Papaioannou *et al*., 1975. See also Chapter 3.
[372] Illmensee, Hoppe and Croce, 1978.

Illmensee considered this comparable to the results published with Mintz in 1976. Illmensee, Hoppe and Croce suggested that this might be a useful technique for studying human gene expression during differentiation.

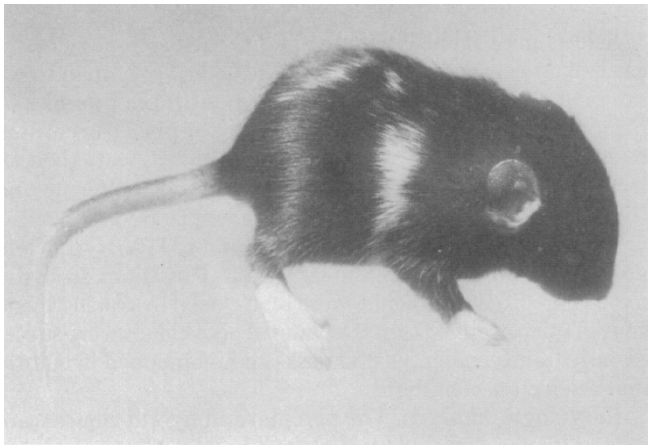

Figure 3: This is the mouse pictured in Illmensee, Hoppe and Croce, 1978. However, during the 1980s, the origins of this mouse were questioned, particularly as the origin of the white patches of fur could not be explained. Joachim Huarte, a graduate student in Illmensee's laboratory, claimed that this mouse did not originate from the described experiment. Illmensee later conceded that he had confused this mouse with another, arising from a testicular cancer cell implanted into an embryo[373].

In the discussion of the 1978 paper, Illmensee explained the unexpected white markings on the coat of the mouse. The teratocarcinoma cell line used was heterozygous for the *steel* gene, and had previously exhibited an *agouti* phenotype[374]. Illmensee suggested that the human-mouse hybrid cells may have become hemizygous for *steel* (i.e. phenotypically white) since a small deletion on chromosome 10 carried the wild-type allele. Spontaneous mutation on chromosome 7 at the *albino* locus could also have caused this change in coat colour. Later, Illmensee wrote about the fate of the human insulin gene in transgenic mice, suggesting that he

[373] Image from Illmensee, Hoppe and Croce, 1978. The allegations of forgery were reiterated in *New Scientist* (31 May 1984). *New Scientist* writer MacKenzie claimed that this mouse was produced when 'a tumour cell from a mouse ovary was implanted into a mouse embryo at the very early blastocyst stage' (p 3). However, the original paper stated that this mouse developed from a mouse blastocyst injected with a human-mouse hybrid cell. The white patches observed were said to have been derived from the injected hybrid cell (approximately 20% of the total coat) (Illmensee, Hoppe and Croce, 1978 p 1916).
[374] Described in Mintz and Illmensee, 1975.

(or at least those working for him) continued work on human-mouse hybrids[375]. This may be considered as a 'fore-runner' to Martin Evans' work in creating mouse models of human disease, which was first proposed by Evans' research group in 1987[376].

In his 1978 textbook on amphibian cloning, McKinnell wrote an interesting summary of where cloning research was at the time:

> "It would be nice to conclude…by stating that the old questions posed half a century or more ago are now resolved. They are not. We still do not know how many cell types (if any) in the adult are totipotent. There is controversy concerning the results obtained by nuclear exchange…There is good reason to believe that egg cytoplasm has the capacity to order a somatic nucleus to mimic a zygote nucleus. …[H]opefully [the rest of this book], will reduce some of the misunderstanding of the results of cloning studies."[377]

In 1979, Illmensee and Croce published together describing the creation of chimaeric mice from rat-mouse hybrid cells; the murine cells were teratocarcinoma cells, and the rat cells were hepatoma cells. Although no visible coat mosaicism was observed, the chimaeric mice were positive for rat-specific liver enzymes. This work was described as more successful than the previous human-mouse hybrid attempts by Illmensee and Croce, and was followed up by a second publication in 1982[378]. This paper examined the protein synthesis of rat-mouse hybrid cells in chimaeric organs. The first author of this paper was Denis Duboule, a PhD student.

Illmensee was clearly interested in teratocarcinomas, and published (alongside Stevens) a substantial review ("Teratomas and chimeras") in *Scientific American* in 1979. The paper described what teratomas were, and illustrated the differentiation of these tumours into a variety of tissues. It also described the strain 129 mice, as established by Stevens, as well as the embryoid bodies and ECCs. Illmensee and Stevens reviewed the work by Kleinsmith and Pierce (1964) as well as Brinster, Gardner and Papaioannou, who created chimaeras from teratocarcinomas and achieved live births[379]. The review ended with some of the work carried out by Illmensee with Croce and Hoppe, in creating hybrid cells and chimaeric mice.

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[375] van der Putten, Botteri and Illmensee, 1984.
[376] Kuehn *et al*., 1987; Evans, 1989. See also Chapter 3.
[377] McKinnell, 1978 p 20.
[378] Duboule *et al*., 1982.
[379] Papaioannou *et al*., 1978; Brinster, 1976.

Following his own hybrid and chimaera work, Illmensee published a series of three papers on the formation of the cytoskeleton during embryogenesis[380]. These appear to be papers produced from the work of Illmensee's laboratory workers and students, one of which was Kurt Bürki. Again these papers appear to indicate that Illmensee, similarly to Martin Evans, saw manipulation of the mouse embryo as a tool for further experimentation, such as creating mouse models or investigating genetics during cell differentiation.

Illmensee and Hoppe continued working together, publishing an important joint paper in the journal *Cell* in 1981. In the introduction to this work, Illmensee and Hoppe suggested that this research might answer questions such as whether changes in gene expression during differentiation restrict the totipotency of the zygote's nucleus. Illmensee and Hoppe described a procedure whereby the nuclear genome of a fertilised mouse egg was replaced by that of an embryonic cell. This was carried out in 363 eggs, of which 48 embryos survived to the pre-implantation stage. Sixteen were transferred into pseudopregnant females, and three mice were born (two female and one male [see Figure 4]). When bred from, these mice produced offspring which also had the nuclear donor phenotype. Illmensee and Hoppe referred back to the experiments carried out by Briggs and King three decades earlier, reporting successful nuclear transfers in frogs (Illmensee and Hoppe also observed however that to date a live adult frog had not been produced derived from the transplanted nucleus of a differentiated adult cell)[381]. Illmensee himself had also previously carried out similar work in fruit flies, also without producing adults[382]. It was reported in *The New York Times* in 1981 that Illmensee and Hoppe had achieved the first scientifically acknowledged cloning of a mammal, and that they would attempt to clone other mammals[383]. A similar report appeared in *The Wall Street Journal*[384].

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[380] Jackson *et al*., 1980; Jackson *et al*., 1981; Krietsch *et al*., 1982.

[381] Illmensee and Hoppe, 1981 p 9. Previously, John Gurdon had attempted cloning of *Xenopus laevis*, and demonstrated that nuclei from the intestinal lining of tadpoles could support development of adult frogs (Gurdon, 1962a). However, after being unable to reproduce this work, a student of Robert Briggs, Dennis Smith, suggested that Gurdon had not isolated intestinal cells, but primordial germ cells, which migrate through the gut during development. Gurdon eventually elected to repeat his experiment using keratinised skin cells, the results of which were published in 1975 (Gurdon, Laskey and Reeves, 1975). Gurdon had succeeded in using an adult donor to support larval development and larval donor to support adult development, but not an adult nucleus to support adult development (i.e. cloning) (Lewis, 2001 p 162-3).

[382] Illmensee and Mahowald, 1974.

[383] Sullivan, 1981.

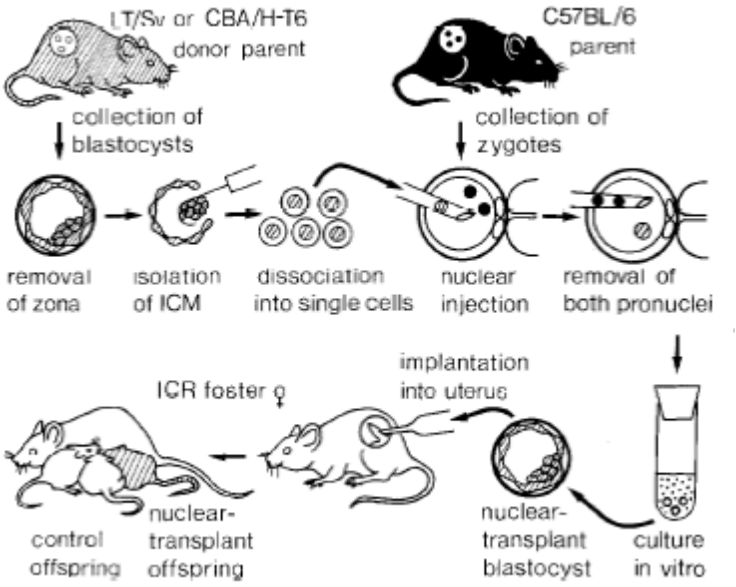

Figure 4: Experimental scheme of nuclear transplantation in the mouse, resulting in a clone. From Illmensee and Hoppe, 1981.

This was followed in 1982[385] by another paper describing the transplantation of parthenogenetic embryonic nuclei into fertilised mouse eggs, also resulting in live births[386]. Diploid parthenogenically activated oocytes from mice, which cleaved spontaneously[387], developed into blastocysts but died within a few days of implantation[388]. Hoppe and Illmensee transplanted the nuclei of these cells (once they had reached the blastocyst stage) into fertilised eggs (which had had their pronuclei removed). When injected with nuclei of cells from the trophectoderm, growth arrested at the morula stage. However, when nuclei of cells from the inner cell mass were injected, live births were achieved. All four female mice born were of the same genotype as the original parthenogenic oocyte, and one was fertile (transmitting the partheonogenic genome to her offspring).

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[384] Unknown author, 1981.
[385] Hoppe and Illmensee, 1982.
[386] Parthenogenesis, usually associated with invertebrates and some fish, birds and reptiles, also occurs in approximately 10% of an inbred mouse strain named LT; unfertilised eggs cleave and develop into diploid blastocysts. Even after transplantation into pseudopregnant females, these early stage blastocysts die and develop no further.
[387] This spontaneous parthenogenic activation occurred only in the LT inbred strain of mouse (in which approximately 50% of females develop ovarian teratomas). The development of the LT strain of mice and the incidence of ovarian teratomas are described in Stevens and Varnum, 1974.
[388] Witkowska, 1973; Tarkowski, 1975.

3.3.1 Claims of fraud

"Good science…presupposed an attitude that one might describe as professional *integrity*. A scientist should not cheat or falsify data or quote out of context or do any other thing that is intellectually dishonest. Of course, as always, some individuals fail; but science as a whole disapproves of such actions. Indeed, when transgressors are detected, they are usually expelled from the community. Science depends on honesty in the realm of ideas. One may cheat on one's taxes; one may not fiddle the data."[389]

The results of Illmensee's and Hoppe's 1981 and 1982 papers were questioned following the claims of fraud made against Illmensee in May 1983. On 2 June 1983, *New Scientist* reported that Illmensee, by then Professor at the University of Geneva, was under investigation for fraud regarding experiments transplanting murine teratocarcinoma nuclei into fertilised mouse eggs. The vice-rector of the university suggested that either there had been negligence in Illmensee's record keeping, or 'intellectual falsification'. The report also claimed that it was a worker in Illmensee's own laboratory who first questioned the results of his research, claiming that they corresponded 'too closely' to the predicted outcomes. Importantly, this research had not been published. This was swiftly followed by a report in *Science* (3 June 1983) describing the same situation, and the withholding of Illmensee's $70000 research grant from the National Cancer Institute (NCI). According to Clement Markert (an embryologist at Yale University), reproduction of these experiments had 'proved difficult'[390]. This news reached the US national press by 4 June; *The New York Times* reported that an internal committee at the University of Geneva had found no evidence of systematic fraud, although an external review was in progress[391]. In addition, another investigation was carried out by a committee at JAX, since Hoppe was an employee of this facility[392]. *The New York Times* stated that Hoppe was sure that the experiments were carried out as described, although he could not exclude the possibility that the embryos had been switched prior to transplantation. The report from Bar Harbor suggested that Hoppe and Illmensee should repeat their experiments, particularly since no other groups had been able to replicate their results. In

[389] Ruse, 1982 p 74 (original emphasis).
[390] Marx, 1983.
[391] Schmeck Jr, 1983.
[392] The JAX archive list an *ad hoc* investigation into Illmensee and Hoppe in the inventory: http://library.jax.org/archives/org_forms/compinvent.html [Accessed October 2011].

defending Hoppe and Illmensee, the JAX report suggested that this might be the case because no other had the microsurgery skills of Illmensee[393]. Amidst this controversy, James McGrath and Davor Solter published a paper in *Science* describing a different method for nuclear transfer (17 June, 1983). McGrath and Solter referred to Hoppe and Illmensee's work, suggesting that many embryos were lost due to disruption of the plasma membrane. Instead, McGrath and Solter described a technique which did not require penetration of the plasma membrane, therefore increasing the success rate[394]. Interestingly this result did not appear to evoke any adverse reaction, despite the high success rate recorded and the forerunner of Illmensee's work still being debated; writing in *The New York Times*, Sullivan even described McGrath and Solter's methods as 'refinement' of Illmensee's work[395].

A report (*New Scientist*, 28 July 1983) published after apparent conversations with Illmensee, stated that the charges were based on five experiments carried out in July 1982; the earlier *Science* report suggested that the University of Geneva vice-rector had received a request from Illmensee to review his previous work (such as that reported in *Cell* in 1981). However, Illmensee had started to produce reasons why the experiments could no longer be repeated, such as changes which would have taken place in the cancer cells whilst frozen.

A commission of enquiry, setup by the University of Geneva in August 1983[396], had found 'no compelling evidence of falsification of data', however did find 'numerous corrections, errors and discrepancies' in the experimental records Illmensee had kept. The commission recommended repetition of the experiments with an outside collaborator. On 23 February 1984, Peter Newmark reported in *Nature* that the fraud charges were unproven. As well as reprimanding Illmensee for his errors (which were clear, even if they did not amount to fraud), the commission noted Illmensee's accusers should have had more documentation to support their accusations, and should have had more rigorously assessed their evidence[397]. It appears that after July 1984 little was published regarding the issue in the mainstream media. The case, however, did appear to continually influence the media's response

[393] Marx, 1983.
[394] McGrath and Solter, 1983.
[395] Sullivan, 1983.
[396] The commission included a law professor, three experts in mammalian development, Anne McLaren of University College London and Richard Gardner of Oxford University.
[397] This was similarly reported in the US national press (*The NewYork Times*) on 26 February.

to other work in the field, even years later. For example, a report in *The Wall Street Journal* titled "Science: How Do We Know Dolly Isn't A Hoax?" suggested that the accusations of fraud at the hands of Illmensee should ensure that serious questions would be asked about the validity of such results[398].

Following the claims of fraud, Illmensee submitted his resignation from his professorship at the University of Geneva in 1985, which came into effect on 30 September 1987 (when his contract expired). Illmensee however expressed his hope that he would be able to repeat the 1982 experiments involving teratocarcinomas (despite the difficulties in re-establishing the cell cultures required) and, if possible, the 1977 experiments, producing live homozygous diploid mice[399].

The papers published in *Naturwissenschaft* (1989)[400] and *Development* (1990)[401] described the transfer of nuclei from teratocarcinoma cells to oocytes and eggs, and their developmental potential. In collaboration with international researchers, Illmensee had repeated the work he had carried out almost a decade earlier, as requested by the commission, with international collaboration. Both Richard Gardner and Anne McLaren both believed that these papers explained that the research in question had been reproduced (albeit using a different cell line). Gardner and McLaren wrote to the University of Geneva in 1991 stating their view that the University should formally advise the scientific community that the controversial findings had been confirmed, as set out by the commission. However, the University had not responded to this request several years later, as highlighted in *Nature* in 1997[402].

4. History of heredity and embryology

In order to examine whether genetics did indeed develop under the paradigm of embryology, and if it did, when it became a separate discipline, we need to look at reasons why this was proposed, and its critics. This section will give an overview of Allen's proposition that genetics developed under the paradigm of embryology initially, before Morgan's work began to divide the disciplines in the early twentieth

[398] Waldholz, 1997.
[399] Newmark, 1985.
[400] Illmensee *et al*., 1989.
[401] Modlinski *et al*., 1990.
[402] Abbott, 1997.

century. This section aims to consider why Allen made such a proposal, and a particular critique of the theory, by Robert Meunier. One of Meunier's claims is that Allen's theory is too Morgan-centric; to examine this, a brief overview of Morgan's relevant work is provided. This section will also include another example, which I claim is useful for looking at Allen's proposal: Weldonian genetics. Greg Radick has provided a history of genetics from the perspective of Raphael Weldon, which I believe is useful here; Weldonian genetics, also developed at the beginning of the twentieth century, offers a view of how genetics, heredity, and development was thought about by some biologists. This should help to determine whether Allen's proposal is too Morgan-centric, as argued by Meunier, and whether, in the light of twentieth century ESC research (examined above), Allen's proposal stands.

4.1 Garland Allen's history of heredity, genetics, and embryology

Initially, as the field of heredity was developing the latter half of the nineteenth century, it emerged under the paradigm of embryology, since the two were "so closely related conceptually and historically"[424]. Late nineteenth and early twentieth century biologists followed the influential work of naturalist Charles Darwin to construct theories of heredity; this was generally done in relation to embryonic development. Weismann and Haeckel were two such individuals who proposed two methods of heredity: firstly, 'transmission' from parent to offspring, and 'translation' of that inherited into traits observed in the adult; both were equally important, and, Allen suggested, Weismann and Haeckel made no distinction between genotype and phenotype. Similarly, there was a belief that the cell nucleus and cytoplasm should also be considered as a whole (as alluded to by Weldon [see below]). This wholist attitude to heredity and embryonic development worked well alongside developing theories of evolution. This led Müller-Wille and Rheinberger (for example) to suggest that all heredity theories up to 1900 were closely tied to theories of evolution, or development, or both[425]. By the turn of the century however, expansion of ideas in philosophy of science and development of the scientific method, meant that younger biologists were keen to develop testable hypotheses – this was important for a new generation who were keen to put biology on an equal footing with the hard sciences of physics and chemistry. Allen observed that Morgan was one of the newer generation

[424] Allen, 1985 p 107.
[425] Müller-Wille and Rheinberger, 2012 p 80.

of researchers keen to identify more rigourous methods for biology[426]. Despite this, the new generation held onto the idea of a unified heredity theory, including genetic transmission and embryogenesis; alongside Morgan, for example, EG Conklin agreed that the problem of heredity was a significant problem in biology:

"Heredity is today the central problem of biology...but the mechanism of heredity can be studied best by the investigation of the germ cells and their development."[427]

For the likes of Morgan and Conklin then, the most appropriate approach to heredity research was through embryology. For instance, learning about transmission between parents and offspring lacked value if it was not considered alongside the development of this trait in the adult. Morgan in particular however began to feel that it was becoming increasingly difficult to maintain this position when it came to experimental testing of hypotheses. Theoretically, it made sense to consider heredity and development together; experimentally, it was more fruitful to separate the two.

Allen opted to look at the separation of transmission (genetics) studies from translation (embryology) through the career of Morgan, who, between around 1910 and 1924, appeared to change his mind about how heredity should be studied[428]. As highlighted above, Morgan initially conceived the study of heredity to involve both transmission and translation together; although this was a useful way of conceptualising heredity, it was less useful for its practical study. Allen identified five factors that not only influenced Morgan, but other biologists of the time to begin studying genetics – a removal of the consideration of heredity under the embryology paradigm:

1) A commitment from biologists of the early twentieth century to the analytic methods of mechanistic materialism (in order to separate complex problems into smaller, testable, components).
2) The growing distinction being made between genotype and phenotype (this, Allen proposed, was highly influenced by Wilhelm Johannsen's work

[426] Allen, 1978.
[427] Conklin, 1908 p 89-90.
[428] Allen, 1985.

published in 1911), and the comparable distinction between genetics and embryology.

3) The research carried out using *D. melanogaster* that had demonstrated chromosomal transmission.
4) The emerging competition developing between fields of study, which, at the time, was advantageous (for obtaining funding and students, for example).
5) The importance of the agricultural context. Late nineteenth-century and early twentieth-century USA was becoming a victim of food shortages, as increasing industrialisation moved workers out of the farms and into the factories. The developing fields of heredity and genetics were harnessed by the agricultural industry as an area where permanent improvements could be made to crops that would improve yield (and profit), leading to significant investment into agricultural genetics (i.e. not embryology)[429].

These claims are also explored in *The cultural history of heredity* by Müller-Wille and Rheinberger; in addition, these authors consider in more detail the social, cultural, and economic factors that heralded the formation of genetics as a discipline. Although social, economic and cultural factors are not a focus of this chapter, it is valuable to note that once again, there was an important contextual influence in the direction of life sciences research[430].

4.2 Thomas Hunt Morgan's separation of genetics and embryology

Concerning the argument that eventually genetics and embryology would separate from each other, there is a different view that the disciplines were never able to split since they were never a singular discipline in the first place. This latter view is supported by Robert Meunier, as a counter-argument to the narrative supplied predominantly by Gar Allen, and supported by Scott F Gilbert. Meunier argued that the separation theory is too "Morgan-centric", and instead suggested that genetics and embryology developed out of different research traditions[431].

In his 1910 publication *Chromosomes and Heredity*, Morgan claimed that:

[429] Allen, 1985.
[430] Müller-Wille and Rheinberger, 2012. In particular, see Chapter 6, 'Disciplining Heredity' (pp 127-160).
[431] Meunier, 2015.

"We have come to look upon the problem of heredity as identical with the problem of development. The word heredity stands for those properties of the germ-cells that find their expression in the developing and developed organism. When we speak of the transmission of characters from parent to offspring we are speaking metaphorically; for we now realize that it is not characters that are transmitted to the child from the body of the parent, but that the parent carries over the material common to both parent and offspring. This point of view is so generally accepted to-day that I hesitate to re-state it".[432]

It is useful, in the analysis of the movement of genetics away from the embryology paradigm, to look closely at what Morgan claimed in this opening paragraph. Firstly, Morgan stated that "the problem of heredity [is] identical with the problem of development". At the turn of the twentieth century then, Morgan is declaring that there is a relationship between the study of heredity, and the study of development, to the point where they are "identical". This has also been highlighted by James Griesemar, who suggested that heredity and development were considered together under the discipline of 'reproduction'[433]. Meunier however argued that through this opening paragraph, Morgan is explicitly moving away from an older, literal view of character transmission, and towards a metaphorical interpretation[434], supporting his claim that genetics and embryology were never a single discipline. As Morgan stated that "heredity stands for those properties of the germ-cells that find their expression in the developing and developed organism", Meunier claimed that here, Morgan is separating himself from the 'transmission of characters' theory of the older, literal interpretation, where characters were transmitted and developed. By rejecting this literal view concerning characteristics then, Morgan has highlighted the distinction between transmission of hereditary material and its expression - transmission only concerns the "properties" of germ cells[435] (i.e. constituents of parent germs cells and characteristics of the offspring are not the same thing). Morgan reserved the term "heredity" for this transmission process; an interpretation of the original legal metaphor of transferring ownership, goods, or property.

Morgan here then is again signalling that he considered heredity and development as separate processes, which was distinct from the older view of

[432] Morgan, 1910 p 449.
[433] Griesemer, 2007.
[434] Meunier 2012 p 196.
[435] Meunier, 2012.

transmission/translation (or heredity/development) being considered as parallel, or at least overlapping, processes[436]. By 1917, Morgan had developed this idea further:

"…it seems desirable in the present condition of genetics and embryology to recognise that the mechanism of distribution of the hereditary units or genes is a process of an entirely different kind from the effects that the genes produce through the agency of the cytoplasm of the embryo".[437]

Here Morgan clearly delineated the distinction between transmission ("mechanism of distribution of the hereditary units or genes") and the manner in which genes affect development, i.e., 'characters'. Those who argue it was Morgan who separated heredity and development, such as Allen and Gilbert, suggest that Morgan abandoned developmental explanations, retaining a view that the presence of characters are explained via genes. This opinion led Gilbert to suggest that studies of inheritance became the discipline of genetics (as defined by Morgan's 1926 accounts of a discipline concerned with transmission of genes)[438]. I prefer the view supported by Meunier, which argued that Morgan actually separated heredity from 'characters', leaving explanation of characteristics to the discipline of development. In *The theory of the gene*, Morgan wrote that "The modern theory of heredity is…primarily concerned with the distribution of units between successive generations of individuals"[439]; 'units' are genes, whilst characters are considered data. Morgan's theory of heredity is therefore concerned with the distribution of genes to successive generations. By excluding characters from heredity, Morgan separated development from heredity[440] (not genetics from embryology).

As previously mentioned, Meunier has argued that the separation narrative is too Morgan-centric. Meunier is not the only researcher to make this claim; in *The cultural history of heredity*, Müller-Wille and Rheinberger also state that the establishment of genetics "cannot be reduced to the relatively narrow realm of pure transmission genetics in the style of Morgan"[441]. Meunier's alternative explanation is that genetics and embryology developed from different research traditions. To

[436] Meunier, 2012 p 197.
[437] Morgan, 1917 p 544.
[438] Gilbert, 1996 p 104.
[439] Morgan, 1926 p 1.
[440] Meunier, 2012 p 199.
[441] Müller-Wille and Rheinberger, 2012 p 159.

support his claim, Meunier developed Rasmus Winther's theory of the two styles of thought in biology: formal (with a focus on mathematical laws and models) and compositional (focusing on parts and wholes, and respective functions)[442]. Winther claimed that developmental biology would be an example of biology in the compositional style, whilst genetics follows the formal. Meunier developed Winther's idea in his PhD thesis by contrasting Winther's 'compositional style' with his own 'differential' way of thinking: in the compositional style, the whole is separated into its constitutive parts. Meunier's differential style instead noted that such parts may be considered as separate, but may be classed together[443].

4.3 Weldonian Genetics

Greg Radick has been concerned with a query regarding heredity and genetics, particularly the Mendelian paradigm and its dominance in the teaching and thinking of genetics. Rediscovered in 1900, Mendel's studies on wrinkled peas and petal colour have shaped the teaching of heredity throughout the twentieth century, with Mendelian genetics dictating that eye colour or hair colour could be explained simply through the inheritance of dominant or recessive characteristics. As geneticists have learned more about the way genes and DNA functions through the latter half of the twentieth century, it became clearer that the Mendelian view of genetics is oversimplified to the point that it is incorrect for anyone requiring any more than a basic understanding of heredity. One of the first to observe this, as Radick points out, was Walter Frank Raphael Weldon (1861-1906), a zoology graduate of Cambridge University. After Mendel's work was rediscovered in 1900, Weldon set-about writing a critique of Mendel's theories of inheritance, which was published in early 1902. Weldon identified that Mendel's results oversimplified the inheritance of hereditary factors (genes), which he noted from observing 'errors' that would crop-up in Mendel's theories. A typical example, as given in Weldon's 1902 paper, is that peas can not be easily separated by colour - although there are green and yellow peas (as

[442] Winther, 2006.
[443] Meunier, 2015.

Mendel claimed), there were also peas that were greenish-yellow, or yellowish-green; there was a continuum of colours[444] (Figure 5).

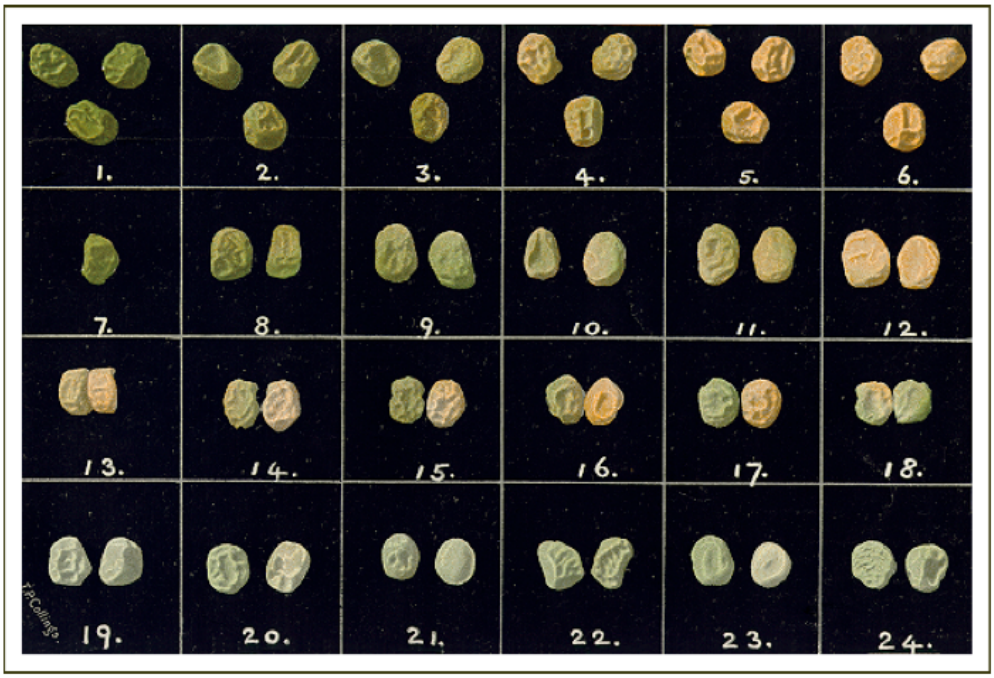

Figure 5: Photographic plate taken by Weldon to demonstrate that not all peas easily fit into a category of 'yellow' or 'green'. From Weldon, 1902.

Radick argued that for Weldon, it was noting those results that were not expected, and checking on what could have caused them, that generated 'good science'[445]. Following publication of his critique of Mendelism, Weldon spent the next four years formulating an alternative explanation. Radick noted that Weldon's work would approach the study of genetics in two ways: firstly, using statistics, and secondly, using experimental embryology. Statistical analysis was useful to Weldon, since it would highlight those outlying results that may otherwise be ignored, and could give clues as to actual underlying mechanisms. Weldon's selection of experimental embryology to study genetics is, according to Radick, surprising; this despite experimental embryology being "one of the premier sciences in biology in the late nineteenth century"[446]. Prior to his untimely death, Weldon was preparing a book which detailed an alternative way of explaining heredity (and genetics), which made

[444] Weldon was a follower of Francis Galton, who had observed a similar phenomena in eye colour - that there was not only 'light' or 'dark', but many different colours that could not be easily predicted based on the eye colour of previous generations.
[445] Radick, 2012.
[446] *ibid*.

important use of experimental embryology. Radick highlighted one such case, where embryos were dissected into three sections, and each section was capable of regrowing and again forming another complete embryo. Such experiments, Radick argued, demonstrated to Weldon that dominance or recessiveness could not be something permanently expressed in tissues (as proposed by Mendelism), but something that could be influenced by environmental or otherwise external factors - gene expression was "fundamentally context dependent"[447]. Throughout his unfinished book, Weldon built a picture of the expressive capacities of hereditary factors that depended on interaction with each other (for example, that characteristics like eye colour were not dependent on the expression of a single gene) and their environment.

In the early twenty-first century, we accept this to be the case - genetic inheritance is not as simple as Mendelian genetics suggests, and in fact interactions between genes, proteins, and other external factors has a significant impact on the characteristics observed. Radick argued that Weldon's premature death allowed his nemesis William Bateson (1861-1926), a firm believer in Mendelian genetics, the opportunity to propagate his own views, and his influence ensured Mendelian genetics would be the way genetics has been taught into the current century[448]. This chapter is not concerned with this point in particular, but it is important that Weldon elected embryology as a way of demonstrating his own theories of inheritance and mechanisms of genetics, whereas Mendel and Bateson did not. It appears that only in the latter decades of the twentieth century, when the complexity of inheritance and the mechanisms of genetics were becoming clearer, that Weldon's ideas, collected in part using embryology, began to appear more accurate than Mendelism. This chapter has previously been looking at how (or whether) the disciplines separated, and how they developed alongside each other; what Radick's work considering Mendelian versus Weldonian genetics shows is that without embryology, a clearer notion of the complexity of genetic mechanisms could not be demonstrated. Early genetics needed embryology to demonstrate not only how genes were transmitted, but that genes should not be considered single 'hereditary units', where each characteristic was 'coded for' by a single gene, inherited from one's parents. Embryology demonstrated that phenotype expression was more complex than this, with gene translation alone

[447] *ibid*.
[448] *ibid*.

being unable to account for development and phenotype. Weldonian genetics needed the embryological paradigm to demonstrate that Mendelian genetics was oversimplified, and that a much more complex explanation was required.

5. Conclusions

What can the 'fantastical experiments' described above tell us about genetics under an embryological paradigm? And why is this relevant to the history of embryonic stem cell research? This chapter has shown that nuclear transfer experiments demonstrate that a form of experimental embryology was still very relevant to genetics throughout the twentieth century.

This chapter began with an overview of conceptualisation of the cell nucleus, since elucidation of its structure and function is relevant to the way a role for the nucleus (and its content) was understood. I use August Weismann's work as an example here to demonstrate that concepts of structure and function of the nucleus were relevant for theoretical work in heredity (and genetics); Weismann's germ plasm theory made use of previous observations on the cell nucleus and heredity to propose that 'id' distribution was important in embryonic development. Here is an important link then with the history of ESC research: in the late nineteenth century, Weismann was combining ideas about heredity, cell biology, and embryology to create a theory concerning the importance of heredity as a mechanism in evolution, and for successful embryological development (this of course is also an important moment in evodevo[449]). What developed from Weismann's theorising was the field of experimental embryology - a way of testing hypotheses such as those set out in the germ plasm theory. For ESC research, the methods of experimental embryology arguably produced the first experimental insights into embryo development at the cellular level (as opposed to the observational work carried out in the earlier nineteenth century, as described in Chapter 1). The earliest work in experimental embryology demonstrated that individual cells of the early embryo were capable of generating new, entire creatures (such as that demonstrated by Hans Driesch), or that the position of certain cells in the embryo would have significant effects on development (Weldon, Spemann, and Mangold).

[449] See Laubichlier and Maienschein, 2007.

Initially, experimental embryology was technically challenging, leading Spemann to, for example, resort to using his young son's fine hair as a tool to separate embryonic cells from each other. Spemann famously suggested that much more could be learned about embryonic development and heredity by transferring the nucleus from one cell to another; an idea previously suggested by Yves Delage. The tools that would be required to successfully transfer nuclei between cells was not available to Delage or Spemann, but the idea for the 'fantastical experiment' was clearly around in early twentieth-century biology. Both Delage and Spemann consider that it would be a useful exercise to transfer the nuclei of one fertilised egg into an enucleated fertilised egg of another species; why? I argue that the proposition of nuclear transfer suggests that Delage, Spemann, and possibly others in the field, had an understanding that the nucleus had two linked functions: a way for information to be passed on from parents to offspring (heredity), and to hold the information required to develop all the different cells needed in a multicellular organism (genetics); this was also a factor considered by Weldon. How were Delage, Spemann *et al*. suggesting that this understanding of nuclear function could be tested? Via embryology. Here then is a clear example of the discipline of genetics developing under the paradigm of embryology (as suggested by Allen). This approach is relevant for this thesis, since the experiments carried out were on the early embryonic cells - those which we now understand to be pluripotent or totipotent. Such totipotency was clearly demonstrated by early experiments (such as by Driesch); although the primary aim of this work was not to explicitly learn more about ESCs, it certainly contributed to the field which would develop later in the twentieth century.

Allen argued that genetics split from its embryological paradigm following the work of TH Morgan in the 1920s. This hypothesis is contested by Müller-Wille and Rheinberger, and by Meunier, who argued that the splitting view is focused too much on Morgan; I am inclined to agree (although Meunier also argued that genetics and embryology were separate disciplines from their inception; this I disagree with). In support of the claim that genetics and embryology did not split, I demonstrate that the fantastical experiments proposed by Delage and Spemann continued throughout the twentieth century. Although of course these are not the only experiments that are being used to learn more about genetic capability of cells, I believe that nuclear transfer experiments demonstrate that a form of experimental embryology was still very relevant to genetics through the twentieth century; furthermore, as experimental

embryology methods were still being used to learn about genetics, it can be argued that genetics was still, to an extent, under the embryology paradigm. The experiments I refer to as examples in this chapter are those carried out by Briggs and King, and Gurdon in the mid-twentieth century, and by Illmensee in the latter decades. Briggs and King began by demonstrating that the fantastical experiment was possible in principle; Gurdon demonstrated that it was an actual possibility, reporting in the early 1960s that frogs created by nuclear transfer had reached adulthood. In the late twentieth century, nuclear transfer methods were being used in mammalian embryology, a focus of Illmensee's work. As highlighted in their published papers, Briggs, King, Gurdon, and Illmensee all utilised nuclear transfer as a way of not only learning more about embryology, but making use of established embryological techniques to learn more about genetics (it also demonstrated that Weldon was correct to suggest that gene expression was dependent on context).

This chapter then has demonstrated, by carefully selecting examples of twentieth-century nuclear transfer experiments, that Allen was incorrect to suggest that the field of genetics split from embryology in the early twentieth century. Instead, I argue that genetics continued to be studied via embryological techniques (such as nuclear transfer), and, as further demonstration, that Weldonian genetics required the experimental results provided by embryology to demonstrate the complexity of genetic mechanisms. This is relevant to the history of ESC research since, as researchers were looking to embryological techniques to examine genetics, they also highlighted the important features of ESCs - i.e. the ability to multiply (and generate all the cells of an entire new organism), and the ability to generate all of the different cell types required (by an entire new organism).

Having now considered in detail the background to stem cell research in the late nineteenth and early twentieth centuries, the thesis now turns to studies that shaped ESC research in the twentieth century. In the following chapter, a brief history of cancer theory is required, before the discussion can move on to discuss parallels between cancer and stem cell studies through the 1900s.

CHAPTER 3:

PATHOLOGICAL AND NON-PATHOLOGICAL DEVELOPMENT: PARALLELS BETWEEN CANCER AND EMBRYONIC STEM CELL CONCEPTS

1. Introduction

Recently, and in particular since the turn of the twenty-first century, several researchers have drawn attention to the historical links between cancer research and developmental biology, in particular that which occurred in the latter half of the twentieth century. For example, Alison Kraft (2009, 2011) and Melinda Fagan (2007, 2010) have highlighted the post-World War II history of haematopoietic stem cell research and bone marrow transplantation. There are few accounts that have generated a comprehensive overview of the link between cancer research and stem cell research, particularly that which goes back further than the mid-twentieth century. Philosopher of science Lucie Laplane has made some steps towards this with her recently published book (2016) on the cancer stem cell theory, however, the focus on philosophy of science is key to this text, and historical context is only provided in the early sections of the book.

This chapter then intends to move towards a more comprehensive history of the links between cancer and embryonic development, with a focus on stem cell research. This will begin with a brief overview of the history of cancer theory, focusing on the nineteenth-century development of the embryonic rest theory. This theory, or, more correctly, cluster of theories, is a key moment in the comparison of embryonic (non-pathological) and tumour (pathological) development. It is possible that the embryonic rest theories were so highly regarded because they highlighted that embryonic cells possessed the "essential factors" of tumour growth[450]. These comparisons are clearest when embryonic development is compared with the development of teratomas – those tumours that, like embryos, contain various tissues originating from all three germ layers.

Teratomas are tumours that can contain several different cell types, including (but not limited to) nervous tissue, skin, bone, and glandular tissue, for example. These types of tumour are believed to form from either germ or embryonal cells (i.e. pluripotent cells), and can grow in almost any region of the body. Since teratomas can arise from germ cells, they are often observed in either the testes or ovaries of adults or children. It is now, according to the journal *Nature*, "clear" that some

[450] Ewing, 1919 p 94.

tumours are sustained by a population of stem cells – cancer stem cells (CSCs)[451]. Just as other stem cells have the ability to divide asymmetrically, so do CSCs, perpetuating and growing tumours with each division. The concept of a cancer stem cell is not a recent idea; in the early twentieth century for example, Boveri interpreted the results of his experiments[452] as demonstrating that tumours "go back to common ancestors…If one extrapolates further, one is bound to conclude that, in general, every tumour has its origin in a single cell"[453]. Boveri referred to this as the 'primordial tumorigenic cell'[454]. Teratoma formation *in vivo* is a useful (and widely used) approach to determine the pluripotent potential of stem cells (such as ESCs), since it enables the cells to demonstrate their ability to differentiate into cell types of all three germ layers. For example, when transplanted into immunodeficient mice, pluripotent stem cells are able to form differentiated teratomas, containing a variety of tissues.

The section of the chapter that discusses teratomas will describe the work of pioneers in mid-twentieth century teratoma research, in particular Leroy Stevens and Barry Pierce. As Stevens and Pierce carried out their experiments, more was learned about the origins of teratomas, and perhaps all cancers (as theorised in the embryonic rest theory, and the later CSC theory). Although in the mid-twentieth century researchers like Pierce and Stevens did not often use terms such as 'embryonic' or 'cancer stem cell', this changed following the work of Gail Martin and Martin Evans, who were able to isolate and culture the stem cells of teratomas. (This is discussed in Chapter 4.)

Following the narrative of teratoma research in the mid-twentieth century, this chapter then explicitly explores the parallels between cancer and embryonic development as currently understood, including, for example, consideration of gene expression and cell biochemistry.

Bringing together the historical conceptualisations of cancer (as explored in this chapter) with the conceptualisation of the embryonic stem cell (as examined in Chapter 1), this chapter concludes with an examination of similar properties and functions between cancer and embryonic development that have been considered throughout history, and how this is relevant to the history of ESC research.

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[451] Passegué, 2006.
[452] These experiments were those carried out to test his hypothesis that genetic changes caused malignancy in cells. For example, see Boveri, 1914.
[453] Boveri, 1914 (transl. Harris, 2008) p 32.
[454] *ibid* p 33.

2. History of cancer theory

As observed by Pamela Sanders-Goebel in her reflections on surgical interventions in cancer, throughout history, cancer treatment has been based on the then prevailing hypotheses, and results following treatment interpreted in light of the popular hypotheses of the time. Treatment also needed to correspond to the personal beliefs of the cancer patient[455]. Lay understanding of cancer by the early twentieth century appreciated cancer as growth, however clinicians and researchers were keen to highlight that there was a difference between normal and pathological (cancerous) growth, and this could be seen most easily in the comparison between embryonic development and cancer[456].

2.1 Ancient

Cancer has been known of for thousands of years and its causes have been debated many times over the course of history. Prehistoric and ancient peoples believed that cancer was caused by supernatural events, such as the alignment of the planets or evil spirits for example. Religious beliefs also played a part, with ancient Greek, Roman and Hebrew writings suggesting that sin and wrath of gods caused cancer. Some scholars have noted that relatively little appears to have been written about cancer at this time, possibly due to its generally incurable nature[457].

Greek-Roman physician Galen (c. AD 129-216) also wrote about various cancers. Cancer [*karkinos*], Galen claimed, developed from accumulation and thickening of the black bile. This theory appeared to be based on Galen's observation that cancer occurred more often in women after cessation of menses, and that rectal cancer was associated with haemorrhoids. Unlike those before him however, Galen proposed treatment for internal, hidden cancers, as well as excision and cauterisation of superficial cancers; Galen had become an active surgeon during his time as physician to the gladiators at Pergamum (AD 158-161), and there is evidence that this practice continued at least into the 180s, and perhaps 190s[458].

[455] Sanders-Groebel, 1991 p 77.
[456] Simonds, 1935.
[457] For example, Riordan, 1949.
[458] Toledo-Pereyra, 1973.

2.2 Middle Ages

After the collapse of the Roman Empire following the fall of Rome in AD 476, the remaining Eastern Roman (or Byzantine Empire) retained some medical knowledge from its predecessor (which was not the case to the same extent for the remaining Western Roman Empire)[459]. For example, physician Paulus Ægineta (c. 625-690) followed Galen's teachings with regards to the description and treatment of cancer. In addition, Ægineta advocated the removal of nearby lymph nodes[460]. Further eastwards, Christian scholars of the Near and Middle East ensured Galen's writings were preserved in churches and monasteries. Some Arabic caliphs encouraged adoption of Greek thought, resulting in translation of Greek medical texts into Arabic, and their use by Arabic physicians. Some such physicians included additional descriptions of cancer and its treatment, such as Avenzoar (1094-1162), who gave descriptions of oseophageal and stomach cancer, and proposed treatments (including feeding via enema). Avenzoar's work became part of standard teaching at several influential medical schools in Europe, including the universities at Padua, Montpellier, and Bologna, by the end of the fourteenth century[461].

2.3 Early Modern and Modern

American pathologist James Ewing (1866-1943) claimed that the first to criticise Galen on the causes of cancer was the Swiss physician Paracelsus (1493-1541). Instead of humoural imbalance, Paracelsus suggested that substances such as salt of sulphur and arsenic could cause cancer if these accumulated in the blood – perhaps building on the previous inclusion of chemical agents in therapeutics. The French surgeon Ambroise Paré (1510-1590) also believed that cancer could be caused by depositing of toxic substances in the blood, which, if overheated, could cause the ulceration observed in some cancers. Such ideas were elaborated on into the seventeenth century, where cancer came to be explained in the context of newly identified anatomical structures or chemistry[462].

The reintroduction of dissection during the sixteenth century aided diagnosis and demonstrated the tumours and ulcers that typify cancer; prior to this point,

[459] Faguet, 2015a.
[460] *ibid*.
[461] *ibid* p 2026.
[462] Sanders-Groebel, 1991.

internalised tumours were either unknown or considered untreatable. Alongside this, the discovery of blood circulation (in 1628), the lymphatic system (in 1656), and red blood cells (in 1661) suggested fermentation and/or coagulation of the blood and lymph could cause cancer (lymph theory)[463]. Lymph theory suggested that a cancerous lump could be caused by coagulation, resulting from obstructed flow around the lymphatic system - where obstruction was in the lymph nodes themselves, coagulation here could also form cancers (and a build-up of acid in these lumps could cause ulceration)[464]. By the early 1700s, many considered the cause of cancer to be stagnation and coagulation of blood, particularly if the blood was contaminated with poisonous substances.

In 1775, the surgeon Percival Pott (1714-1788) suggested that chimney sweeps were susceptible to cancer due to the accumulation of soot on the skin[465]. Prior to Pott, it may have been Bernadino Ramazzini (1633-1714) who had first suggested that cancer may be linked with occupations, behaviour, environment. Working particularly in Modena (1700) and Padua (1713), Ramazzini studied the incidence of cancer in nuns revealing that, compared with the general population, there was a lower risk of cervical cancer, but a higher risk of breast cancer[466]. In 1761, botanist and physician John Hill (1714?-1775) would also link tobacco snuff and cancer[467]. Surgeons believed that the most effective treatment was immediate excision of the ulcer or tumour; physicians however did not agree[468].

With the cause, nature, and treatment of cancer still provoking a variety of theories and practices, the Academy of Lyon in France offered a prize in 1773 for the most enlightening report on *Qu' est ce que le cancer?*. The competition was won by French surgeon Bernard Peyrilhe's (1735-1805) doctoral thesis, published in 1776[469]. In his book, Peyrilhe suggested that cancer was caused by a toxin, and resulted in

[463] Ewing, 1919; Javier and Butel, 2008. The lymph theory suggested that fermenting and degenerating lymph were the constituents of cancers (proposed by George Ernst Stahl and Friedric Hoffmann in 1695, and supported by the Scottish surgeon John Hunter in the eighteenth century).
[464] Sanders-Groebel, 1991.
[465] After Pott's work, some chimney sweeps guilds would advise bathing every day to help prevent 'chimney-sweepers cancer'. Faguet, 2015a; Young, 2005.
[466] Faguet, 2015a.
[467] Hill, 1761.
[468] Hadju, 2006 p 1644.
[469] Hadju 2006; Ewing 1919; Faguet, 2015b.

'virus' formation[470], although the precise causative agent was unknown. It was Peyrilhe's experimental approach that was particularly novel – Peyrilhe proposed injecting samples from cancers underneath the skin of other mammals[471]. Peyrilhe also considered why relapses occurred after surgery, suggesting that the cancer had spread unseen through blood or lymph, or that the entire tumour was not removed by the surgical procedure[472].

Meanwhile, the introduction of improved microscopy was allowing more detailed observational records of cancer to be made. For example, Giovanni Morgagni (1682-1771) contributed to the understanding of cancer pathology via his book *On the seats and causes of diseases as investigated by anatomy* (1761), which included careful descriptions of autopsies including many who died of cancer[473].

In the early nineteenth century, there re-emerged the concept that cancer was like a parasite[474], mirroring earlier ideas of cancer eating the flesh of the patient, clinging to it with claws like a crab. This parasitic view of cancer suggested that a tumour could live an autonomous existence in the body of the patient.

Cell Theory of the nineteenth century therefore had a significant effect on cancer theory. As pathologists began applying cell theory to cancer, they began suggesting that cancer cells existed. In 1845, anatomist Hermann Lebert (1813-1878) described 'cancer cells' when referring to surgical removal of a breast cancer tumour[475]. Lebert, an influential clinical pathologist, had previously also asserted that cancers may contain "heterologous" elements (i.e. that tumours contained cells that were not typical of the tissue of cancer origin) – an idea that became popular (especially in France) in the mid-nineteenth century[476]. Professor of anatomy and physiology, and later pathology, at Utrecht, Jacobus Coenraad Schroeder van der Kolk (1797-1862) also subscribed to the cancer cell theory, claiming in 1853 that cancer cells could be present in the body far removed from the original tumour[477]. William Halsted (1852-1922), an American surgeon, proposed that cancer could arise

[470] Peyrilhe's reference to a 'virus' uses a historic definition of 'virus', similar to a poison. It did not refer to a virus as we would currently understand it.
[471] Faguet, 2015a.
[472] *ibid*.
[473] *ibid*.
[474] Bauer, 2004.
[475] Sanders-Groebel, 1991.
[476] Bauer, 2004.
[477] Sanders-Groebel, 1991.

from a single proliferating cell, which would result in cancerous cells spreading throughout other tissues in the region; surgery was one option to remove all of the cancer cells from an area, regardless of which tissues they had become present in. The cancer became inoperable when the tumour cells spread beyond the locality[478]. In addition, development of Cell Theory and understanding of cytology led to the view that cancer was not generally caused by any type of infection by micro-organisms (with the exception of the virus found to cause some sarcomas)[479]. It was also during the nineteenth century that French physician Joseph Claude Anthelme Recamier (1774-1852) coined the term 'metastasis'[480].

A further theory that gained in popularity during the nineteenth century was the proposal that cancer risk could be inherited. One such physician who noted the greater incidence of cancers in some families was the physician Sir James Paget (1814-1899); many who took this view also believed that the cause of cancers may have been inherited due to an "underlying constitutional defect"[481]. Paget suggested that previously undetected (developmental) defects in organs may also leave the affected susceptible to malignancy[482]. Boveri would help later researchers demonstrate a link between genetics and cancer through the results of his experiments on the artificial fertilisation of sea urchins. Boveri observed that when he fertilised a single sea urchin egg with two spermatozoa, the resulting fertilised egg often had more chromosomes than normal, and would multiply to form not embryos, but unorganised masses of tissue[483]. From such experiments, Boveri would conclude that tumours may be the consequence of "a certain abnormal chromosome constitution", although was aware that during the first decades of the twentieth century, his ideas were often met with scepticism[484].

Between the 1920s and 1960s, radiation treatment became increasingly popular, particularly after research had demonstrated how radiation could kill cancer cells[485]. In the context of studies on the effects of radiation, James Till and Ernest McCulloch of the Ontario Cancer Institute developed (accidentally) the first

[478] *ibid*.
[479] Simonds, 1935.
[480] Faguet, 2015a.
[481] *ibid* p 2029.
[482] Ewing, 1919.
[483] Calgins and Boveri, 1914; Baltzer, 1967; Faguet, 2015a.
[484] Boveri, 1914 (transl. Harris, 2008) p 5.
[485] Sanders-Groebel, 1991.

qualitative assay for generating haematopoietic cells (from haematopoietic stem cells). They did this by irradiating mice to kill all bone marrow cells (mirroring radiation poisoning), before attempting to re-populate the bone marrow. Some of the injected cells would create a 'colony-forming unit' in the spleen, effectively creating tumours from single cells[486].

2.3.1 Embryonic rest theories

The embryonic rest theory suggests that cancer can develop from residual embryonic cells. It is a theory that has been proposed since the early 1800s; the pathologist Jean Frederic Lobstein (1777-1835) and Recamier both compared the growth of tumours to embryonic growth in the 1820s. In particular, Lobstein noted the similarity of tumour growth with embryonic tissue, conceiving that neoplastic growths were tissues that were no longer under the control of the organism[487]. Paget also suggested that 'invisible defects' in organ formation may be prone to malignancy. As noted in Chapter 1, in the early nineteenth century, Schwann proposed that cells were formed from the cytoblastema. Schwann's mentor Müller, in his work *On the fine structure and forms of morbid tumors* (1839), agreed that cells would form from condensation of the cytoblastema, and that cancer had a similar origin: cancer would develop from crystallisation of *semen morbi* (germ of disease) of the cytoblastema[488] – i.e. the cancer 'germ' was in the cytoblastema, and cancerous cells would develop out of this diseased cytoblastema (although Müller did not see evidence for this from the microscopical studies carried out)[489]. Remak refuted Schwann's theory however, suggesting instead in the mid-nineteenth century that cells divide (forming two cells where there was once one), as opposed to any spontaneous generation theory[490]. Tumour cells, therefore, must also arise in this fashion[491]. As an alternative to Müller's suggestion that cancer cells emerge from the cytoblastema, Adolf Hannover (1814-1894) suggested that tumours would arise from a specific cancer cell [*cellula cancrosa*], which was morphologically distinct from other non-pathological cells. The anatomist and surgeon Alfred Armand Louis Marie

[486] Lancaster, 2009.
[487] Krebs, 1947.
[488] Laplane, 2016, p 49.
[489] Faguet, 2015a.
[490] Remak, 1852; Remak, 1855.
[491] Faguet, 2015a.

Velpeau (1795-1867) also proposed that a cancer cell existed, but that it was not the cause of the cancer; instead Velpeau suggested that there must be "some more intimate element" that caused cancer[492]. Virchow was also unable to find evidence of the *cellula cancrosa*[493]. Initially, in *Die krankhaften Geschwülste*, Virchow proposed that cancer occurred due to changes in the connective tissue (see below), rejecting Remak's suggestion that cells multiplied by division. It did not take long for Virchow to reverse his decision however, making use of Raspail's phrase *omnis cellula e cellula*[494]. Virchow would also disagree that cancers could be heterologous, since there was no analogy for this in the healthy body (see below for Virchow's reasons for finding equivalents in pathological and non-pathological tissues)[495].

Professor of Surgery at the University of Rome, Francesco Durante (1844-1934), was likely to be one of the first to clearly state that all tumours arise from embryonal cells in 1874[496]. This said, it was the German pathologist Julius Friedrich Cohnheim (1839-1884) who popularised the theory (possibly through his influence as a biologist[497], presumably having more scientific influence than Durante as a surgeon). Ewing (1919) has also remarked on the comprehensive nature of Cohnheim's theory, which would increase its appeal. In addition, Durante's papers and books were published only in Italian[498], which is likely to have restricted his readership. Cohnheim worked at several universities in Germany, including Berlin, where he studied under Virchow. Although this theory was not his most famous work, Cohnheim is often credited with the 'embryonic rest' theory of cancer – i.e., that cancers form from embryonic cells which had not migrated to the appropriate region of the body. The model for this theory was the teratoma, which could contain tissues from all three germ layers, as found in the developing embryo (see section 3).

Hugo Ribbert (1855-1920) was a German pathologist who agreed with most of the 'embryonic rest' theory, but attempted to update it in light of some criticisms (for example, that adult cells were also capable of forming tumours under appropriate circumstances). Ribbert believed that cancer could arise from cells which 'had a

[492] Velpeau, 1853 (transl. Faguet, 2015a p 2029).
[493] Faguet, 2015a.
[494] *ibid*, p 2029.
[495] Bauer, 2004 p 8.
[496] Eyre, 1896; Ewing, 1919.
[497] Krebs, 1947.
[498] Eyre, 1896.

disturbed relationship with their neighbours'[499], causing them to grow and divide abnormally.

In 1854, Remak had proposed what Laplane has referred to as the 'delocalised' embryonic rest theory. Remak's view was inspired by Virchow's detailed description (above), in an effort to explain why a tumour of ectoderm origin appeared in tissue of mesodermal origin. Remak's explanation was that the tumour had arisen from an ectodermal embryonic cell, which had become 'delocalised' during the early stages of embryogenesis[500] – hence Laplane's terminology. This was not intended to be a general theory of cancer development, but was intended to explain how a tumour of a different tissue type to the organ of origin could emerge. For example, Remak would also note that cancer could emerge from islands of epithelial cells, in tissues that would not normally contain epithelia[501]. Louis Bard (1829-1894) expanded Remak's work, by identifying a method by which different cell types could arise: differentiation. Bard proposed that normal cells would divide and then mature into different functional cell types. In cancer development, defects in the cells would result in tumour formation instead of non-pathological mature cell types[502].

Virchow himself had a different view however, proposing what Laplane referred to as the 'connective tissue' theory[503]. Virchow believed that all new cells came from division of cells in connective tissue, therefore connective tissue cells could generate cells of all three germ layers, both normal and pathological. Virchow was convinced that the underlying goal of Cell Theory was correct – that it was a unifying theory, explaining that all cells originated from inside the body, including pathological cells. Virchow rejected any ontological pathologist view that the entity of a disease was outside the patient's body; likewise, the tumour was always part of a patient's body (and subject to the same 'laws of biology'). Virchow believed that the cell was the seat of physiological processes, pathological and non-pathological, and that Cell Theory could be preserved if he could show that tumour cells emerged from non-pathological tissue[504]. Unlike Remak's theory, Virchow believed that this theory

[499] Witkowski, 1983 p 271.
[500] Remak, 1854 p 172.
[501] Ewing, 1919.
[502] Faguet, 2015a.
[503] Laplane, 2016 p 52.
[504] Bauer, 2004 p 3.

could be applied to all cancers. Virchow's connective tissue theory certainly had its critics however.

In January 1861, the *British Medical Journal* published detailed reviews of Virchow's *Cellular Pathology*, which had been translated into English, and published in 1860. In addition to refuting Schleiden and Schwann's Cell Theory to concur (unreferenced) with Remak's views, Virchow suggested how morbid growths were generated. Virchow proposed that cancer cells must arise from pre-existing cancer cells; since cancer could develop in any tissue, Virchow formulated the 'histological substitution' argument, stating that it was possible "to find one tissue at a certain fixed point of the body replaced by an analogous one belonging to the same group, or, in other words, by an *histological equivalent*" (original emphasis)[505]. The *BMJ* reviewer was not wholly impressed by Virchow's histological equivalent theory, concluding that obvious failures in Virchow's initial theory of cancer generation were too easily explained by a claim that tissues could be mixed up[506]. Considering the connective tissue theory, the *BMJ* reviewer first noted that the term 'connective tissue' is not particularly specific, since collectively, German anatomists referred to many fibrous tissues, including ligaments, as connective tissue[507]. The reviewer called into question Virchow's assertion that the connective tissue had "the common stock of germs (*Keimstock*) of the body, and [it is possible to] directly trace to it, as the general source, the development of new formations"[508], including benign and malignant morbid growths[509]. The reviewer stated that the evidence Virchow supplied for this was "very unsatisfactory", and that "Nowhere have we seen any evidence that tubercule forms within fibre-cells, and still less cancer", despite the frequency with which such diseases occur[510].

A further type of embryonic rest theory, proposed by Julius Cohnheim, has been referred to as the 'superabundant rest' theory[511]. Cohnheim's theory can be seen as an adaptation of Remak's idea – in contrast to the restrictions Remak placed on the types of cancers caused by embryonic rests, Cohnheim believed that the theory could explain the development of all cancers. In addition to applying the theory to all

[505] Virchow, 1860 p 70.
[506] British Medical Journal, 1861a p 45.
[507] British Medical Journal, 1861b p 94.
[508] Virchow, 1860 p 398.
[509] British Medical Journal, 1861b p 94.
[510] *ibid* p 95.
[511] Laplane, 2016 p 53.

cancers, Cohnheim proposed that the cancerous cells were not just those that had become 'detached' (like Remak), but also those that the organism did not require for development (i.e. 'superabundant'). Instead of focusing on the displacement of embryonic cells in the causation of cancer, Cohnheim gave more weight to the "inherent disposition" (the developmental ability intrinsic to the cell) to explain potential tumour development. In addition, the cell may have the potential to become cancerous, but cancer would not develop without other developmental factors[512].

Cohnheim was the individual whose name became attached to the embryonic rest theory in general - for example, in his 1935 review of cancer, Northwestern University's professor of pathology James Simonds refered to the potential for hereditary causes of cancer: "In the development of the human embryo certain faults in reproducing the normal pattern may occur. Some of these faults, such as Cohnheim's embryonic rests, may be a factor in the later occurrence of cancer"[513]. Ewing, in 1919, described Cohnheim's theory as 'modern', and highlighted the details of the theory: that tumours develop from masses of tissue misplaced during embryonal development, or from 'small groups of superfluous cells', which retained their embryonic characteristics (and were not necessarily misplaced). The 'embryonal character' of the cells however was central to the theory. Cohnheim believed that these cells were the result of overproduction of the germ layers prior to organ formation. These cells were then distributed throughout the body, with a tendency to gather in certain regions. Sudden development of these cells occurred due to changes in blood supply[514]. Ewing stated that "the present support of Cohnheim's theory is extensive", since it explained the presence of different tissue types in tumours; this is most obvious in teratomas (see below)[515].

Anatomist Ernest Krebs (1911-1996) suggested that the embryonic rest theory was a "logical consequence" of German physiologist Wolff's theory of epigenesis[516]. Epigenesis, as opposed to preformationism, proported that multicellular organisms develop from seeds or eggs through a sequence of development and differentiation. Initially, Wolff's eighteenth-century proposal struggled for popularity, since it

[512] *ibid* p 54.
[513] Simonds, 1935 p 536.
[514] Ewing, 1919 p 94.
[515] Ewing, 1919 p 94-95.
[516] At the time of its inception (*Theoria generationis*, 1759), Krebs suggested this theory supplanted the previous theories of spermists, ovists and homunculists. Krebs, 1947 p 270. See also Roe, 1979.

suggested that development required the gradual formation of organised parts from unorganised matter[517]. This surpassed theories which suggested that the baby was pre-formed in either the egg or sperm prior to conception. It also suggested that an organism would develop as different parts were added. As Krebs observed, epigenesis enabled embryonic rest theory; if, as the creationists or preformationists asserted, offspring were already fully developed in the germ cell, then there would be no opportunity for cells of this embryo to become misplaced. In epigenesis however, embryos were not pre-formed in the germ cell, and were generated anew; this opened up the possibility for newly emerging cells to become misplaced in the embryo, and later develop into cancers in the adult. Krebs used the metaphor of a house, being assembled brick-by-brick, then posing the question: "[What if] some of these "bricks" be misplaced in the process of building?"[518]. Similarly, Spemann, in experiments carried out in the late nineteenth century, demonstrated that when regions of the embryo (which have not yet differentiated into a specific organ) are transplanted to another 'organ field', the transplanted piece begins to conform to the 'morphological pattern' of the host region[519]. More differentiated tissue does not conform in the same way. This could account for 'embryonic rests', but probably suggests the possibility that embryonic cells are capable of conforming to a new environment, and that therefore there would be no 'rests'.

Krebs also reported French pathologist Charles Oberling's (1895-1960) view opposing the embryonic rest theory: Oberling argued that although embryonic cells and cancer cells do somewhat resemble each other, embryonic cells all eventually lose their proliferative potential and differentiate. Malignant cancer cells however continue to multiply[520]. This said, Spemann's and Oberling's theories were restricted to embryos; Oberling stated that in the body of an adult, embryonal cells may behave differently. Boveri also stated in the early twentieth century that he was sceptical of the "embryonale Reste" theory, claiming that "tumours are often found in circumstances that make one think that they may have arisen from arrested embryonic cells, but in most cases a connection of this sort can be excluded"[521]. In addition, although Ewing initially praised Cohnheim's theory, he stated that experiments

[517] Roe, 1979 p 2.
[518] Krebs, 1947 p 270.
[519] *ibid*.
[520] *ibid*.
[521] Boveri, 1914 (transl. Harris, 2008) p 56, 55.

testing the isolation of these cell groups were not always followed by tumour formation. In fact, tumour formation was one of five possible outcomes (no changes, cyst formation, limited growth, or normal development then atrophy may also occur)[522]. It is also likely, Ewing claimed, that tumours grew where no embryonic cells existed[523].

3. Teratomas

"We killed it, and looked at the testes, and they had strange things inside."[524]

3.1 History of teratomas

As early as the Roman period, tumours of the testes were known. For example, Galen described seven types of scrotal tumours, and prescribed their surgical removal[525].

In the eighteenth century, Pott was credited amongst his contemporaries for his understanding of cystic disease, which we would refer to today as teratomas of the testes (with cysts); Pott understood that these tumours could be malignant; this is in contrast with Pott's contemporary Sir Astley Cooper (1768-1841), a surgeon and anatomist, who believed that testicular teratomas were benign. Cooper authored one of the first books solely on the topic of testicular cancer (*Observations on the structure and diseases of the testis*), published in 1830[526]. Dr Samuel Gross also described several types of testicular tumour, including the teratoma, in his 1857 book, *Elements of pathological anatomy*[527].

According to Melinda Cooper, teratomas were first described in 1822 by Étienne Geoffroy Saint-Hilaire (1772-1844) in the second edition of *Philosophie Anatomique*[528], although he did not use the term 'teratoma'. It was his son Isidore[529]

[522] Ewing, 1919.
[523] Ewing's chapter on 'Theories and Nature of Cancer' also highlighted other important factors required for tumour formation, which may hint at the possibility of a cancer stem cell. For example, "The period of isolation of the cells is an important factor. The earlier its occurrence the less is the differentiation and the greater the capacity for growth. Early embryonal rests when starting to grow meet conditions which do not favor normal development" (Ewing, 1919 p 97).
[524] Don Varnum, speaking to Ricki Lewis; Lewis, 2001 p 132.
[525] Toledo-Pereyra, 1973 p 373-374.
[526] Young, 2005.
[527] *ibid*.
[528] Étienne Geoffroy Saint-Hilaire was a French naturalist, completing *Philosophie Anatomique* whilst Professor of Zoology at the University of Paris.

(1805-1861) who extended his father's work and used the term 'teratology' to describe the study of 'monstrosity' in anatomy[530]. The pair had also unsuccessfully attempted to reproduce 'monstrosities' by manipulating fertilised chicken eggs. Cooper claimed that the first to successfully induce teratomas was Camille Dareste (1822-1899)[531], as described in 1871[532]. Cooper noted that Dareste realised the potential of such work in studying evolution and development:

"[My research] demonstrates in the most complete manner the possibility of modifying, by the action of external physical causes, the evolution of a fertilised germ. The demonstration of this fact is of interest not only for the production of monsters but also for biology in its entirety. In effect, if it is possible to produce monstrosities by modifying the evolution of a fertilised germ, we must consider it possible to produce simple varieties, in other words slight deviations from the specific type, which are compatible with life and the generative functions."[533]

In *Neoplastic Diseases* (1919), Ewing devoted a chapter to ovarian teratomas. Previous observations were described in detail, particularly with regard to the different structures and cell types observed. In addition, there were also several images of dissected tumours. With regards to the etiology, Ewing stated that many currently believed the origin of ovarian teratomas to be the ovum:

"The essentially tridermal character of these tumors requires that the originating material be totipotent. Only two possible sources of such material have been seriously considered, the isolated blastomere of Marchand-Bonnet, and the primitive unfertilized ovum."[534]

Ewing stated that the blastomere theory[535] was not generally accepted since the frequency of tumours was too high, and these tumours had also been observed in

[529] Isidore Geoffroy Saint-Hilaire was appointed assistant naturalist to his father at the University of Paris in 1824, after completing studies in natural history and medicine.
[530] Cooper, 2004.
[531] Dareste was a French zoologist and specialist in embryology, holding doctoral degrees in both medicine and science.
[532] Dareste's *Recherches sur la Production Artificielle des Monstruosités ou, Essais de Tératogénie Expérimentale* also used the term 'teratogeny' to describe the 'experimental counterpart' of teratology. Cooper, 2004.
[533] Dareste, 1891; in Cooper, 2004.
[534] Ewing, 1919 p 603.
[535] Felix Marchand (1846-1928, Professor of pathology at Marburg) and the Greifswald anatomist Robert Bonnet (1851-1921) developed the 'blastomere theory', which stated that tumours could arise from misplaced embryonic cells that had become separated from the rest

testes. Support for these tumours originating from the unfertilised ovum came from the frequent observation of multiple dermoid cysts, the totipotent nature of ova, and the fact that teratomas occured during the fertile period in a woman's life.

In 1939, the French histologist Félix Albert Peyron (1884-1947) described the histology of the teratoma, including embryoid bodies; Peyron's contribution to teratoma histology would total more than 17 papers, including further descriptions of embryoid bodies and polyembryomas[536]. Peyron was sent his first sample of a testicular tumour (including embryoid bodies) from Professeur Limousin, a former colleague of Peyron's from the Institut Pasteur. The polyembryoma, described by Peyron in 1936, is a neoplasm comprising embryoid bodies; this was a topic of particular fascination for Peyron, having started his research on polyembryomas in 1919, and publishing his last paper on the topic in 1941[537]. All of Peyron's contributions were published in French; the first English description of the polyembryoma was written by uropathologist Meyer Melicow (1894-1983) in 1940, describing a fatal case[538]. Of all of the polyembryoma cases described during the first half of the twentieth century, none were pure, often having a teratoma or mixed germ cell element associated with them[539]. More recent thoughts on the polyembyroma tumours described by Peyron and others suggest that these tumours may be primitive forms of teratoma, since the embryoid body (and presence of embryonal epithelium) are characteristic of both polyembryomas and immature teratomas[540].

Drs Nathan B Friedman (1912-2009) and Robert A Moore published their work on testicular teratomas in 1946; this was a collection of 922 cases collected by the Armed Forces Institute of Pathology in the early 1940s. Friedman was working with the Institute at the time, whilst Moore held the Chair in Pathology at Washington

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of the developing embryo prior to the blastocyst stage. These cells did not develop normally, and therefore had the potential to become cancerous later on. Marchand had also hypothesised that tumours (and in particular perhaps teratomas) may develop from polar bodies. Ewing, 1919; Marchand, 1898; Bonnet, 1901; Maehle, 2011.

[536] Young, 2005.

[537] In 1942, Peyron was sacked from the Institut Pasteur; this is likely to be because of the conflict between Peyron and the Minister of National Education, as the Vichy Government requested Peyron's dismissal. Peyron however found another position, studying syphilis at the Institut Prophylactique Arthur Vernes; this may explain why no further papers on testicular tumours or polyembryonas were forthcoming. Service des Archives de l'Institut Pasteur, n.d.; Young, Stall, and Sevestre, 2016.

[538] Young, Stall, and Sevestre, 2016.

[539] *ibid*.

[540] *ibid* p 95; Friedman and Moore, 1946.

University School of Medicine. The military was a useful resource for testicular tumours, because of the age range of the men in the armed forces. Friedman and Moore suggested four classifications of testicular tumour: seminoma, embryonal carcinoma, teratoma, and teratocarcinoma[541].

The case studies collated by the Armed Forces Institute of Pathology was revisited by Moore alongside University of Pittsburgh researcher Frank J Dixon (1920-2008) in the early 1950s. Pathologist Robert H Young noted that the 1950s *Cancer* papers by Dixon and Moore were more clinical in nature than the previously published work, such as that in the *Armed Forces Institute of Pathology* journal[542]. In the late 1950s, Barry Pierce (see section 3.3) would work alongside Dixon to demonstrate the similarities between developing teratomas and early embryos. In his review of cancer research from 1959, Charles Oberling quoted from recent research into gonadal tumours, reporting that testicular tumours could be 'female'[543] - a result of the tumour arising from germ cells, which have either an X or Y chromosome. This was part of another aspect of cancer research growing in the 1950s - cell culture[544]. For example, Alice E Moore of the Sloan-Kettering Institute for Cancer Research at Cornell University, published research results regarding the culture of cells in the laboratory - it was possible for non-cancerous cells in culture to develop chromosomal abnormalities[545]. Critically however, the reason cells in culture became cancerous remained elusive[546].

Whilst Moore, Friedman, and Dixon were developing their classifications of testicular tumours in America, a British group were working on the same problem. In 1964, the British Testicular Tumour Panel published their suggestions in a supplement to the *British Journal of Urology*; the British team also identified four classifications: teratoma differentiated, malignant teratoma intermediate, malignant teratoma anaplastic, and malignant teratoma trophoblastic. This work, claimed Young, was easily comparable to the classifications suggested by Friedman and

[541] Young, 2005.
[542] *ibid*.
[543] Sohval and Gaines, 1955; Oberling, 1959.
[544] In his 1959 review, Oberling updated the reader on several results from cell culture studies, including behaviour of the cell membrane, studies on invasiveness, and the role of some hormones on metastases.
[545] Moore, 1957; Oberling, 1959.
[546] Oberling, 1959.

Moore[547]. Eventually, the World Health Organization (WHO) would publish their own testicular tumour classification system; the gaps between the American, British, and WHO classification systems were in part due to WHO initially also classifying ovarian tumours, in an effort to standardise terminology and classification of tumours common to both ovaries and testes. The WHO system, based mainly on the suggestions of the American classification system, was published in 1976[548].

3.2 Stevens' contribution

3.2.1 JAX history

The Roscoe B Jackson Memorial Laboratory (known as "Jackson Laboratories" or "JAX") was founded in 1929 by Clarence Cook Little (1888-1971), upon leaving the presidency of the University of Michigan[549]. Throughout his academic career, Little had been interested in mammalian, and in particular murine genetics, and had many years experience in creating inbred strains of *Mus musculus*[550]. This was an aim of JAX – to create 'pure' mouse lines for research – particularly for cancer research, as was Little's interest at the time[551]. The difficult economic situation of the period made it necessary for JAX to sell inbred mice for research elsewhere[552]; it now annually provides around 2.5 million mice to laboratories worldwide[553]. By the 1950s, JAX was already considered to be the supplier of the 'ultimate' laboratory mouse[554]; this would also have created an environment where purity of strain and successful upkeep of substantial numbers of mice was paramount. The drive of research into the genetic causes of cancer was championed by Little, and this provided Stevens (see below) with the ideal

[547] Young, 2005.
[548] *ibid*.
[549] Griesemer and Gerson, 2006. Jackson Laboratories was named after Roscoe Jackson, founder of the Hudson Motor Car Company of Detroit, who supplied a great proportion of the funding needed in order to found the laboratory.
[550] For a biography of Little detailing his work with mice before, during and after his time at JAX, see Rader, 2004.
[551] This was followed after WWII by interest in the effect of radiation damage, giving further use to the inbred laboratory mouse. Rader, 2004, Kraft, 2009 and Lancaster, 2009.
[552] Rader, 2004.
[553] The Jackson Laboratory, 2016.
[554] In 1941, 'JAX Mice' were registered with the US Patent Office. Griesemer and Gerson, 2006.

environment for research following his identification of testicular teratomas in strain 129 mice[555].

3.2.2 Stevens' introduction to teratomas

"Today, it is widely held that Stevens's lucky break and all his science thereafter went a long way toward launching the field of stem cell biology."[556]

Leroy Stevens (known as Roy to his colleagues) graduated with a biology degree from Cornell University in 1942. The same year, he married and joined the army. Stevens began his research career with a PhD in experimental embryology at the University of Rochester, under the supervision of embryologist J Holtfreter; Barry Pierce has highlighted that this makes Stevens a 'scientific descendant' of Hans Spemann[557]. Later the same year as completing his PhD (1952), Stevens began as a postdoctoral assistant to Little[558]. Stevens remained at JAX until he retired in 1989.

Stevens was working with mice at JAX, particularly strain 129 mice. These were inbred mice maintained through brother and sister matings[559]. Six months into his work, Stevens observed a single mouse with an abnormal testis. Through histological examination, JAX histological stalwarts Elizabeth Fekete and Katherine P Hummel identified a teratoma; Stevens recalled that this piqued his interest as no murine testicular teratomas had been previously described[560]. Stevens also recalled that he had the freedom to pursue such interests: "I felt perfectly free to do anything I wanted, and didn't have to account to anybody"[561]. It was another six months before another testicular teratoma was observed in the same mouse strain, then a third was found two months later. Stevens would have been accustomed to visually scanning mice, and would have probably been swift to observe these abnormalities[562]. At the

[555] First described in Stevens and Little, 1954.
[556] Parson, 2004 p 26.
[557] In 'An Appreciation' of Stevens presented in 1987, Barry Pierce highlighted Stevens' military career, stating that he served in Italy, Africa and Sicily, and landed on the Normandy beaches on D-Day. Pierce stated that despite being decorated twice during his time in the army, Stevens was more keen to discuss how he graduated from Cornell with more demerits than anyone else (Pierce, 1988).
[558] Parson, 2004; Pierce, 1988.
[559] For more details of the genetic background of these mice, see Stevens and Little, 1954.
[560] Stevens, 1984.
[561] Stevens, 1986.
[562] Ricki Lewis suggested that at this time, embryologists would spend many hours scanning "dozens, hundreds or even thousands of animals", instantly alert when one "stood out from the crowd". Lewis, 2001 p 132.

time, Stevens was testing different cigarette components on mice, which he did not find particularly interesting, so was pleased when he saw the teratoma: "I was pretty good at reading slides", Stevens boasted in 1990[563]. This prompted an investigation into the genetic susceptibility of strain 129 mice to testicular teratomas, resulting in publication of Stevens' first paper in 1954[564]. It has been suggested that Stevens appreciated the resemblance of teratomas to embryos, due to his previous experience as a PhD student[565].

"I very slowly got this thing off the ground. I mean, what do you do when you find something as *rare* as that!"[566]

In Stevens' first publication on the subject, a short review indicated the research into teratomas Stevens and Little were familiar with[567]. Spontaneous murine testicular teratomas had not previously been reported, although ovarian teratomas had. One such publication was by Fekete and Ferrigno, also working on inbred mice at JAX (see above)[568]. A spontaneously occurring ovarian tumor was described in which differentiated cells and tissues where observed as well as "undifferentiated embryonal cells"[569]. Serial transplantation into other mice demonstrated a pluripotent

[563] Lewis, 2001 p 132.
[564] Stevens and Little, 1954.
[565] Parson, 2004.
[566] (original emphasis) Stevens in Parson, 2004 p 25.
[567] Stevens and Little, 1954.
[568] Fekete and Ferrigno, 1952. As Pierce and Dixon observe, the first ascites tumours of teratomas were produced using a 'Fekete's teratoma' (Pierce and Dixon, 1959b, referring to Leighton, 1954). If this was a development useful to research (as it later proved to be), why was Fekete's teratoma ascites ignored in favour of developing another ascites tumour from strain 129 teratomas? Was this because of popularity of Stevens' publications in comparison? Was it that Stevens encouraged others (like Barry Pierce and Beatrice Mintz) into his laboratory to learn from him? Undoubtedly, the work carried out by Pierce was essential in allowing strain 129 teratomas to be used in research elsewhere, but if research into or using teratomas was considered so important, why were Fekete's teratomas, with ascites available (as produced by Leighton) years prior to 129 teratomas, not more widely utilised? This is probably due to Elizabeth Fekete's retirement in 1956 (Hummel, 1980). Since Fekete retired as the importance of teratomas was only just being realised, her work was less likely to be recognised in favour of her more 'current' colleague, Roy Stevens.
[569] This paper appears to be the model on which Stevens and Little based their publication on testicular teratomas two years later. However, on the whole this contribution appears to have been largely ignored by later researchers, with only a handful of citations compared to the 320 citations of Stevens' and Little's 1954 publication (estimated by Google Scholar; available at: http://scholar.google.co.uk/scholar?hl=en&lr=&cites=16851338284840483051&um=1&ie=UTF-8&sa=X&ei=i26OUKLRKKjL0QWtt4G4CA&ved=0CCUQzgIwAA [Accessed September 2016]).

element, from which many other cell types could arise whilst maintaining growth of the tumour. Testicular teratomas had been described in both humans and horses; studies had shown that some similarities in tissue type had been identified. Both embryonic and adult tissues which were not usually observed in the testis were observed in these tumors. Furthermore, Stevens and Little highlighted a few studies in inducing teratomas, which usually involved injecting an inorganic compound in fowl or autografting different regions of a newborn rodent to the testes.

3.3 Barry Pierce

3.3.1 Barry Pierce's introduction to teratomas

Gordon Barry Pierce was born in 1925 in Alberta, Canada. He was raised on a remote farm, and stated that it was his experiences regarding his ill father (Pierce describes him as a 'cardiac invalid') that made him want to train as a physician. After serving in the Canadian Army during World War II, Pierce completed a BSc in biology, MSc in anatomy and an MD at the University of Alberta[570]. Whilst at medical school, Pierce became particularly interested in medical science. So much so, Pierce took a year out to work in a research laboratory – the project he was assigned was related to breast cancer. Pierce described this as "one of the most wonderful years of my life"[571]. After graduating and during his two year pathology residency, Pierce remembered treating a young boy with testicular cancer. Pierce recalled that "He died, which was terrible, but what disturbed me was our ignorance of testicular cancer. It bothered me that we did not even know the diagnosis of the tumor that killed him. So, I decided then that I was going to be a scientist and work on testicular cancer"[572].

This led Pierce to Frank Dixon, who had written about testicular cancer for the *Armed Forces Institute of Pathology*[573]. By this time, Dixon was Professor of Pathology at the University of Pittsburgh, and Pierce joined him in 1955. Dixon believed that teratomas had a multipotential embryonal carcinoma precursor cell, but Pierce admitted that this was not a widely accepted idea at the time, particularly in Britain. Pierce claimed that his late 1950s work with Frank Dixon made him realise

[570] Pierce, 1993.
[571] *ibid* p 8.
[572] *ibid* p 8.
[573] Dixon and Moore, 1952.

he needed a better understanding of embryology[574]. To this end, Pierce went to work with Stevens[575]. Pierce learned the technique of producing embryoid bodies; an important technique Pierce felt, since it allowed mass production of the ascites variant[576] of the solid tumour[577]. Pierce was excited to tell Stevens of this technique, and when Stevens published a paper about such work months later[578], Pierce said "[Stevens] was very gracious to me, and we became very close friends"[579].

3.3.2 Ascites tumours and cancer cell differentiation

"The remarkable manner in which early embryonic life is recapitulated in neoplasia surely is one of the most stunning examples of the numerous fascinating aspects of microscopy which on a daily basis provides interesting images…"[580]

Embryoid bodies are rounded structures that, histologically, become clear in sections because of the dark staining of ectodermal cells, which form a disc. This disc is associated with a thin layer of yolk sac epithelium, and a cavity (similar to the amniotic sac), lined by flattened endodermal epithelium. Human embryoid bodies also stain for human chorionic gonadotrophin and α-fetoprotein. Usually, the embryoid bodies are singular (i.e. do not usually form in pairs or in greater numbers), but can be found in large groups, with each embryoid body separate from others. Where a cancer has metastasised, embryoid bodies may be observed at the secondary site. Polyembryomas, as mentioned previously, include blastocysts and embryoid bodies similar to day 17 or 18 embryos[581].

The pathologist Max Askanazy (1865-1940), professor at Geneva, was an expert in teratomas working in the early twentieth century, and his experiments were

[574] Pierce, 1993.
[575] Pierce states that he started working with 'the Fekete ovarian teratocarcinoma' in 1956, before moving onto Stevens' strain 129 testicular teratomas. Pierce, 1993 p 9.
[576] Embryoid bodies form from teratoma cells transplanted into the ascitic fluid; these are so-called since, morphologically, they appear similar to early embryos – there forms a layer of cells on the outside, and a mass of cells on the inside. Embryoid bodies differ from embryos however as the endoderm, normally part of the inside mass of the developing embryo, becomes part of the outer layer of cells on the embryoid body. Effectively, the embryoid body looks like an embryo, however those cells on the inside of the normal embryo are on the outside of the embryoid body, and *vice versa*. The embryoid body is also referred to as the 'ascitic form' of the solid tumour, since it develops in ascitic fluid.
[577] Pierce, 1993 p 9.
[578] Stevens, 1960.
[579] Pierce, 1993 p 9.
[580] Young, Stall, and Sevestre, 2016 p 104.
[581] *ibid*.

the beginning of a series of similar experiments that would begin again in the mid-twentieth century. In 1907, Askanazy gave an overview lecture on the topic to the German Pathological Society, which has become a highly cited source for understanding of the teratoma in the early twentieth century. In this lecture, Askanazy suggested that teratomas arise from an *eiwertige* [egg equivalent] stem cell remaining in the adult body. To demonstrate that the embryonic rest theory was correct, Askanazy would inject embryonic tissue into the peritoneal cavity of rats. The experiments (as far as Askanazy was concerned) were successful – teratoma-like tumours would develop from the injected embryonic tissue[582].

In 1954, a paper describing the conversion of a murine ovarian teratoma to an ascitic form was published[583]. This was part of a research project with the aim of determining to which extent different tumours produced aggregates of cells[584]. The ascites form was later shown to lose more differentiated tissues and remain as a small cluster of cells, or monocellular carcinoma[585]. Those cells remaining then must be, argued Pierce, highly malignant. Pierce and Dixon observed that these tumours would be especially useful in determining whether malignant teratoma cells were capable of successfully differentiating into "adult forms" (as proposed by Askanazy in 1907)[586].

In two papers published in 1959 (see below), Pierce and Dixon demonstrated that ascites tumours could be made from Stevens' strain 129 teratomas, and that the undifferentiated cells obtained using this method could form other teratomas (including several differentiated cell types). What Pierce and Dixon demonstrated was that embryonal carcinoma cells (ECCs) were multipotent, and that these cells were similar to those of the preimplantation embryo[587]. Pierce submitted his paper to *Cancer* – a clinically-oriented journal, in keeping with Pierce's background in pathology. Pierce said he received the card to state that his paper had been received by the journal, but it was under review for six months – a long time. Eventually an associate editor of *Cancer* telephoned Pierce, and he was immediately told that there was nothing wrong with the data presented. Pierce claimed he was relieved, however

[582] Maehle, 2011.
[583] This was the ovarian teratoma described by Fekete and Ferrigno (1952), and referred to by Leighton as the 'Fekete teratoma'.
[584] Leighton, 1954.
[585] Goldie, 1956; Klein and Klein, 1956.
[586] Pierce and Dixon, 1959b p 584.
[587] Pierce and Dixon, 1959a.

was then told that the paper could not be published. Pierce enquired as to why, and was told that "everyone knows that cancer cells can't differentiate". Pierce protested, stating that if the data was sound, and the data showed that cancer cells could differentiate, then presumably they could. After a pause, the assistant editor agreed to publish the paper with a change to the title. Pierce agreed. The original "Teratocarcinogenesis by differentiation of multipotential cells" (described by Pierce as 'a jawbreaker') became "Teratocarcinogenesis by metamorphosis of multipotential cells"[588].

In this paper, eventually published in 1959, Pierce and Dixon showed that murine teratomas developed from undifferentiated cells. This had already been postulated by other groups, however Pierce and Dixon were the first to publish experimental data to support the claim. Pierce and Dixon minced solid teratocarcinomas and injected the resulting thick suspension into the peritoneal cavity of weanling mice (i.e. infant mice that had recently begun feeding independently of their mother). After a period averaging 35 days, with their bellies distended, the mice were killed and an incision made into the peritoneal space. The fluid was aspirated and stained. Pierce and Dixon observed distinct growths in this fluid, including cysts and free-growing tumour cells. Teratocarcinomas were also observed. These tumours contained tissues differentiated from all three germ layers, as well as undifferentiated tissue. This elegant experiment then showed that minced teratocarcinomas were capable of producing other teratocarcinomas with differentiated tissues from all three germ layers. The 'stem cells' of these tumours then had to be multipotent (or pluripotent, to use current terminology). However, the concept of cancer cells which were capable of differentiation was not particularly well received (see above).

In order to refute the argument he was continually faced with (i.e. that differentiation does not occur in cancer cells), Pierce demonstrated that differentiation occurred not only in teratocarcinomas, but in other cancers as well. In addition, Pierce continued to work on demonstrating the multipotentiality (pluripotency) of teratocarcinoma cells specifically. In 1964, Pierce published a paper with Lewis Kleinsmith, a Cancer Research Fellow student. Taking the 1959 work a step further, Kleinsmith and Pierce isolated single cells from embryoid bodies (from ascites

[588] Pierce, 1993 p 10.

tumours of teratocarcinomas), and transplanted these into mice[589]. Those mice which developed tumours were killed, and 44 clonal cell lines obtained from these tumours. Kleinsmith and Pierce reported that between four and eleven different tissue types were observed from each clonal line over five generations. This then very clearly demonstrated that single teratocarcinoma cells were capable of producing many different cell types[590]. As a result of this work, Pierce suggested an appropriate future therapy for cancer may be differentiation therapy – a therapy which would force the cancer cells to differentiate, and become benign, reducing the growth rate[591].

3.4 Primordial germ cells – 'stem cells of teratomas'

Stevens' first paper in 1954, co-authored with Little, followed several months work with strain 129 mice by Stevens, who observed their susceptibility to testicular teratoma formation (see above). Testicular teratomas occurred spontaneously in approximately 1% of strain 129 males, and contained "undifferentiated embryonic tissue", glandular tissue, cysts, nodules of bone (with marrow) and/or cartilage, cuboidal epithelium, fat and nervous tissue; the larger the teratoma, the more tissue types were observed[592]. Stevens transplanted fifteen of these teratomas into other mice and whilst all grafts took, only one developed into a "rapidly growing transplantable tumor", which contained undifferentiated cells[593]. Stevens and Little ascribed the maintenance of the transplanted teratoma to "pluripotent embryonal cells"; this concurred with Dixon and Moore's previous suggestion that totipotent, undifferentiated cells of germ cell origin had the potential to become embryonal carcinomas[594], and Stevens and Little considered their own findings were in accord with this theory, stating this in their 1954 paper.

In 1964, Stevens published a paper summarising his experimental work producing testicular teratomas in mice, and how these results supported the theory

[589] In 2000, the generation of human embryoid bodies *in vitro* was achieved by removing pluripotent stem cells from the feeder layer of the suspension culture. Itskovitz-Eldor *et al*., 2000.
[590] For example, see Pierce and Wallace, 1971.
[591] The beginning of this research was already underway, with Niu, Cordova and Niu demonstrating that ectopic ribonucleic acid induced differentiation in ascites tumours (Niu, Cordova and Niu, 1961).
[592] Stevens and Little, 1954. Not all tissue types were observed in all teratomas examined.
[593] Those which did not grow remained as small nodules of highly differentiated cells, which had the growth characteristics of homogenised 13-14 day foetal tissue, injected subcutaneously (Stevens and Little, 1954).
[594] Dixon and Moore, 1952.

that testicular teratomas were derived from primordial germ cells (PGCs)[595]. In order to demonstrate this, Stevens investigated the induction of teratoma formation from transplanted genital ridges of mice. The optimal time for this transplant was at twelve days after conception, where the genital ridges were transplanted out of the foetus, and grafted onto either the spleen or testes of adult mice. Approximately 80% of testicular grafts developed teratomas, identical to spontaneous teratomas; Stevens suggested that this development was via PGCs. The grafts into the spleen however were less successful. Where teratomas developed in the spleen (ten times less frequently than testicular grafts), the tumours were smaller and simpler; Stevens suggested that the spleen provided an environment that promoted differentiation of the "undifferentiated embryonal cells", which were described as "the stem cells of these tumors"[596].

Following repetition of the 1964 experiment, John T Aldrich (also at JAX) and Stevens described the effect of 5-Fluorouracil (FU), an anticancer agent used in chemotherapy, on murine testicular teratomas[597]. The paper championed the use of their induced testicular teratomas in cancer studies, noting that the system was unique; the "carcinogenetic process" was known to take place within 24 hours of grafting, and no other factors were required to induce tumour formation[598].

As well as continuing work on teratoma development, Stevens continued testing chemotherapies on induced teratomas with other researchers[599]. Using Stevens' induced teratoma model meant that drugs could be administered at a precise stage in tumor development; again, a particularly useful research tool. Aldrich and Stevens suggested that two types of cell were potentially exposed to the effects of FU: the "teratomatous tissue" contained in the grafted genital ridges, and the normal cells

[595] Stevens, 1964. Boveri had originally named primordial germ cells in 1892, suggesting that these cells may be directly descended from the fertilised egg, and retain the 'character' of the spermatozoa or egg in its chromatin. PGCs, which would then differentiate into either egg or sperm cells of the new organism, would then retain the 'character' of the offspring's parents in the offspring's germ cells, and so on. Baltzer, 1967; Maehle, 2011.
[596] Stevens, 1964 p 659.
[597] FU functions as an antimetabolite for uracil in nucleic acid synthesis. It therefore affects rapidly proliferating cells.
[598] In continued use of strain 129 mice for this work, histocompatibility was also not an issue (since the mice were inbred), which may have been a problem for other researchers (Aldrich and Stevens, 1967 p 945).
[599] For example, see Mount, Stevens, and Whitmore, 1970.

of the grafted genital ridge[600]. Aldrich and Stevens noted that other studies had shown that teratomas could arise from a single cell: the PGC[601]. Stevens was confident enough to agree:

"This influence [of the testicular environment] results in the initiation of development of male primordial germ cells. They proliferate and give rise to undifferentiated embryonic cells which in turn give rise to the primary germ layers. The primary germ layers differentiate into disorganized mixtures of many kinds of tissues characteristic of teratomas."[602]

Stevens' 1968 paper described the use of grafted fertilised eggs (instead of genital ridges) to induce testicular teratomas. Where undifferentiated cells were observed, Stevens attributed this to their environment: the influence of sex hormones and the disorganised nature of the tumour[603]. Alternatively, Stevens suggested that "undifferentiated cells may have arisen from cells with characteristics of primordial germ cells"[604], although which cells these could be (if not PGCs) is unclear. Serial transplantation highlighted a sub-set of cells which were capable of proliferating and remaining undifferentiated for 165 days. Stevens suggested that as these cells were able to divide asymmetrically and had a high proliferative potential, they were likely to be the "stem cells of teratomas"[605].

Earlier examination of grafted eggs showed that early development was very similar to normal embryogenesis, including development of extra-embryonal cells. This was the case for grafts up to 14 days post-grafting, when some regions of the tumour started to become disorganised. Tumours examined at later stages of growth showed increasingly differentiated cells alongside some undifferentiated embryonic cells, demonstrating that differentiation of some cells "may be delayed for remarkably

[600] Aldrich and Stevens, 1967 p 946. I believe this implies that, as highly proliferative cells are susceptible to FU, the cells in genital ridges are stem cells; Aldrich and Stevens make no explicit note of this however. More recently, it has been suggested that primordial germ cells colonise the genital ridge (for example, see Sutton, 2000), as implied by the work carried out by Stevens in the 1960s.
[601] In particular Pierce, Dixon, and Verney, (1960) and Kleinsmith and Pierce (1964).
[602] Stevens, 1968 p 329.
[603] Later, Stevens further investigated the effect of environment by culturing genital ridges at either 32°C or 37°C prior to transplantation. One observation was that ridges cultured at 32°C induced many more teratomas following transplantation than those cultured at 37°C, confirming that environment did have a role in teratocarcinogenesis (Friedrich, Regenaas, and Stevens, 1983).
[604] Stevens, 1968 p 330.
[605] *ibid* p 332.

long periods of time"[606]. As well as this, Stevens suggested that these cells "…give rise to differentiated tissues and to more undifferentiated proliferating cells like themselves"[607]. Note the similarity here to current definitions of stem cells – capable of proliferation and differentiation. Stevens suggested that these cells were indistinguishable from the pluripotent "stem cells of teratocarcinomas" described separately by both Pierce and Stevens in 1967. Stevens noted the relevance of the "misplaced blastomere" theory in his research, which suggested that teratomas developed from "embryonic totipotent cells", which were no longer influenced by "embryonic organizers"[608]. However, Stevens preferred to believe that teratomas developed from PGCs, citing his previous work[609]. Stevens explained that the results of his study (i.e. that teratomas can develop from eggs at the two-cell stage) demonstrated that teratomas derive from cells destined to become PGCs, or from disruption of normal cell-cell relationships[610].

Later, Stevens described the induction of teratomas from more developed embryos, at 3 and 6 days following fertilisation. Some grafts contained undifferentiated cells which remained pluripotent and proliferated indefinitely through several serial transplants. Stevens now had an altered view on the cells from which teratomas develop. Spontaneous teratomas, Stevens still believed, were derived from PGCs, however teratoma development could be induced in the laboratory by grafting embryos/fertilised eggs[611]. The term "undifferentiated stem cells" also seems to replace "undifferentiated embryonic cells" in this paper[612], which also contained Stevens' first reference to an "embryonic stem cell"[613]. Stevens however does not appear to use this phrase any differently to similar phrases used throughout previous publications; the 'undifferentiated embryonic stem cell' is the cell responsible for the continued growth observed following serial transplantation of tumours – the theory that such a cell existed had been mooted many times previously. The 'embryonic stem cell' is not referred to again in this paper. The shift in terminology may be a reflection of the general shift in terminology for such cells,

[606] *ibid* p 336.
[607] *ibid* p 337.
[608] *ibid* p 338.
[609] For example, Stevens 1967b.
[610] Stevens, 1968.
[611] Stevens, 1970.
[612] For example, Stevens, 1970 p 375.
[613] Stevens, 1970 p 380.

with a greater number of researchers understanding the concept of an embryonic stem cell in the context of Stevens' work.

4. Parallels between development and cancer

Several approaches have been taken to examine the parallels between normal development and cancer development in mammals. Referring to the study of developmental biology in the context of regenerative medicine, John Gurdon (see Chapter 2) stated that "As we get older, the normal processes of cell renewal...may become uncontrolled, leading to cancer"[614]. Here Gurdon is asserting that the usual processes of development and regeneration are 'hijacked' by cancer; better understanding of one should therefore aid in better understanding of the other. Immune tolerance, invasiveness, and proliferative mechanisms that are essential in pregnancy and early development are exploited by malignancies to evade the host's immune response to spread, and to grow[615].

Some of the earliest investigations in this area were carried out in the early twentieth century, and made use of the increasingly precise measurement of cellular components available; this led to several researchers examining the biochemistry and metabolism of both developing embryos and cancers. Other research has been concerned with what determined cell fate (a continuation perhaps of the early twentieth century interest in cell lineage studies in the USA [see Chapter 1]). As demonstrated by nuclear transfer in the mid-twentieth century (see Chapter 2), the genome is stable, and no changes are made during development. What changes, however, are the genes that are expressed at any one time. This consistency is key, argued Gurdon, since it ensured that by changing the pattern of gene expression, any cell type could become any other cell type (see also Chapter 6)[616].

4.1 Biochemistry

In a report from 1972, W Eugene Knox, then Professor of Biological Chemistry at Harvard Medical School, summarised the findings of his own experiments and those of others, who had compared cancer and foetal cells of various organs, and quantified their similarities. Biochemistry was utilised as an approach to

[614] Gurdon, 1999.
[615] Holtan *et al*, 2009.
[616] Gurdon, 1999.

determine those substances (such as proteins) present in the cytoplasm of various cell types; this, Knox argued, would enable detailed examination of both gene products and their rates of degradation in the cell. He admitted however that such information would only be a snapshot of the cell's cytoplasm at a particular moment in time, and that the components of the cytoplasm would be affected by factors such as age, and stress, for example. Such research was not necessarily part of any "ambitious quests" to identify either the cause or the cure of cancer, Knox noted, preferring that the research he and others were carrying out be considered as "defining…the relation between normal animal tissues and their neoplasms"[617]; a further indication that parallels between cancer and normal tissue were becoming appreciated and, to an extent, exploited.

Making use of various cell types of the rat, Knox (and others) isolated and quantified the components of each cell type, in particular normal adult tissues, foetal tissues, and neoplastic tissues (especially kidney, liver, and brain). In 1947, the biochemist JP Greenstein had published similar research, concluding that tumours from various tissues resembled each other more closely than their tissue of origin; this, Knox suggested, presented a paradox: experts knew that "the outstanding characteristic of tumors was not their uniformity but their diversity in nature and behavior"[618]. Instead, Greenstein's results suggested that tumours were rather similar to each other. Perhaps, Knox argued, noting that Greenstein would have tested undifferentiated tumours, tumours would be less similar to each other in highly differentiated tumours. Knox's own research agreed with Greenstein's results, and furthermore, showed that foetal tissues were more similar to each other than the adult tissues they would develop into. There was a parallel emerging; foetal tissue was more similar to other foetal tissue than its differentiated adult state. Similarly, undifferentiated tumour tissue was more similar to other undifferentiated tumour tissue, than the tissue it had arisen in. (More differentiated tumours however were more similar to the tissue of origin than undifferentiated tumours.) These results led Knox to ponder over whether tumour cells became *un*differentiated through (genetic) change, or whether normal development was reversed, resulting in

[617] Knox, 1972 p 480.
[618] Knox, 1972 p 485.

*de*differentiation[619]. Why was such work useful? Because, suggested Knox, "Neoplasms become less mysterious as they are recognized to be similar to a familiar type of normal tissue and as they become susceptible to some logic"[620]. This contrasts with some of the earlier descriptions and explanations of cancer (see section 2), conceptualising cancers as crab-like monsters, eating away at their victims. Knox is suggesting here that in the laboratory, experimental work on neoplastic tissue should be carried out in a similar fashion to those experiments on normal tissue. In addition, Knox is proposing that cancer perhaps should not be considered as something abnormal or alien, but should be considered as a regular function of cell fate (through either undifferentiation or dedifferentiation). Is this then an indication of the normalisation of cancer, as it became more prevalent through the twentieth century?

In the 1920s, biochemists began investigating metabolism in organisms and, eventually, individual tissues and cells. Nobel prize winner Otto Heinrich Warburg (1883-1970), for example, described the aerobic glycolysis[621] that cancer cells used to generate energy in the form of ATP (adenosine triphosphate) (now referred to as the Warburg Effect[622]). Cells other than cancer cells have also been shown to create ATP in this less efficient way: rapidly proliferating cells, such as those in the pre-implantation embryo[623]. It has been suggested that one reason for this may be that the blastocyst requires a lot of ATP, since the cells are rapidly dividing; much of the ATP requirement is met through the regular glycolysis pathway in the mitochondria (via the tricarboxylic acid, or TCA, cycle), however extra ATP can be generated in the cytoplasm. As mentioned prior, the aerobic glycolysis cycle is less efficient at generating ATP from glucose than the TCA cycle, leading biologists to enquire as to

[619] *ibid* p 486. Knox also proposed the term "fetalism" to describe the resemblance of undifferentiated tumours to foetal tissue (p 487).
[620] *ibid* p 487.
[621] Normally, mammalian cells prefer to generate energy by glycolysis in the mitochondria – the cell's 'power station'. In cancer cells, however, glycolysis occurs in the cytoplasm, which results in a build-up of lactic acid (or lactate). This process normally only occurs when there is a lack of oxygen available (for example, after exercise), and is not a metabolic pathway that is designed to continue for extended periods of time (due to its inefficiency, and the build-up of acid in and around the cell).
[622] This is the Warburg Effect as it refers to oncology. Warburg's research was not limited to animal biochemistry however, and there is also a Warburg Effect that refers to plant physiology, and the decreased rate of photosynthesis in environments containing a high concentration of oxygen. For example, see Turner and Brittain, 1962.
[623] Fridhandler, 1961.

why cancer cells, or the early embryo, generate a significant proportion of their ATP using this method. It has been suggested that the different pathways, with different products (or intermediates), may be useful to the cancer cells or embryo. Below, two such examples are given: the products of lactic acid, and glucose-6-phosphate[624].

In cancer, aerobic glycolysis occurs regardless of the amount of oxygen available (i.e. through vascularisation), and cannot be accounted for by mitochondrial defects[625]. It has been suggested that the cancer cells may actually thrive in the highly acidic environment created by this glycolytic pathway, giving cancer cells an advantage over surrounding non-cancer cells, which would find the increased acidity toxic. For example, the localised acidosis may result in the breakdown of junctions between cells, and of the extracellular matrix reinforcing tissue structure; this may enable the invasion and metastasis of cancer cells, resulting in cancer spreading and secondary tumours[626].

In the blastocyst, like in cancer, aerobic glycolysis also results in the production of lactic acid, and the acidosis of the local environment. It has been suggested that just as the increased acidity of the local environment aids in the invasion and metastases of cancer, the increased acidity of the local environment around the pre-implantation embryo may enable implantation, mirroring tumour invasion[627].

The aerobic glycolysis pathway produces another intermediate not generated by the TCA cycle: glucose-6-phosphate. Glucose-6-phosphate can be utilised in a further metabolic pathway: the pentose phosphate pathway (PPP). The PPP is important for rapidly dividing cells, as it increases the amount of carbon available to cells where there may otherwise be a shortfall. The PPP generates ribose for nucleic acid synthesis, and NADPH (nicotinamide adenine dinucleotide phosphate), essential for lipid (and therefore membrane) synthesis[628]. NADPH is also important for

[624] There are several other examples available in addition to lactic acid and glucose-6-phosphate. For example, there is increased production of glutamine in cancer cells and the early embryo, which is useful in generation of ATP. For a review, see Smith and Sturmey, 2013.
[625] Genetic defects of mitochondrial proteins only occurs in a few cancers, however almost all cancers make use of the alternate glycolysis pathway. Smith and Sturmey, 2013.
[626] *ibid*.
[627] Gatenby and Gilles, 2004; Smith and Sturmey, 2013.
[628] Vander Heiden, Cantley, and Thompson, 2009; Smith and Sturmey, 2013.

antioxidant production, and therefore maintaining the redox status[629] of cells[630] – particularly important since, as established above, cancer cells and the early embryo are generating a lot of lactic acid. There is an increase in the amount of glucose in the PPP cycle from 7% in the cleavage-stage embryo, to around 20% in the blastocyst[631].

The above examples demonstrate the similarities between cancer cell and early embryo metabolism; there are further parallels observed between cancer cell and embryo regulation. The example of pyruvate kinase will be used to highlight this.

In the final step of the TCA cycle, pyruvate (and ATP) is produced from the action of an enzyme, pyruvate kinase (PK), on phosphoenolpyruvate (PEP). A different isoform of the pyruvate kinase enzyme, named PKM2, is expressed in both cancer cells[632] and early embryos (at the cleavage stage). The dimeric form of PKM2 (induced by interaction of PKM2 with some oncoproteins) is less active than PK, resulting in the production of less pyruvate from PEP[633]. An alternative reaction then takes place, due to the increased amount of PEP in the cell. Independently of PK, PEP acts as a phosphate donor (to the enzyme phosphoglycerate mutase 1 [PGAM1]), which also results in PEP becoming pyruvate[634]. This action, side-stepping the usual TCA cycle pathway, reduces the amount of ATP produced, but generates more pyruvate for the PPP, which, as noted above, is important in rapidly proliferating cells.

In addition to its cytoplasmic function, PKM2 has a role in the nucleus, where it acts as a transcription factor. In this role, PKM2 functions as a regulator in histone modification[635] and epigenetic regulation. In particular, the PKM2-dependent histone modification affects genes involved in tumourigenesis and proliferation[636]. PKM2 has been shown to function as a transcription factor on genes that have roles in the

[629] 'Redox' is the term given to chemical reactions that involve both *red*uction (gain of electrons) and *ox*idation (loss of electrons). The TCA cycle is an example of redox reaction in the cell. Other such redox reactions can result in the production of 'free radicals', or electrons, which attach to other molecules almost instantly after their production. For some molecules, this can be harmful, reducing or preventing their function altogether. Other molecules, such as NADPH, function as antioxidants, which free radicals can attach to without causing harm.
[630] Leese, 2012.
[631] Javed and Wright, 1991.
[632] The amount of the dimeric form of PKM2 in tumour cells correlates with the degree of malignancy. Eigenbrodt *et al*., 1992.
[633] Mazurek *et al*., 2005; Cortés-Cros *et al*., 2013.
[634] Vander Heiden *et al*., 2010.
[635] Histones are proteins that 'package' and organise the DNA inside the cell nucleus.
[636] Yang *et al*., 2012.

progression of cancers, and many that are needed for normal embryonic development[637].

4.2 Cell fate

As demonstrated by the success of nuclear transfer experiments in the mid-twentieth century (see Chapter 2) and the generation of induced pluripotent stem cells (iPSCs) in the early twenty-first century (see Chapter 6), every cell contains exactly the same DNA[638]; what causes one to be muscle and one to be brain (for example) are the genes that are being expressed. Differentiated cells are encoded by <10% of the total number of genes in nuclear DNA[639]. Gurdon has argued then that cell fate cannot be determined by genetics, but must instead be influenced by the cell's environment. Cell lineage studies have demonstrated that there is no simple linear relationship between the position of a particular cell in the early embryo, and the fate of its descendants. Removal of any such cell in the embryo would simply require a compensatory action of the neighbouring cell[640].

A useful example of the sort of cell lineage changes that Gurdon refers to is in epithelial and mesenchymal cells of the early embryo. During organogenesis, epithelial and mesenchymal cells switch phenotypes depending on their function at the time. This is referred to as epithelial-mesenchymal transition (EMT) or mesenchymal-epithelial transition (MET), both of which are required for complex tissue patterning and morphogenesis[641]. The plasticity afforded to epithelial cells via EMT is essential for development, such as enabling cell migration during embryogenesis (in particular, for example, morphogenesis of the mammary gland) and for cancer (in particular, invasiveness). Once the epithelial cells have migrated and no longer require their mesenchymal characteristics, MET returns the cells to a completely epithelial state[642]. In cancer, epithelial cancer cells acquire some mesenchymal features in order to metastasise; for example, EMT results in

[637] These genes include *Oct4*, *Myc*, *KRAS*, *HIF*, *TFAM*, *SLC2A1*, *STAT3*, and *p53*. Luo and Semenza, 2012; Smith and Sturmey, 2013. It is also of interest to note that both *Oct4* and *c-Myc* are two of four genes (the others being *Sox2* and *Klf4*) required to dedifferentiate somatic cells into iPSCs (induced pluripotent stem cells). Takahashi and Yamanaka, 2006.
[638] The exception of course being erythrocytes (red blood cells), which do not contain any nuclear DNA.
[639] Gurdon, 1999.
[640] *ibid*.
[641] Micalizzi, Farabaugh, and Ford, 2010.
[642] *ibid*; Nieto, 2013.

delamination of the primary tumour, a first step required for metatstasis[643]. The genes expressed during EMT in development include those that are also expressed in cancer, their expression correlating with poor clinical outcomes[644]. Comparing developmental processes with their parallels in cancer provides targets for therapeutics.

4.2.1 The niche

Large organisms contain many microenvironments referred to as niches; in particular, these are regions where stem cells reside, and retain their potential for both self-renewal and differentiation (see Chapter 6). Another such niche is the microenvironment created by the early embryo during implantation – the developing placenta creates a microenvironment that supports immunological privilege (i.e. the immune system of the host/mother, will not attack the invader/preimplantation embryo)[645].

So whilst the embryo generates a microenvironment or niche, so do adult stem cells, residing in niches around the body (such as the intestinal crypts or the hair follicle bulge). The microenvironment of adult stem cells again permits asymmetric division, enabling regeneration (such as wound repair). The microenvironment has also been seen to affect the developmental path of ESCs and iPSCs; non-pathological ESCs and iPSCs transplanted into the peritoneal cavity of immunodeficient ('nude') mice develop into teratomas[646].

This extrinsic determinant of cell fate has not gone unnoticed by those who propose that there are populations of cells that give rise to cancer cells, whilst also being able to self-renew; these are referred to as CSCs, and are also thought to generate their own niches, a microenvironment that determines cell fate, proliferation, migration, and vascularisation (for example)[647]. The CSC concept is described in detail below.

[643] *ibid*.
[644] Micalizzi, Farabaugh, and Ford, 2010 p 117.
[645] Holtan *et al*., 2009.
[646] For example, Hanna *et al*., 2009.
[647] Ruiz-Vela, Aguilar-Gallado, and Simón, 2009.

5. Comparison of CSC and ESC properties and functions

5.1 The CSC concept

"As differentiated normal and cancer cells can reenter an undifferentiated stemlike state, another level of cell plasticity has become apparent".[648]

CSC theory has evolved from research such as that highlighted previously in this chapter. Currently, it is a popular theory – it works for both for practical experimentation and as an explanation for successful or unsuccessful theories. Recently, Laplane has published a book on the CSC theory, examining its roots and explaining, using philosophy of science, how the theory has become practically useful for biologists and clinicians.

In her book, Laplane noted that the first international meeting specifically addressing the topic of CSC theory was held in 2006 by the American Association for Cancer Research. At this meeting, a functional definition of the CSC was decided on:

"[A CSC is] a cell within a tumor that possesses the capacity to self-renew and to cause the heterogeneous lineage of cancer cells that comprise the tumor"[649]

Laplane unpacked the definition provided, highlighting the four propositions implied:

1) CSCs are capable of self-renewal
2) CSCs are capable of differentiation
3) CSCs represent a small subpopulation of cells (these are distinct, and therefore can potentially be isolated)
4) CSCs initiate cancers[650].

These propositions however are burdened with currently-held assumptions about stem cells and cancer. In an attempt to circumnavigate this problem, Laplane separated the propositions. Propositions 1) and 2) refer to stem cell properties – stem cells are cells that have the ability to both self-renew and differentiate (see Chapter 1, Chapter 6). Proposition 3) is a relational property; CSCs are only distinct if compared with other

[648] Nieto, 2013.
[649] Clarke *et al*., 2006 p 9340; in Laplane, 2016 p 28.
[650] Laplane, 2016 p 28.

non-CSCs (i.e. those without the ability to self-renew and differentiate). This proposition is required to show that not all cancer cells are CSCs[651].

Although Laplane's work on CSCs is useful, it does not explain where CSCs arise: from normal adult stem cells that "run amok", or somatic cells that dedifferentiate[652]? Such questions are important for the goal of treating cancer: which cells need to be eliminated? One possible explanation is that there are several changes required in any cell (leading to mis-controlled differentiation and growth) prior to oncogenesis, and the first of these events is likely to occur in stem cells (since these cells undergo sufficient numbers of cell divisions to acquire oncogenetic changes)[653].

Furthermore, the CSC theory may not be as clear as Laplane's work (and in particular the four propositions) suggests. In 2008, Quintana *et al*. reported that, far from the low numbers of CSCs purported to be present in each tumour (0.0001-0.1%), one in four tumour cells, when implanted into immunodeficient mice, were capable of initiating a further tumour. One possible explanation for this involves the cell's microenvironment, such as ECM-rich areas (where increased levels of ECM components, like laminin, improve tumour-cell viability)[654].

5.2 The ESC concept

The history of the ESC concept was discussed in Chapter 1, however relevant aspects of the ESC concept will also be briefly included here for clarity.

The growth in cell biology studies that led up to development of the ESC concept began in nineteenth-century Germany, where there was funding and microscopy tools available. At Berlin, Schwann published his Cell Theory; although soon repudiated, the attempt to unify several disciplines would continue to influence how biological sciences studies were carried out well into the twentieth century.

In the first decades of the nineteenth century, Barry concluded that the first divisions observed in the fertilised egg were actually the generation of the first cells

[651] This is an important distinction to make. The 'classical', or 'stochastic' model of cancer looks at the properties of all the cancer cells as a whole. Based on this, some of these cells (although they are not distinct) must be able to contribute to tumour growth and secondary tumour formation / metastases. Only in the CSC model are these cells conceptualised as separate to other cancer cells (non-CSCs). Laplane, 2016.
[652] Passegué, 2006 p 754; Krivtsov *et al*., 2006.
[653] Eaves, 2008.
[654] Quintana *et al*., 2008; Eaves, 2008 p 582.

of the new organism – embryonic cells. Around twenty years later, Haeckel would use the term *Stammzelle*, influenced by Darwin's 'stem trees', initially to refer to the earliest (single-celled) organisms that arose from *Moneren*, the ancestors of multicellular organisms – i.e., a phylogenic, evolutionary developmental context. Later, *Stammzelle* would also refer to the fertilised egg. In 1892, Boveri suggested that it was not only the fertilised egg that should be considered as a stem cell, but the daughter cells of the next few cell divisions as well.

In the USA, Wilson translated Haeckel's *Stammzelle* into the English 'stem cell', and make use of this term in his popular book *The Cell*, which was produced in several editions well into the twentieth century. The popularity of *The Cell* meant that the idea of stem cells became habitual in relevant discourses (particularly referring to the embryo) throughout the twentieth century. Meanwhile, experimental techniques had improved, enabling embryos of some species to be created and observed in the laboratory.

As highlighted by Stevens' infrequent use of the phrase 'embryonic stem cell', it was perhaps still not a concept many were comfortable with, in regard to a definition. Stem cells had been defined previously, however the ESC had not – it is possible that even by the mid-twentieth century, some researchers were not confident to claim knowledge of the lineage of each cell, as it comes into existence, in the early embryo. The concept of an ESC then needed to wait until more experimental work could be carried out to demonstrate the stem cell properties of embryonic cells (see Chapter 4).

5.3 Comparison of CSC and ESC concepts

The nineteenth century was a significant period for research comparing pathological and non-pathological development, as parallels were drawn between tumour growth and embryonic growth. In the late 1820s, both Recamier and Lobstein compared the growth of tumours to the growth of the embryo, with Lobstein suggesting that tumours occurred when the organism lost control of tissue growth. It appears that the ability of tumours to grow so quickly, and apparently separately from the rest of the organism, that led to the comparison with embryonic development.

As discussed in Chapter 1, Barry's work in the first half of the nineteenth century clearly showed that he appreciated that the continuous matter between the embryo and the adult were cells, and Cell Theory would have supported Barry's

ideas. It would also renew conversations about the origins or causes of cancer. For some, such as van der Kolk, the explanation of tumours as clumps of cells would enable the suggestion that metastases could arise from the movement of cancer cells around the body. The ancient view that any surgical treatment should remove the entire tumour, leaving nothing behind, was reinvigorated with the suggestion that tumours were made-up of cells, and that leaving any cancer cells behind would result in tumour re-growth (as Lebert advised in 1845).

Cell Theory would also suggest how cells would arise in the first place: that both non-pathological and pathological cells arise from the cytoblastema (in the adult). For Virchow in particular, the tumour needed to be considered as part of the body (even though it was pathological), refuting the view that tumours could originate from a source separate from the body. Remak would also agree that tumour cells must arise from other (non-pathological) cells.

Where the comparison of tumour and embryonic growth appeared again in the later nineteenth century, Italian surgeon Durante would suggest that all tumours arise from embryonic cells. This idea then takes the premise of Cell Theory and *omnis cellula e cellula*, together with the older observation that tumour growth mimics the speed and individuality of embryonic growth, to generate direct comparisons between pathological and non-pathological development.

In 1895, Durante published a paper explaining how he worked out this idea; he observed two cases where he would surgically remove dermoid cysts, only for secondary tumours to reappear later. He then examined other similar tumours that had been removed, and saw that these tumours contained various, disorganised tissues. When considering how all of the tumours he examined contained both mature and embryonic tissue, he was led into consideration of development, and in particular pubertal development. During puberty, Durante reasoned, some tissues that had been dormant in an otherwise adult body, would spring into life. Durante suggested that an external stimulus may then be responsible for other dormant embryonic cells to become active, becoming cancerous growths[657].

As suggested by Eyre, the other embryonic rest theories that followed, were a variation on Durante's initial ideas. The notion that tumour growth was like

[657] Durante, 1895 (transl. Eyre, 1896).

embryonic growth – so much so that the latter could induce the former – was not at issue.

In 1947, Krebs claimed that modern embryology had 'disposed' of the embryonic rest theory of cancer. There was an individual who would later suggest an alternative to the embryonic rest theory – Charles Oberling. Oberling did not agree that embryonic cells remaining in the adult may suddenly lose control of their growth and become cancerous. Oberling's view is projected in discourses currently occurring in the philosophy of stem cell biology, such as Fagan's argument that stem cells are capable of either proliferating *or* differentiating, but not both at the same time[660]. Oberling observed that both embryonic and cancer cells were morphologically similar. However, Oberling argued that for tumour growth, cancer cells needed to continue to multiply. Embryonic cells, Oberling said, did not have this continuing proliferative potential, instead becoming differentiated cells[661].

"It is true that embryonal cells do somewhat resemble cancer cells in appearance, but the two are entirely different in nature. For whereas the proliferative vigor of the former gradually flags as they differentiate to form normal tissues, their malignant prototypes continue to multiply indefinitely and end at last in anarchy and ruin.
But, it may be said, in the embryo growth is restricted by controlling and directing influence; in the body of the adult, where these are missing, the embryonal cells behave quite differently. Experiment does not confirm this objection. Embryonal tissues in all stages of development have been inoculated into countless adult animals, and always with the same outcome; they never changed their character, but continued to act as they do in the embryo, growing for a time but ending as mature tissue".[662]

Oberling's consideration that malignant cells did not stop proliferating, and embryonic cells did, was supported by the experimental results suggesting that transplanted embryonic tissue would not result in tumour formation. For Oberling, this was conclusive – no resting embryonic cells were the cause of malignancy in the adult.

In the latter half of the twentieth century, an updated version of the embryonic rest theory emerged – the concept of the cancer stem cell. Through the twentieth century, it became clear that stem cells could not be restricted to the embryo, as the

[660] Fagan, 2013a.
[661] Oberling, 1944.
[662] Oberling, 1944 p 31.

adult body would also need a means of repairing and regenerating – stem cells must also be required in the adult body. If stem cells continued to exist in the adult, then 'embryonic rests' were no longer needed to explain how tumours emerged, even if their tissues were different to that of the tissue of origin. The cancer stem cell then was the stem cell that was 'went rogue', and became the stem cell of the tumour. Laplane's recent exploration of the CSC concept highlights its practical use for clinicians and researchers: it offers an explanation for whether certain therapies are either successful or not[664].

In comparison, the original ESC theory was theoretical. Developed from the early nineteenth century onwards, the ESC theory developed through microscopical observations and attempts to explain how a single fertilised egg could develop into an enormous, organised, multicellular organism. Although the term *Stammzelle* or stem cell became used throughout Europe and North America in the late nineteenth and early twentieth centuries, its application in embryonic discourses appears lacking. For example, as noted previously in this chapter, Stevens only used the term once as late as 1970. Practically then, ESCs were not as immediately popular as its CSC counterpart. Further evidence for this is the increasing popularity of the term after the experimental (i.e. practical) work of Martin and Evans; a search on PubMed for "embryonic stem cell" reveals no papers were published containing this term until the early 1970s (demonstrating that Stevens' use of the term was an early example). In the twenty years following the publication of Evans and Kaufman, and Martin's work isolating mESCs, the use of the term increases drastically, with hundreds of papers published on the topic (in the twenty-first century, this number increased into the thousands).

6. Conclusions

The aim of this chapter was to explore the parallels between cancer and embryonic development, with a focus on the nineteenth and twentieth centuries. This began with a brief history of concepts of cancer, narrating the influence of Hippocratic thinking (via Galen) in Ancient Europe, and the importance of Christian and Islamic scholars in the upkeep on these ideas about cancer and its treatment into the Middle Ages. It was not until the early modern period that the humoural theory of

[664] Laplane, 2016.

health and wellbeing in general (including cancer) became to be questioned; the cultural and technological changes of Renaissance Europe further enabled the creation and dissemination of alternative ideas. The discovery of blood circulation (in 1628), the lymphatic system (in 1656), and red blood cells (in 1661) suggested fermentation and/or coagulation of the blood and lymph could cause cancer, and resulted in the lymph theory in the latter half of the seventeenth century. Other potential causes of cancer came to light in the eighteenth century, such as Pott's observation that cancer could be caused by the accumulation of soot on the skin, and Ramazzini's study regarding the incidence of different cancers in nuns.

Re-emerging concerns about the parasitic nature of cancer in the early nineteenth century were refuted by those believing in the unifying ideas of Cell Theory, maintaining that all organisms were made up of cells, which can only arise from the division of other cells. This idea included both pathological and non-pathological cells, encouraging researchers such as Virchow to argue against extenal causes of cancer, and focus on the possibility that cancer cells could arise from non-pathological cells or tissues.

Durante was possibly the first to suggest that cells from the embryo may remain in the adult, suddenly emerging from a dormant state, and initiating tumour formation and growth. A variety of European researchers would agree with the concept of left-over cells from the embryo ('embryonic rests') being able to cause cancerous growths, several theories developed from this theme, including those of Ribbert, Remak, Virchow, and Cohnheim. The embryonic rest theory, or a version of it, would survive well into the twentieth century. Oberling was a vocal opponent to the theory in the mid-twentieth century, citing experiments that demonstrated transplanting embryonic tissue into an adult did not result in tumour formation.

At this time, through the studies beginning with Stevens at JAX, teratomas became a useful research tool for the study of cancer origin and, later, development (see Chapter 4). Stevens was not the first to describe testicular teratomas, however developed a strain of mice with a high incidence, which could be easily maintained and stocks sent to various laboratories around the world; this popularised the strain 129 mice as the model of study for teratoma research. Barry Pierce became interested in teratoma research from his experiences as a clinician, and in particular the lack of understanding regarding human testicular tumours in the mid-twentieth century. His studies with Frank Dixon demonstrated that human teratomas, like their murine

counterparts, would differentiate into a variety of tissue types from all three germ layers. Pierce worked with Stevens precisely to gain a better understanding of embryology, and how this compared with the teratomas he had observed. Pierce was amongst the first to controversially conceive that some cancer cells were capable of differentiation in the late 1950s; this idea was only slowly accepted following the publication of results by researchers like Stevens and Pierce, clearly showing that this was the only explanation for tumour growth and cell differentiation in the tumour.

Throughout the late 1950s and 1960s, Stevens also worked to elucidate the cause of teratomas, carrying out experiments to test his hypothesis that PGCs were responsible. This would, apparently unknown to the current generation of researchers (as it was not mentioned in their publications), mirror the suggestion made by Beard in 1902 that the trophoblast cell (a precursor to germ cells) could be the misplaced 'embryonic rest' that could initiate cancer generation in the adult.

After closely narrating the important work of Stevens, Pierce, and others in describing the nature and origin of mammalian teratomas, parallels between development and cancer have been discussed in this chapter. Several approaches have previously been taken comparing development and cancer, such as Gurdon's reflections cumulating in the claim that the processes of normal renewal become uncontrolled, leading to cancer. Some of the earliest investigations comparing non-pathological and pathological development were carried out at the beginning of the twentieth century, including, for instance, scrutiny of the cellular biochemistry of cancerous and non-cancerous cells. Another research avenue was the examination of cell fate, possibly as an off-shoot of the concern with cell lineage studies in the USA during this period.

In a further attempt to characterise cancer cells, Greenstein and Knox (for example) carried out a series of biochemistry experiments in the mid-twentieth century. In 1947, Greenstein's published results suggested that biochemically, cancer cells were more similar to each other than the tissues they arose in. Knox suggested that this presented a paradox, since up to this point, tumours had been characterised by their diversity. Knox repeated Greenstein's experiments and observed the same results; furthermore, Knox also showed that foetal cells were more similar to each other than the organs they would differentiate into. This led Knox to ponder whether tumour cells became undifferentiated, or dedifferentiated, in order for tumours to grow. Knox also advocated further study of cancer, since the less mysterious cancer

was, the more susceptible to logic (for treatment) it became. In fact, Knox went a step further to suggest that cancerous tissue should be "recognized to be similar to a familiar type of normal tissue"[665]; a clear further indication that in the twentieth century, parallels between pathological and non-pathological tissue were sought out. In the case of Knox, the clear aim was to improve understanding. More recent work into cell metabolisms and pathways has provided further information regarding the ATP (energy) generation of pathological cells (in comparison to non-pathological), and how the preference for various pathways results in the production of intermediates, useful for cancer growth and invasion. Interestingly, these same pathways and intermediates also appear to be responsible for growth and implantation of the early embryo. There are further similarities in the genes expressed, and by the creation of niches, by cancer and embryonic cells *in vivo*. It is noteworthy that at this time there is still no clear, defined origin of the CSC (unlike the fertilised egg origin of the ESC).

The apparent ability of cancer cells to self-renew and differentiate was not lost on the research community, and, possibly aided by the clear biochemical and metabolic similarities between cancer cells and ESCs, a concept of the CSC emerged. In her recent book, Laplane linked the history of the CSC concept to the embryonic rest theories of the nineteenth century. Her focus is on the practical uses of the CSC theory for research and therapies, and to do this, she noted four premises of CSCs for their definition: CSCs are capable of self-renewal and differentiation; they represent a small subpopulation of cells (these are distinct, and therefore can potentially be isolated), and CSCs initiate cancers. The capability of self-renewal and differentiation are, of course, properties that CSCs have in common with all other stem cells (including ESCs). Although more is becoming understood regarding the properties of CSCs, there is still no clearly defined origin of CSCs.

Lastly, comparison of CSC and ESC concepts explains that the nineteenth century was a key period for the development of comparative links between normal and pathological development. Influential researches included Barry's initial work suggesting that the cells of the embryo were equivalents of cells in the adult; Virchow's interpretation of Cell Theory (and *omnis cellula e cellula*) requiring that pathological and non-pathological tissues must arise from inside the body, (although

[665] Knox, 1972 p 487.

there is no precise definition of pathological origin); Durante's idea that misplaced embryonic cells might lie dormant in the adult, before suddenly beginning to proliferate (and differentiate) uncontrollably, forming a tumour; further variations on the embryonic rest theory; Oberling's rebuttal; and eventually the observation that PGCs were able to initiate teratomas in mammals.

Initially, the ESC concept was theoretical in nature, relying on the observations and interpretations of microscopists in the nineteenth century. Experimentally (or practically), the ESC concept was initially of little use, and perhaps stalled its acceptance into embryological and developmental research. (The ESC concept became populariased in the 1980s and beyond by the research carried out by Gail Martin and Martin Evans [see Chapter 4].) Alternatively, the CSC concept appears to have been born out of practical requirements for explaining results of research and therapeutics. ESC theory provided an explanation of how ESCs could arise, and their specific origin. CSC theory in contrast does not specifically address the problem of how CSCs arise, or define their origin.

I argue that the CSC theory developed from embryonic rest theories through the practical work carried out in the mid-twentieth century. This practical work was only possible because of the theoretical background provided by ESC concepts in the late nineteenth and early twentieth centuries. The ESC concept suggested that cells in the early embryo were capable of two functions: self-renewal, and differentiation. This explained how an entire multicellular organism could arise from a zygote – self-renewal enabled physical growth, whilst differentiation enabled a multitude of specialised tissues to be created. Parallels were seen with tumour development – tumours could physically enlarge (showing that self-renewal was required), and include a range of tissue types (demonstrating differentiation potential). Embryonic rest theories were then, generally, an attempt to explain how:

1) the unifying nature of Cell Theory could be applied to pathological as well as non-pathological tissues (and there was a continuum);
2) tumours could contain various tissues (possibly different to the tissue of origin);
3) stem cells (or cells with the ability to both proliferate and differentiate) could exist in the adult.

This would also encompass Beard's later trophoblast theory of cancer origin. Experimental attempts to test the embryonic rest hypotheses emerged in the early

twentieth century, with researchers transplanting sections of embryonic tissue into other organs, and examining the results. These experiments demonstrated that 'misplaced' or 'reactivated' embryonic cells were not responsible for tumour formation. As addressed by Oberling, if embryonic cells, with their properties of proliferation and differentiation, were not responsible for tumour formation, it implied that other cells with these properties were. This inferred that CSCs existed, however gave no suggestion as to their origin. The embryonic rest theory had implied that cells with the properties of self-renewal and differentiation (i.e. stem cells) were present in the adult, and that a subset of these cells were capable of causing cancer. Tumours, with their ability to grow uncontrollably, and contain both undifferentiated and differentiated tissues, were the clear result of the stem cell 'running amok'. The definition of CSCs currently in use, and helpfully unpacked by Laplane, shows that CSC theory emerged from embryonic rest theories, and therefore from the ESC concept. The diffence between ESCs and CSCs however is that whilst the origin of ESCs are firmly defined, the CSC origin is not.

This chapter concluded with the work of researchers in the mid-twentieth century comparing pathological and non-pathological cytology. The following chapter continues chronologically, demonstrating how the use of teratomas became essential for the isolation and culture of mESCs.

CHAPTER 4:

AN EXAMINATION OF BRITISH AND AMERICAN SCIENCE IN THE 1970S AND 1980S: CASE STUDIES OF EMBRYONIC STEM CELL RESEARCH

1. Introduction

Following on from the teratoma research discussed in Chapter 3, the teratocarinomas and their cells identified in the 1950s and 1960s were recognised as promising models for both embryo development and cancer research. The early embryonic stem cell work that started in the 1970s began particularly by taking methods and techniques from cancer research. Throughout the twentieth century, techniques for embryo culture and *in vitro* manipulation were improving, and the early embryonic cell researchers took full advantage of this.

In the 1970s and 1980s, two prominent researchers, Gail R Martin, and Martin J Evans, began working to develop a line of embryonic stem cells that could be used in the laboratory. Martin and Evans worked together during the 1970s, developing a line of embryonal carcinoma cells. By the mid-1970s, it had also been shown that mouse embryos could complete some of their development *ex utero*. In the early 1980s, Martin and Evans' continuing work individually resulted in the first isolation of embryonic stem cells, both researchers independently publishing methods for isolation and culture of murine embryonic stem cells: Martin in the USA, and Evans in the UK. This milestone in stem cell research, and in particular ESC research, therefore requires particular attention in this thesis. Although Martin's and Evans' work in isolating mESCs has been noted previously, there as yet has been little consideration of the reasons or motivations for Evans or Martin to isolate mESCs in the first place. This prompted the selection of these case studies for this chapter, particularly as an opportunity to demonstrate the role of social, political, and economic factors on what may generally considered to be such 'milestone' research. This is an interesting time period for looking at potential effects on science by social, economic, and political factors, since the 1980s in particular were a time of change – changes in social attitudes and priorities (such as high unemployment), political changes (with the election of Margaret Thatcher in Britain and Ronald Reagan in the USA), and changes in economics, as the downturn hit. This period also matches one of the key timepoints in stem cell history – isolation of mESCs. Instead of simply reiterating the history of Evans' and Martin's achievements, this chapter utilises these well-known experiments in stem cell history as a way of examining the effects of changing social, political, amd economic factors on research in this field.

The research directions Martin and Evans elected to pursue were rather different. Evans, in the UK, concentrated his research on the more clinical and therapeutic applications of the stem cells he had cultured in the laboratory. Half a world away on the west coast of the USA, Martin used her stem cells to learn more about animal development. It is unlikely that these selections were arbitrary choices on the part of Martin and Evans. This chapter therefore examines what encouraged or discouraged the research directions of Martin and Evans through the 1980s in the USA and UK. This will include global and national economics and politics, as well as the research traditions Evans and Martin were working in at their respective universities. Although it is expected that the capitalist ideologies in late twentieth century USA would encourage more applied research than the UK, this chapter demonstrates that in the 1980s, this was not the case. Instead, Evans, in the UK, was encouraged to find more practical applications for mESCs, whereas Martin in the USA could focus on fundamental research into development.

Biologist Chris Graham claimed that from the mid-1960s onwards there was a strong sense of international collaboration in the fields of fertility and developmental biology research, making it "impossible to isolate a uniquely British contribution to the field"[666]. In this chapter, I seek to demonstrate that this is not necessarily the case. I agree that there is more movement and collaboration of individuals and groups between Europe, Britain, and North America through the latter half of the twentieth century than there had been previously; however, I will also demonstrate that such influence and collaboration has not completely merged European and American research into a single 'Western pool'. Instead, I intend to show that whilst collaboration and travel were important and influential, other factors determined how embryonic stem cell biology developed separately on each side of the Atlantic.

1.1 Science in the UK and USA: 1970-1981

As early as the 1970s, UK and US governments began to reduce their monetary support for scientific research. This said, the biomedical sciences were spared from the worst of this initial austerity – their clear application to public health and wellbeing was too important. Eventually, funding in this sector too would be scaled back by British Prime Minister Margaret Thatcher (1925-2013) and US

[666] Graham, 2000 p 51.

President Ronald Reagan (1911-2004) (see below)[667]. At this time however, biomedical sciences were increasing their demand for resources – new areas of research (such as genetic sequencing and molecular biology) meant an increase in requirements for equipment and specialists, that would provide further improvements in medicine and new ways to generate knowledge. The increased requirements and decreasing funds led to biomedical science becoming a highly competitive field.

Political scientist Dietmar Braun reported that there were large increases in the number of scientists and academics working in the biomedical sciences through the 1970s and 1980s. In Great Britain for example, Braun reported a 30% increase in senior lecturers, readers, and professors between 1972 and 1980[668].

As demonstrated below, there was some public disenchantment with science in the 1970s UK and USA; coupled with growing economic difficulties throughout the decade, the US and UK government budgets on scientific research in general was squeezed[669].

1.1.1 UK science: 1970-1981

Following World War II, it was becoming more obvious that there was a developing relationship between scientific and technological progress and power; this relationship continued into the late twentieth century, becoming inexorably linked with the Cold War. Science could no longer be considered "value-free"[670]. Polish bacteriologist and political commentator Ludwig Fleck (1896-1961) had suggested this as early as 1935, warning that Western power was too great, and scientific discoveries were becoming dependent on social framing (e.g. networks, prestige)[671].

During the 1960s, the general public became much more aware of research science, and were developing strong opinions on what procedures or experiments should and should not be carried out. As predicted by Fleck, science was becoming implicated in social and political interests[672]. This was further emphasised since the state funded most biomedical science research in Britain[673]. This can be seen in the debates concerning animal experimentation and *in vitro* fertilisation, used here as

[667] Braun, 1993.
[668] *ibid* p 269.
[669] *ibid*.
[670] Jacob, 1992 p 488.
[671] Fleck, 1979 (1935); Jacob, 1992.
[672] Jacob, 1992 p 488.
[673] Johnson *et al*., 2010.

examples to demonstrate the increasingly complex interactions between science and the public in the late twentieth century.

Animal experimentation

By the end of the 1960s and through the first half of the 1970s in particular, animal experimentation was becoming increasingly contested by the general public. Letters to the Home Office concerning vivisection increased ten-fold between 1972 and 1975[674]. Growing public concern became political concern, with both the House of Commons and the House of Lords meeting to bring in more restrictive legislation on animal testing. Several pressure groups and societies were formed, keen to promote the abolition of vivisection in particular and animal experimentation in general; most wanted to highlight the advances made in tissue culture to show that it was a viable alternative to animal testing. In 1977, British Prime Minister James Callaghan (1912-2005) announced that it was his Labour government's policy to encourage research to move away from animal experimentation as soon as possible[675]. The announcement was generally welcomed by animal welfare groups and the public; however, only two years later, with a general election looming, animal welfare disappeared from political debate. The global economic downturn and strikes dominated discussion on the run up to the election, with Margaret Thatcher (Conservative) being elected as Prime Minister on the back of her promises to decrease union powers and improve the economy.

In vitro *Fertilisation*

There was a significant increase in interest in medical and biomedical ethics from the late 1960s onwards, with publications contributing to influential journals such as the *New England Journal of Medicine* and the *British Medical Journal*. The reproductive sciences, which had previously fallen between the divisions of other disciplines (such as agriculture and medicine), were also beginning to establish themselves as legitimate fields in their own right[676]. One area of research that concerned many interested in bioethics was that being carried out by obstetrician and gynaecologist Patrick Steptoe (1913-1988) and physiologist Robert Edwards (1925-

[674] Wilson, 2011 p 85.
[675] *ibid* p 87.
[676] Johnson *et al*., 2010.

2013), researchers who were attempting to work on a method of carrying out human fertilisation *in vitro*. Although fertility research was not looked on unfavourably by the UK research councils, it was not a priority. Instead, as concerns regarding the effects of radiation were fading in popularity, this was replaced by another increasing concern: over-population. Infertility was considered to be a relatively low priority in comparison with research into contraceptive methods and birth control[677]. Steptoe and Edwards had failed to obtain funding from the Medical Research Council (MRC) (who, Jon Turney suggests, had therefore tacitly proscribed the research), but had obtained money for their work from private American funding[678].

In a paper detailing why the MRC did not fund Edwards and Steptoe's work, it is again made clear that social and political agendas affected scientific funding and therefore research direction. The MRC were positive about Edwards' proposal at first; at the beginning of the 1970s they had significant amounts of money to spend on establishing specific research institutes, and Edwards was invited to participate. Edwards preferred to carry out the work at the University of Cambridge however. Furthermore, given the research priorities on over-population, infertility was considered a low priority. This was not only a British consideration, but one which had warranted the establishment of the United Nations Fund for Population Activities in 1968, and the population control-focused Human Reproduction Programme of the World Health Organization in 1972[679].

As Edwards and Steptoe continued their work with American funding, it was becoming clear that there was very little legislation concerning their work in the UK, again highlighting the need for ethical, legal, and philosophical discussion concerning embryo research. The British legal system was relatively slow with regards to creating and implementing new legislation, so, in the meantime, the Warnock Committee was set-up to look carefully at research on embryos in the UK[680]. Again, since legislation was slow, their recommendation for licensing research appeared to be the most viable option.

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[677] Graham, 2000; Johnson *et al*., 2010.
[678] Turney, 1998 p 175.
[679] Johnson *et al*., 2010.
[680] The report by the Committee of Inquiry into Human Fertilisation and Embryology (also known as the Warnock Report, after its Chair Mary Warnock [1924-]) led to the Human Fertilisation and Embryology Act 1990, and the establishment of the Human Fertilisation and Embryology Authority. For more on the ethics of the Human Fertilisation and Embryology Authority, and bioethics in general in twentieth century Britain, see Wilson, 2014.

As demonstrated by these two brief examples, scientific endeavor was being bound by the goals and interests of politics; the effect on scientific research was going to be significant, since the governmental funding of research was vital in British universities. Similar agendas could be observed in American politics and science.

1.1.2 USA science: 1970-1981

The increased concern for the welfare of laboratory animals and the ethical debates concerning embryo experimentation were not limited to the UK; in the USA, similar discussions were appearing from the late 1960s through the 1970s. This led to some areas of biomedical research becoming particularly politicised. The USA would react slightly faster to new technological capabilities; for example, in October 1971 the Federal Government deliberated human experimentation, with a particular focus on research carried out on the foetus or embryo. Linked with the abortion debate, particularly after elective abortions became legal in 1973, the American government was keen to show that such a step was not taken in order to encourage abortion[681]; this led to the Department for Health, Education and Welfare (later the Department of Health and Human Services) releasing a statement, advising that there should be no attempt to implant a human ova fertilised *in vitro* until further guidelines had been developed. Initially, this was a moratorium, only made obligatory in 1974, and even then only for a year. In 1974, Congress established the National Commission for the Protection of Human Subjects of Biomedical and Behavioural Research, who recommended that an ethical board be put in place[682]. In 1975, the Ethics Advisory Board was set-up, however did not approve any research until after the birth of the first IVF baby, Louise Brown, born in Britain, in 1978. Newspaper reports from the time suggest that there was some resentment amongst American researchers, since research in the USA could not benefit from those achievements made in embryology elsewhere[683].

From 1973, federal funding was not available for research on embryos; the private sector however could fund whatever research they wished; since the seventeenth century, free enterprise has been a cornerstone of American values,

[681] Wertz, 2002.
[682] *ibid*.
[683] Turney, 1998 p 182.

preventing the government from legislating against such companies pursuing their own goals[684]. The Ethics Advisory Board would only eventually approve research funding for basic research - not therapeutic or infertility research. This continued until the Ethics Advisory Board charter expired in 1980. It was never replaced, technically leaving no lawful body to approve (or disapprove) embryo research[685].

2. Embryoid carcinoma cells to embryonic stem cells: 1970-1981

The period 1945-1965 in biology is particularly noted for establishing the mouse as an experimental model[686]. The era was also defined for its research into the effects of radiation (see Chapter 3). Other research interests were catered for however; in Britain, for example, the Marshall School of Reproductive Physiology in Cambridge and Waddington's Institute of Animal Genetics in Edinburgh were also well-funded at the time. By the late 1960s, mammalian physiology, genetics, and embryology had been linked with clear applications – Graham refers to this as "a powerful cocktail"[687]. In Britain, this would lead to the mouse being adopted as the animal model for developmental biology; in the USA, *Drosophila* was the preferred model, due to the better understanding of its genetics at the time[688].

2.1 Martin J Evans

Sir Martin J Evans (1941-) is probably most well known as the winner of the 2007 Nobel Prize in Physiology or Medicine (alongside Mario R Capecchi and Oliver Smithies)[689]. Evans, Capecchi and Smithies were awarded the Nobel Prize "for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"[690]. Much of Evans' life has been well documented, particularly since being awarded the Nobel Prize. Evans' recollections of his childhood and early university career suggest his constant interest in biology, and some fortunate instances which led him to meet and/or work with influential scientists.

[684] Wertz, 2002 p 143.
[685] *ibid*.
[686] For example, see Myelnikov, 2015.
[687] Graham, 2000 p 51.
[688] *ibid*.
[689] Nobel Foundation, 2007.
[690] *ibid.*

Evans was born on 1st January 1941 near Gloucestershire, and soon moved to Hertfordshire, where Evans recalls his 'first experiment': mixing sand cement with water, because he could not understand how the mixture could become solid. After suffering from a burst appendix on what should have been his first day of school, Evans remembers being treated with one of the first antimicrobial drugs (M&B 693), without which he may not have survived; this said, Evans then went on to suffer from several other childhood infections, such as mumps[691]. Evans suggested that recollecting on the time spent in bed at home as a child, reading, playing with his chemistry set and electric experiment set, he was 'naturally' a scientist[692]. After electing to study Chemistry, Zoology and Botany at sixth form, Evans won a scholarship to Christ's College, Cambridge, where he found the Natural Sciences options tempting, giving him an opportunity to elect courses he enjoyed; this included biochemistry (taught by noted plant biologists David Coombe (1927-1999), Malcolm Dixon (1899-1985) and Don Northcote (1921-2004)[693]) and molecular genetics. In the academic year 1962-63, Evans recalled a series of lectures by Jacques Monod (1910-1976)[694] and Sidney Brenner (1927-)[695] about mRNA; from this point Evans claimed he was resolved to work in either developmental biology or plant biochemistry[696]. Evans never had the opportunity to sit his final exams however, becoming ill with glandular fever[697]. Although disappointed at the lack of opportunity to embark on a postgraduate research career at the time, Evans considerd himself fortunate to have been employed as a research assistant with Elizabeth Deuchar at University College London (where he was also able to complete his PhD). Evans described the atmosphere as somewhat relaxed, stating that Deuchar encouraged but did not direct, allowing Evans the freedom to experiment, innovate, develop techniques and learn a wide range of skills, whilst working on *Xenopus* development.

[691] *ibid.*
[692] *ibid.*
[693] Evans, 2001.
[694] Monod was to win the Nobel Prize in 1965 for work on genetic control of virus synthesis.
[695] Brenner was another future Nobel Prize winner (2002), and one of the first to see Watson and Crick's 1953 model of DNA.
[696] Evans, 2001.
[697] Nobel Foundation, 2007; Evans, 2001.

During this time, Evans was attempting to isolate developmentally controlled mRNA[698]. Evans was able to use blastula and gastrula ectoderm, providing an early insight into embryo biology. Evans observed that there were two limiting factors for his research: lack of foreseeable genetics, and difficulty in obtaining enough material for research[699]. This latter Evans discussed with colleague Robin Weiss (1940-), who suggested using mouse teratocarcinomas, following the 1966 publications by Stevens and Pierce (see Chapter 3). Evans noted that Stevens had presented these rapidly dividing cells which could divide asymmetrically, whilst Pierce had demonstrated their clonality. Stevens sent Evans stocks of mice from JAX, and Evans was taught the tissue culture techniques required by Weiss and Pavel Vesely (visiting from Prague)[700].

2.2 Gail R Martin

Gail Martin (née Zuckman) was born in New York, USA, in 1944, and went to the University of Wisconsin before beginning her research career at the University of California at Berkeley. She obtained her PhD in 1971. Martin then moved to England with her English husband Steven Martin (also a biologist), after he had been offered a job there. Martin herself found work in Martin Evans' biochemistry group at University College London (UCL), where she credited Robin Weiss with introducing her to teratocarcinomas[701]. Martin was not the only American at the time to visit the UK and Europe after completing a PhD; the National Institutes of Health (NIH) and March of Dimes provided funding for fellowships. In fact Graham estimated that such funding doubled the research in mammalian developmental biology in the UK between 1960 and 1980[702]. Martin returned to the USA in 1976 to work at the University of California at San Francisco (UCSF), where she has remained until the present. Martin is currently Emeritus Professor in the Department of Anatomy, working particularly on development and organogenesis in mice and chickens. Martin was also President of the Society for Developmental Biology in 2006-2007 after winning the Edwin Grant Conklin Medal from the Society in 2002.

[698] Nobel Foundation, 2007.
[699] *ibid.*
[700] *ibid.*
[701] Robin Weiss was working in a shared laboratory alongside Steven Martin at the Imperial Cancer Research Fund's Laboratories in London in 1971. It was Robin Weiss (whose primary interest was retroviral biology) who introduced Gail Martin and Martin Evans.
[702] Graham, 2000.

In 2007, Martin received the Pearl Meister Greengard prize (along with Beatrice Mintz and Elizabeth Robertson), celebrating women in science. She is also a member of the American Academy of Art and Sciences and the National Academy of Sciences (cellular and developmental biology), and in 2015 was elected to be a Foreign Member of the Royal Society.

Gail Martin began her research career investigating collagen, in particular its use in laboratory tissue culture and its cross-linking properties[703]. It was likely to be this experience in cell culture and associated techniques which made Martin a promising postdoctoral addition to Evans' laboratory at UCL.

2.3 The SIKR Cell Line

Publications from Evans' laboratory suggest that the group were spending almost all of their time and resources on mouse work, including their ongoing studies into teratocarcinomas. However, if one is to believe Graham's interpretation of the era, this was relatively unheard of. Graham instead proposes that few groups invested in mammalian developmental biology full-time, instead preferring to top-up funding for their mouse work with other areas of research in the 1970s[704]. It is possible that Evans, who was only beginning to establish his own small group in the early 1970s, could garner enough funding for his laboratory to concentrate solely on murine cell biology.

In 1972, Evans published "The isolation and properties of a clonal tissue culture strain of pluripotent mouse teratoma cells" in the *Journal of Embryology and Experimental Morphology*. In this paper, Evans described a clonal culture of cells isolated from a teratoma of strain 129 mice, obtained from Stevens. Evans made it clear that tissue culture work using mouse teratoma cells was already well underway, citing the work by Stevens and Little (1954), Stevens (1964, 1967, 1968 and 1970) and Kleinsmith and Pierce (1964). In 1970, two further groups had demonstrated that teratomas had a single cell origin (Kahan and Ephrussi, 1970; Rosenthal, Wishnow and Sato, 1970[705]). Evans differentiated his work from his predecessors by stating that his paper demonstrated the 'isolation of pluripotent stem cells from a solid

[703] For example, *The nature of the collagen synthesized by cultured human fibroblasts* and the review *Recent progress in collagen research*, both published in 1971.
[704] Graham, 2000.
[705] These papers had described homozygous teratocarcinoma lines, whereas Gail Martin and Martin Evans described a heterozygous cell line.

teratocarcinoma derived from the implantation of an early embryo into the adult testis'[706]. This then is continuing a trend highlighted previously – the comparison of cancerous growth and normal development (i.e. that teratomas arise from initially non-pathological cells), and studying their parallels. The paper described the nature of "SIKR" cells[707] (see Figure 6), which were capable of producing teratomas in mice when reintroduced (demonstrating differentiation into ten tissue types).

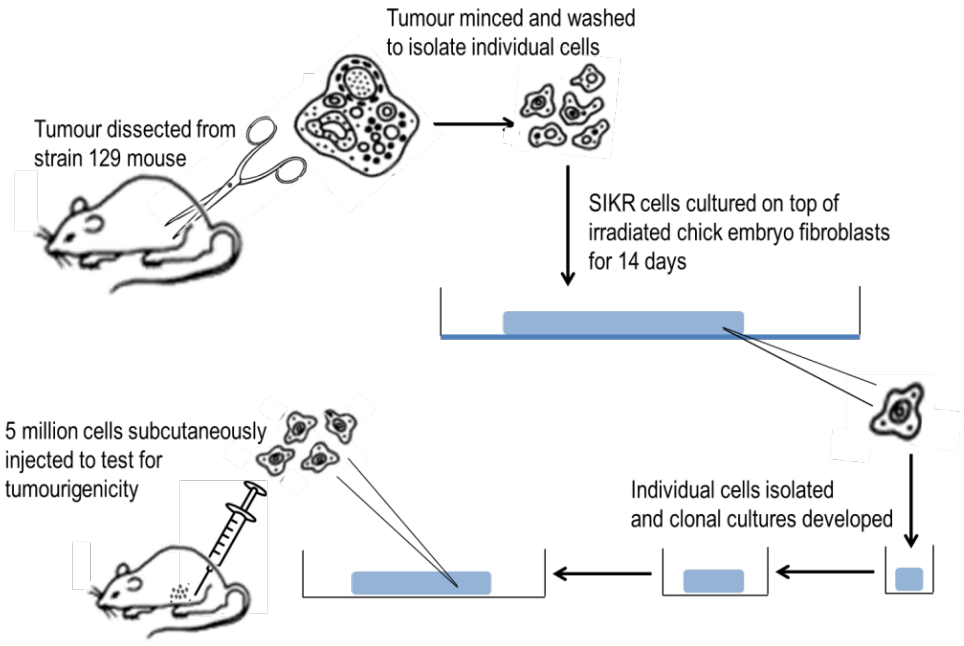


Figure 6: Isolation, culture and tumourigenicity testing of Evans' SIKR cell line. Constructed using information in Evans, 1972.

In culture, two sub-clones were isolated: C-type (tumourigenic, pluripotent and comparable to SIKR cells in differentiation range) and E-type (non-tumourigenic and with lower differentiation capacity). C cells grew as monolayers on E cells; C cells were unaffected by colony density, whereas E cells would not proliferate once the culture had reached a certain density. The C cells were considered to be more primitive than E cells; E cells alone were not tumourigenic, but could become so

[706] Evans, 1972 p 164.

[707] "SIKR" appears to be a shortened version of the longer name given to the cell line, OTT 5568S/1/KR; OTT 5568 was a transplantable tumour obtained from Stevens in May 1969. The tumours were maintained by continuous transplantation, with the slowest growing tumours selected for Evans' project. These were named OTT 5568S. One particular clone of OTT 5568S, OTT 5568S/1/KR, grew well *in vitro*, and demonstrated good differentiation *in vivo*. This was selected for use by Evans, and the name shortened to SIKR. See Evans, 1972.

following spontaneous transformation *in vitro*. C and E cells were also pluripotent[708]. The ratio of C and E cells in culture could also be manipulated by seeding the cells at different densities. When mentioning previous reports of teratocarcinoma cell line establishment, Martin and Evans suggested that:

"These reports primarily have been concerned with demonstrating that these in vitro teratocarcinoma lines are pluripotent. This evidence was obtained by reinjecting the cells of these in vitro lines into mice and making a histological examination of the tumors formed. There has, however, as yet been no detailed description of the characteristics in vitro of these cell lines."[709]

Martin and Evans also suggested that *in vitro* cell lines would be useful as a research tool, as large numbers of these cells could be cultured in controlled environments. This would allow more detailed study, and an understanding of the "biological and biochemical characteristics of pluripotency" to be established[710]. What Martin and Evans had done was to produce a heterogenous teratocarcinoma cell line, unlike homogeneous lines described by Kahan and Ephrussi (1970) and Jakob *et al*. (1973)[711]. Importantly, Martin and Evans concluded that the C cells were 'the stem cell line of teratocarcinomata'; this was supported by evidence including morphological examination and molecular biology techniques (in this case, experiments testing alkaline phosphatase levels)[712]. The cells were described as having large clear nuclei with prominent nucleoli, and a minimal, dark cytoplasm. In culture, these cells formed small, tight colonies. In bacteriological dishes (where the cells had no opportunity to adhere to the plastic base of the plate), embryoid bodies developed, noted as morphologically similar to early post-implantation embryos[713]. Martin and Evans also concluded that C cells gave rise to E cells and that this process

[708] Martin and Evans, 1974.
[709] *ibid* p 163.
[710] *ibid* p 163.
[711] BW Kahan was at the Department of Zoology, University of Wisconsin and Boris Ephrussi, a Russian geneticist was at Laboratoire de Génétique physiologique, Gif-sur-Yvette, France, in 1970. The Jakob *et al*. group were based in France; H Jakob was at the Institut Pasteur, Paris.
[712] Martin and Evans (1974) suggest that these homogeneous cultures consisted of only C cells. In 1973, a group working across the UK, USA and France published a paper specifically describing alkaline phosphatase activity in murine teratomas (Bernstine *et al*., 1973). This publication demonstrated that a correlation between alkaline phosphatase activity and embryonal carcinoma cells (the stem cells of teratomas) had been established.
[713] Martin and Evans, 1974; Martin and Evans 1975a; Martin and Evans 1975b; Martin, 1975.

was not reversible; this was identified as a possible route to studying cell determination (although this was difficult *in vivo* with E cells as they were not malignant).

To follow this, Martin and Evans published another paper less than twelve months later, describing the nature of SIKR subclones[714]. Where C cells were subcloned, the cultures were homogeneous, consisting of only embryonal carcinoma cells. When injected into strain 129 mice, these cells gave rise to teratocarcinomas with differentiated tissues present, such as nervous tissue, cartilage and epithelium. With appropriate cell culture techniques, the C cell colonies could be induced to differentiate *in vitro*. The first stage of this was the development of aggregates of cells which produced an endodermal outer layer – these formations were found to be identical to the embryoid bodies observed in the ascites fluid of mice with intraperitoneal teratocarcinomas[715] (and also similar to developmental events which had been shown to occur during normal murine embryogenesis[716]). If allowed to continue growing for several weeks *in vitro*, a variety of cell types could be observed, including keratinising epithelium, cartilage, endodermal cysts, neural cells, muscle, fibroblasts and pigmented cells. Martin and Evans had identified that the early differentiation processes of teratocarcinoma cells that occurred *in vitro* were identical to those which occurred *in vivo*[717]. In addition, this process was shown to be highly organised, and paralleled early development of the mouse embryo; in their conclusions however, Martin and Evans did not suggest that this would be a useful tool for studying the early stages of embryogenesis. Three months later, Martin published a review describing how teratocarcinomas might be useful for studying embryogenesis and neoplasia. This detailed paper described teratomas, embryoid bodies, embryogenesis, embryonal carcinoma cells, and the development of derived cell lines. Here then, Martin seems to be highlighting herself the parallels between normal and pathological development[718]. Martin also described techniques used to learn more about these cell lines, including morphology, karyotyping, the biochemical

[714] These subclones were produced by culturing colonies from a single cell of the original SIKR cell line.
[715] As described by Teresky *et al*., 1974.
[716] This was described byTarkowski and Wroblewska in 1967; this is the reference Martin and Evans refer to in their description of the process.
[717] This is further investigated and published in a separate paper: Martin and Evans, 1975b.
[718] Since Martin appears aware of the significance of the parallels in the 1970s, this observation may have driven her focus towards development, as seen in her later work.

marker alkaline phosphatase, immunological properties and some genetics, focusing on the T locus[719]. A section was also devoted to describing the similarities between embryonal carcinoma cells and normal embryonal cells:

"No significant differences have yet been detected among embryonal carcinoma cells found in tumors from different sources…There are at least two possible ways in which embryonal carcinoma cells could arise from their progenitor cell types. First, some malignant change occurs in the progenitor cell types. Second, that embryonal carcinoma cells are normal undifferentiated embryonic cells (or primordial germ cells) which behave abnormally because they are not in their normal environment"[720].

Martin commented that this latter hypothesis was first suggested by Cohnheim and Ribbert in the nineteenth century (see Chapter 3). Ivan Damjanov and Davor Solter also discussed this embryonic theory in two papers published in 1974. It was an attractive theory as the genetic and chromosomal stability of embryonal carcinoma cells was demonstrated over many generations both *in vitro* and *in vivo*; tumours also developed from early embryos transferred to extra-uterine sites, suggesting that malignancy would occur readily in this situation. Martin then reasoned that if embryonal carcinoma cells were normal pluripotent embryo cells (and if pluripotent embryo cells could be cultured from early embryos), then these cells should have the same characteristics of embryonal carcinoma cells *in vitro*, and should form teratocarcinomas *in vivo*. Martin highlighted however that Michael Sherman (at the Roche Institute of Molecular Biology, New Jersey) had so far been the only researcher to produce cell lines from mouse embryos, and these cells appeared

[719] Mutations at this locus had been previously shown to affect mouse embryogenesis and the antigen expression of spermatozoa. Artzt, Bennett and Jacob (at Laboratoire de Genetique Cellulaire, Institut Pasteur et College de France, Paris) (1974) reasoned that this could therefore also affect the antigen expression of early embryos and embryonal carcinoma cells. In addition to this, in a paper published in late 1975, Peter Stern (at the Neuroimmunology Unit at University College London) alongside Martin and Evans, described the investigation of the antigens expressed by teratocarcinoma cells. As the cells differentiated, their surface antigen profile changed. In the conclusions, there were hints that the importance of such work would be eventually realised in developmental biology, if cell determination could be identified by changes in cell surface antigen expression. This suggests that Martin may not have been the only researcher at the time to have recognised the significance of the parallels between pathological and non-pathological development, and the importance of this for a variety of research projects.

[720] Martin, 1975 p 240. This could be seen as a development of earlier work by Knox, although Martin does not reference this previous research, so it is unknown whether she was aware of it or not.

somatic, not pluripotent, and in no way similar to embryonal carcinoma cells[721]. Evans and Matthew Kaufman (see below) would later develop a method to improve on Sherman's results.

2.4 Embryonal carcinoma stem cells

Martin's next set of experiments revealed that there were common proteins in both embryonic carcinoma cells and the embryonic ectoderm (which were not observed in other cell types)[722]. Furthermore, it appeared unlikely that embryonic carcinoma cells and preimplantation embryonic cells were homologous. This led the group to suggest that whereas embryonic carcinoma cells begin to differentiate by developing an endoderm outer layer, they were more biochemically similar to normal embryonic cells which had already developed this layer – i.e. these embryoid bodies were developing 'inside-out'. It was therefore suggested that the embryonic carcinoma cells were a better model system for studying the embryonic ectoderm than earlier embryonic development (since it was more easily accessible)[723]. In addition, later 1970s work studied the expression of a protein in both embryonic carcinoma cell lines and cells isolated from the ICM (inner cell mass) of the blastula at 4 days post-conception. The protein investigated (large external transformation-sensitive protein, or LETS protein) was expressed correlating with differentiation. A comparable result was observed – that teratocarcinoma cells were similar to the embryonic ectoderm[724].

This work led Martin to write another, updated, review comparing teratocarcinomas and embryogenesis, again highlighting the importance of the parallels between abnormal development and embryogenesis for researchers in this field.

"There is…some uncertainty about the normal embryonic equivalent of embryonal carcinoma cells, and whether pluripotent embryonal carcinoma cells isolated from different tumors are all derived from the same embryonic cell type. Nevertheless, these tumor cells, particularly those cell lines that synchronously form embryoid

[721] Sherman, 1975. Sherman had developed a culture medium which promoted the hatching of mouse blastocysts; the free cells would then adhere to the culture dish. These cells would swiftly differentiate however. In 1978, Mintz, Cronmiller, and Custer also published a paper describing the potentially somatic cell origin of teratocarcinomas.
[722] Martin, Smith and Epstein, 1978.
[723] *ibid.*
[724] Zetter and Martin, 1978.

bodies, can provide a model system for studying differentiation during the early postimplantation period"[725].

Martin explained that such a model was useful because this is a period in time when the embryo is least accessible. This is also the time when the most important steps in cell determination and differentiation are occurring. For Martin then, the parallels between the normal and abnormal were essential for this research.

Evans' and Martin's work on ECCs initiated a trend amongst developmental biologists, who also elected to use ECCs; for example, Virginia Papaioannou and Richard Gardner both attempted to generate germ cell lines from ECCs (this line of research was not successful). What became clear however was that it was possible to manipulate ECCs in culture, then combine them with a developing blastocyst, to generate a chimeric mouse[726].

* * *

After becoming lecturer at UCL, and approaching the salary bar from lecturer to senior lecturer, Evans applied for a post in the Genetics Department at Cambridge University; eventually he was offered the post (and began working there in 1978)[727]. During his interview for the Cambridge post, Evans stated that his aim was to use mouse teratocarcinoma cells as a vector for the study of mouse genetics[728]. As early as 1972, Evans suggested how useful isolated (and easily cultured) pluripotent cells could be:

"It would be very useful if this process could be experimentally manipulated. Cell lines of this type should prove extremely useful in the studies of the control of cellular determination and differentiation."[729]

[725] Martin, 1980 p 769-70.
[726] Graham, 2000.
[727] Evans, 2001.
[728] Evans had the opportunity to make his point when in 1983, a collaboration between researchers at the Clinical Research Centre in Harrow, the Laboratory of Human Molecular Genetics in London and Evans at the Department of Genetics at the University of Cambridge led to a publication describing the use of immunoblotting to study glycoprotein production in differentiating cells. The cells used included several different EC and EK cell lines (Childs *et al*., 1983; Stacey and Evans, 1984).
[729] Evans, 1972 p 176.

This proved to be a useful move for Evans' goal as he met Matthew Kaufman, who showed Evans how to use delayed blastocysts, a technique that was then used to isolate ESCs (see below).

Once at Cambridge, Evans' interest in the use of biochemical markers to identify points of differentiation in ECCs continued. Evans' first work was with Ten Feizi, with whom Evans demonstrated that the main surface antigen on ECCs were carbohydrate epitopes of glycohalix[730]. Meanwhile, Peter Stern had moved to Brenner's laboratory (also at Cambridge), and produced an antibody against another cell surface glycolipid (named the Forssman antigen[731]). Evans saw the potential of this and used the antibody on ECCs and early embryos for comparison[732]. This complimented further work by Evans' PhD student Robin H Lovell-Badge (1953-), who established that many changes in protein synthesis occurred in the 12 hour period following embryoid body formation[733]. Lovell-Badge and Evans used two-dimensional electrophoresis to ascertain protein expression and post-translational modifications, which was important for two reasons: firstly, the method is capable of resolving large numbers of proteins at once, allowing very precise changes to be noted without first needing to identify which proteins to specifically observe. Secondly, this was a change from identifying differentiating cell types morphologically[734].

"…2D electrophoresis would seem to be a valid means of establishing homologies amongst different cells. Theoretically, it should be better than techniques that rely on morphology, histochemistry, or the detection of individual cell products, as it is monitoring a significant fraction of the genetic and epigenetic activity of the cells under comparison."[735]

This meant that differentiation could be identified far earlier in the process than had been achieved previously, and monitored in comparison to normal cells. Lovell-Badge and Evans used mouse ICM (inner cell mass) cells as controls. Since the 2D

[730] Kapadia, Feizi and Evans, 1981.
[731] Stern *et al*., 1978.
[732] Stinnakre *et al*., 1981.
[733] Lovell-Badge and Evans, 1980.
[734] Histology was still being used to identify cell types in teratomas. It was late in Stevens' career for example when he was an author on a published paper describing the usefulness of creating biochemical profiles of teratomas (Blüthmann *et al*. 1983).
[735] Lovell-Badge and Evans, 1980 p 202 (likely to have been written to justify the use of a new technique).

electrophoresis method was relatively new (first described in 1975[736] and modified by Lovell-Badge in 1978), few proteins had been specifically identified by the spot pattern produced.

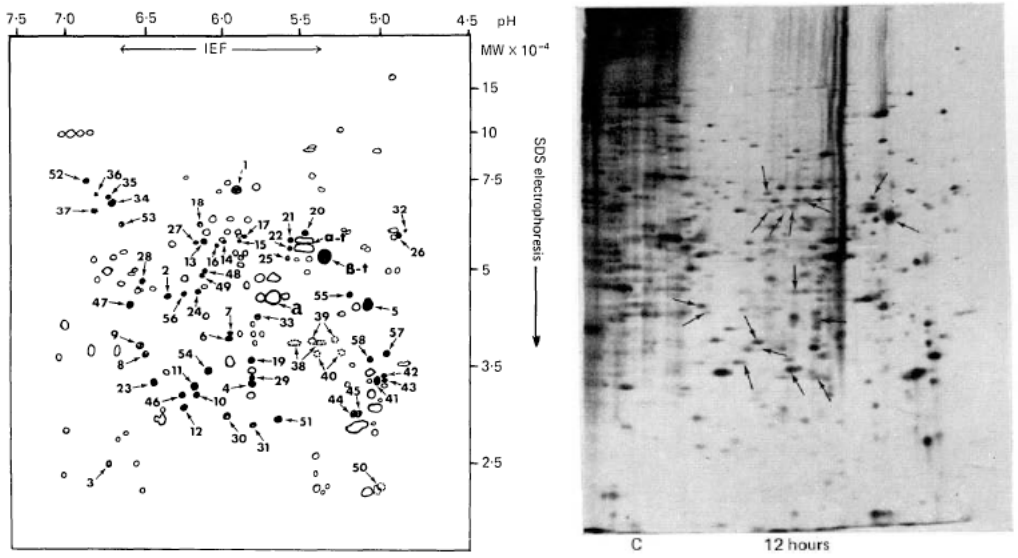


Figure 7: A 2D electrophoresis pattern from EC cells at 12 h post embryoid body formation, and a key to the numbers assigned to different spots. From Lovell-Badge and Evans, 1980.

This meant that few specific proteins were identified from this work (one example however was α-foetoprotein, tentatively identified as spot 1 in Figure 7). What the 2D electrophoresis could show however were differences in the protein profiles between cell types. Many proteins, required for regular cell function, will be expressed by all cells; some cells however have specialist functions which require production of specialist proteins. The differences in the proteins created by each cell type could be observed using the 2D electrophoresis technique. Therefore, as cells differentiated, slightly different protein profiles would result[737].

[736] O'Farrell, 1975.

[737] Lovell-Badge and Evans continued to describe the results by noting changes in spot appearance or movement between cell lines and times. It was also clear that there were few protein spots that appeared to change even between cell types – for example, Lovell-Badge and Evans also analysed fibroblasts using 2D electrophoresis, and when compared to ECCs, only approximately 5% of the detectible proteins differed between these cell types. (This said, Lovell-Badge and Evans noted that if a 2D gel resolves 1000 polypeptide spots of mostly 'abundant' and 'intermediate' proteins, extrapolation of the 5% may be as many as 400 proteins when post-translational modifications were considered.) It was also observed that the EC cells used may not be differentiated enough, as differences of approximately 25% had been noted between adult organ-specific cells and embryonic cells (Klose and Von Wallenberg-Pachaly, 1976).

In 1981, Evans published his first paper with Matthew Kaufman, who was working in the Anatomy Department at Cambridge. This was an extremely influential paper, published in *Nature*, describing the isolation and culture of pluripotent murine embryonic cells[738]. These were named 'EK' cells (after 'Evans' and 'Kaufman'). Early embryos were removed from mice pre-implantation, and cells from the blastocyst isolated and cultured, using Kaufman's 'delayed blastocyst' technique (Figure 8): removal of the ICM cells of the blastocyst before they developed to the egg cylinder stage. The ICM cells could then be disaggregated, separating the cells, prior to culture[739]. What Kaufman had done was to take a phenomenon that occurred normally in rodents, and develop this into an *in vitro* technique[740].

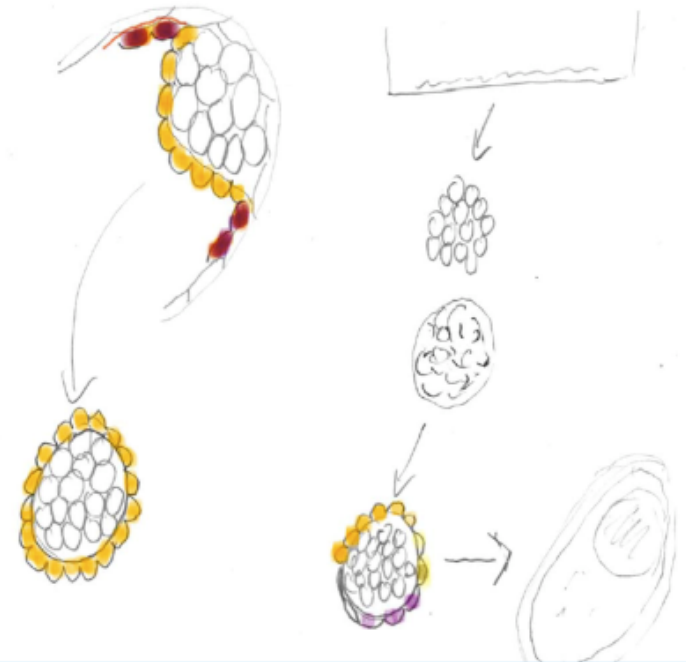
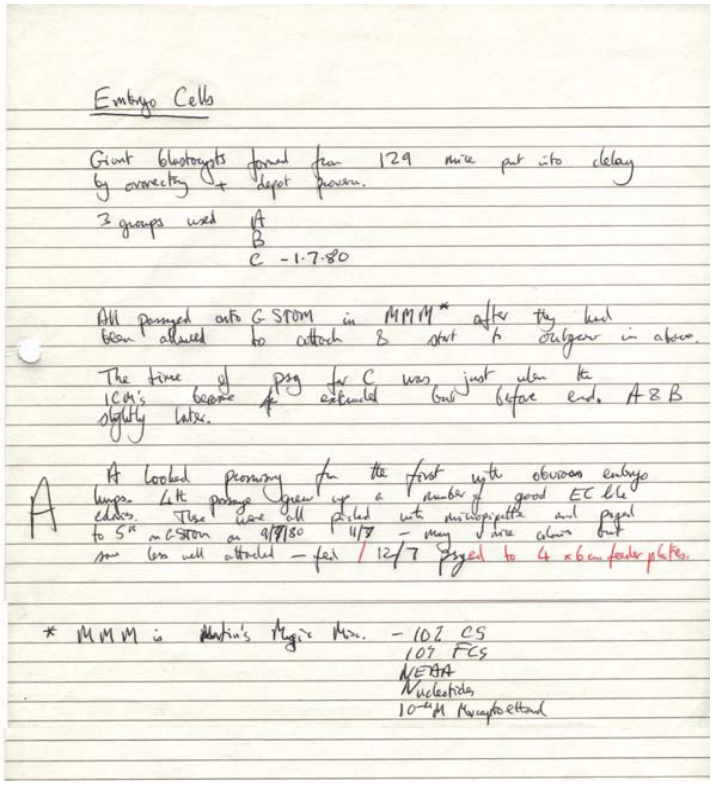


Figure 8: A sketch from Evans' laboratory notebook showing the isolation and culture of ESCs. From Evans' Nobel Lecture, 2007. Evans shows the removal of the ICM, their *in vitro* culture, and their re-aggregation and differentiation into an embryoid body.

[738] Evans and Kaufman, 1981. According to Google Scholar, this paper has been cited 7159 times (up to 8 September 2015).
[739] Kaufman *et al*., 1983.
[740] *In vivo*, this is referred to as diapause, allowing rodents to generate a new litter of fertilised eggs whilst remaining pregnant with a previously conceived litter. The newer batch arrest development at the blastocyst stage until the previous litter is born; the 'delayed blastocysts' can then implant and continue their development.

EK cells, obtained by this method, were defined as 'pluripotential embryo-derived' cells[741], and Evans and Kaufman claimed them to be more simple to use than ECCs; they could be grown entirely *in vitro*, and were capable of participating to normal development in chimaeric mice[742] (Figure 9). The cells had normal karyotypes, and could form embryoid bodies (a technique learned from ECC cells). It was hoped that further work may elucidate how teratocarcinoma cells could be 'transformed' into normal embryonic cells and tissues[743]. Arguably, this is a further example of the importance of the parallels between normal and tumerigenic development for research; techniques used in the fields of both developmental and cancer biology to demonstrate the properties of both.


Embryo Cells

Giant blastocysts formed from 129 mice put into delay by ovariectomy + depot progesterone.

3 groups used A
B
C - 1.7.80

All passaged onto G STOM in MMM* after they had been allowed to attach & start to outgrow in above.

The time of pssg for C was just when the ICM's became ~~4~~ extended but before end. A & B slightly later.

A looked promising from the first with obvious embryo like passage grew up a number of good EC like colonies. These were all picked into micropipette and passed to 5th in G STOM on 9/9/80 4/8 - many mice about but some not well attached - fed 12/7 passed to 4 x 6cm feeder plates.

* MMM is Martin's Magic Mix. - 10% CS
10% FCS
MEM A
Nucleotides
10^{-4}M Mercaptoethanol

Figure 9: Notes on growth of EK cells in culture, including a note on "Martin's Magic Mix" for culturing cells, and dates of media changes and passages (from July 1980). From Evans' Nobel Lecture, 2007.

[741] Kaufman *et al*., 1984 p 75. Evans later suggested that these were the same cells described by Martin as embryonic stem cells (Evans, 2001).
[742] Unpublished observations by Robertson, Kaufman and Bradley, as described in Kaufman *et al*., 1984. These observations were published as Evans *et al*., 1985.
[743] Kaufman *et al*., 1984.

Gail Martin was also continuing her work on ECCs in San Francisco. Just as Martin had been one of many American early career researchers to move to Europe for research experience in the early 1970s, she also followed the trend of many researchers in Britain moving to North America, which began only a few years later. Martin may have been one such researcher; however, her husband's work in England would have also had a significant effect on her decision to move. This general movement, Graham suggests, was due to lack of adequate funding, and something of a bottleneck in career progression. UK mammalian biologists moved to North America and continental Europe, following the potential of funding for their research. Those staying in the UK found that research priorities were moving away from development, and more towards care for an aging population[744] (following on from practical concerns regarding over-population).

In December 1981, Martin published a paper describing the development of a teratocarcinoma cell line which, when injected into an adult mouse, gave rise to a teratoma. When injected into a blastocyst, the cells contributed to normal development of a mouse (Figure 10).

[744] Graham, 2000.

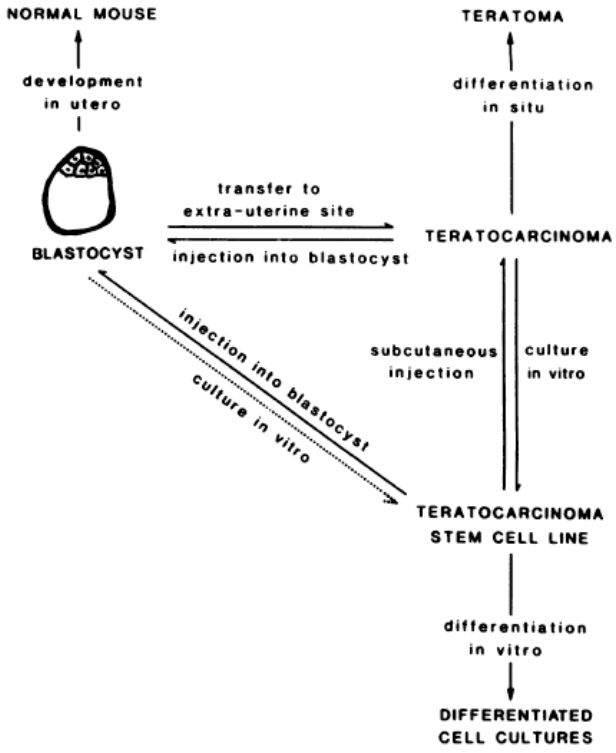


Figure 10: The relationship between normal embryos and teratocarcinoma stem cells. From Martin, 1981. Here, Martin shows how teratocarcinomas and the teratocarcinoma cell lines were utilised in several ways, creating differentiated cells (*in vitro* and *in situ*), and their ability to contribute to normal development after injection into blastocysts.

Martin referred to the important publication by Evans and Kaufman, published earlier the same year. Evans and Kaufman had used the same strain 129 mice as in Stevens' experiments. Martin created her own cell line by using a feeder layer[745] and conditioned media for the first five passages, naming these cells embryonic stem cells (ESCs) to denote their embryonic origin. The cells were also genetically tested to ensure that they had not been contaminated by ECCs (as the two appeared to be morphologically similar). The ESCs were able to produce teratomas (containing all three germ layers) when implanted into mice, and could produce clones (although this ability was reduced to only around 10% of that observed in ECCs). These clones could become teratocarcinomas when implanted, again differentiating into several cell

[745] 'Feeder layers' are cells (such as fibroblasts) that are cultured alongside other cells. These fibroblasts (often irradiated to prevent proliferation) produce proteins which 'condition' the media, providing the co-cultured cells with, for example, cytokines essential for growth.

types, demonstrating the pluripotency of these cells. Differentiation could also be induced *in vitro* and protein markers of stem cells were shown to be present. Martin highlighted that the method she described, as well as the alternative described by Evans and Kaufman (1981), negated the requirement for producing a tumour from an embryo *in vivo* to isolate pluripotent stem cells. Furthermore, Martin noted that the development of this method would allow for production of mESC lines with specific genetic alterations, providing an additional research tool for mammalian biologists, in particular those interested in development.

Importantly, both Martin (1981) and Evans and Kaufman (1981) described methods for the culture of ESCs which no longer required tumour induction. It is possible that this is indicative of research moving on from the parallel paradigm in the laboratory. Martin highlighted that ESCs would now be available for genetic manipulation, something also mentioned by Evans and Kaufman. In his discussion published in 2006, the immunologist Davor Solter concluded that the isolation and culture of ESCs in the early 1980s was most widely perceived as a new tool for gene function analysis. Solter continued to suggest that the application of ESCs for therapeutics and regenerative medicine had not yet been realised; it was only once this happened that a wider scientific community and the general public became aware of these cells[746]. As previously mentioned, Evans was aware of the usefulness of ECCs in the study of embryonic development as early as 1972[747]; it seems realistic that Evans would also have realised the same potential for ESCs. He may not however have realised the potential therapeutic benefits of such cells just yet. In part, this may have been because Evans is a scientist as opposed to a clinician, or that he did not have any significant links with clinical research, which may have made him more aware of potential therapeutic benefits.

3. Science in the UK and USA: 1982-1989

As previously hinted at, there was an economic slow-down in the later 1970s; this peaked at the turn of the decade, and had significant global impact. Both British PM Thatcher and US President Reagan needed to reduce government expenditure. This set the tone for science in the UK and USA over the next decade.

[746] Solter, 2006.
[747] Evans, 1972.

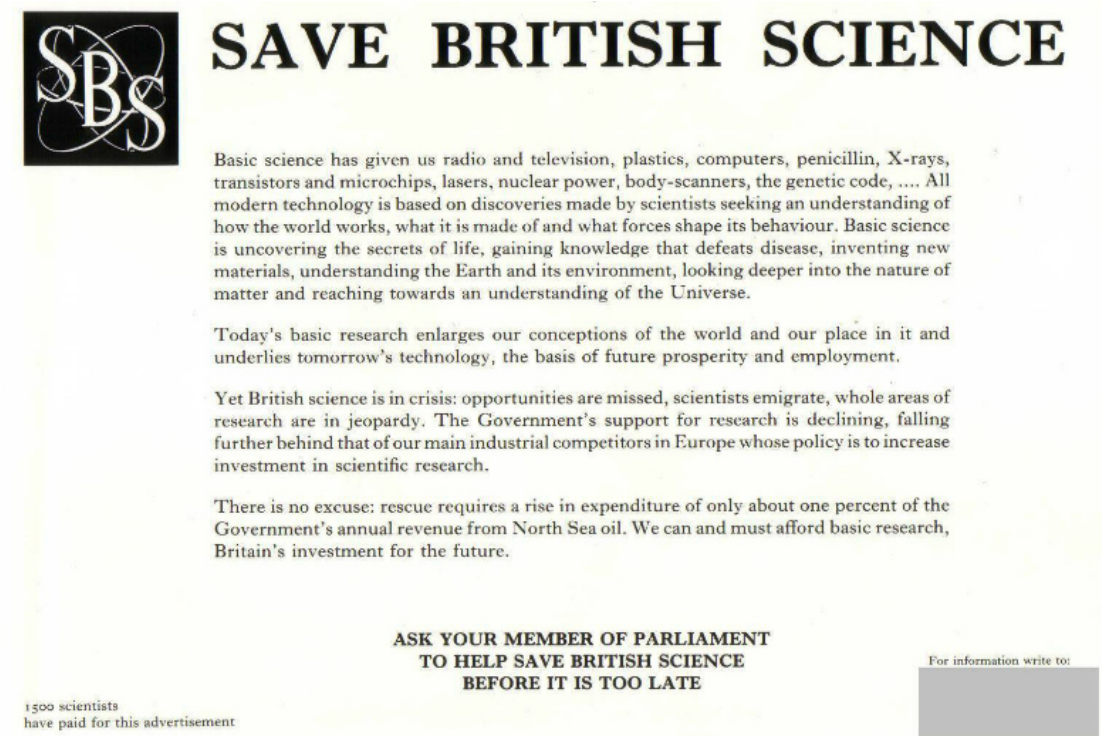
3.1 UK science: 1982-1989

In the UK, Thatcher did not explicitly state that science funding would be cut; science funding did however fall into a category of government-supported activities that would be financially affected by a global recession. Such activities were supported by 40% of gross domestic product (GDP) in 1981, falling to 29% by 1988[748]. Regarding scientific endeavor specifically, Thatcher would comment – being a trained chemist herself, this is unsurprising: Thatcher stated that scientists needed to contribute to social and economic needs. Any funding for research had to represent value for money, meaning that research grants were now more often handed out to those with specific applied outcomes. This did not mean that there was no money for other research at all however; some of the deficit was made-up by other sources of funding, including charities and industry. There was a catch here though: unlike government grants, such funding would not provide new or improved research facilities[749]. A more politicised perspective of science was developing in the UK. Developmental biologists had learned to describe their work in this framework by, for example, deferring to the breakthroughs of IVF, and highlighting the increasing importance of understanding congenital abnormalities. As Graham described it, "mammalian developmental biologists had learned to explain themselves"[750]. Such a skill was key under a Thatcher government keen to emphasise science links with industry and economic output.

The decline of funding for scientific research in the 1980s led to concerns in the scientific community - how would this affect the country's future prosperity, and, more immediately, would they be able to keep working as research scientists[751]. In response to this, a group of researchers thought an advertisement in *The Times* might help to alert the general public to the plight of science. In order to pay for this advertisement, the small group solicited scientists from around Britain to contribute, and contribute they did. On 13 January 1986, a half-page advertisement appeared in *The Times* asking readers to encourage their MPs to 'save British science' (Figure 11). The generosity of the scientific community not only paid for this advertisement

[748] Braun, 1993 p 270.
[749] *ibid*.
[750] Graham, 2000 p 54.
[751] Weir, 2014.

and a press-conference[752], but the founding of a 'Save British Science' (SBS) movement[753]. Politicians of the time claimed that the movement became useful for them in understanding research and establishing scientific policy; the SBS movement were not simply a group of 'whinging scientists', but were persuasive and informative[754].



SAVE BRITISH SCIENCE

Basic science has given us radio and television, plastics, computers, penicillin, X-rays, transistors and microchips, lasers, nuclear power, body-scanners, the genetic code, ... All modern technology is based on discoveries made by scientists seeking an understanding of how the world works, what it is made of and what forces shape its behaviour. Basic science is uncovering the secrets of life, gaining knowledge that defeats disease, inventing new materials, understanding the Earth and its environment, looking deeper into the nature of matter and reaching towards an understanding of the Universe.

Today's basic research enlarges our conceptions of the world and our place in it and underlies tomorrow's technology, the basis of future prosperity and employment.

Yet British science is in crisis: opportunities are missed, scientists emigrate, whole areas of research are in jeopardy. The Government's support for research is declining, falling further behind that of our main industrial competitors in Europe whose policy is to increase investment in scientific research.

There is no excuse: rescue requires a rise in expenditure of only about one percent of the Government's annual revenue from North Sea oil. We can and must afford basic research, Britain's investment for the future.

ASK YOUR MEMBER OF PARLIAMENT
TO HELP SAVE BRITISH SCIENCE
BEFORE IT IS TOO LATE

For information write to:

1500 scientists
have paid for this advertisement

Figure 11: The 'Save British Science' advertisement from *The Times*, 13 January 1986.

Thatcher's neo-liberal government of the 1980s also made some steps towards allowing professions more economic freedom; this included looking more carefully at market lines and resulted in many professions becoming more accountable to the 'end user'. With regard to tissue culture for example, some patients were becoming wise to the idea that their doctors may be removing tissue, culturing it in the laboratory, then selling it on. The Thatcher government in fact encouraged such commercial incentives. From this point of view then, it can be argued that the Thatcher

[752] Much to the organiser's frustration, coverage of the press conference was relatively muted; on the same day, Michael Heseltine resigned from his position in the Cabinet, a story that trumped saving British science.
[753] Weir, 2014.
[754] Weir, 2014. In 2005, SBS became CaSE (Campaign for Science and Engineering), which continues to be an influential communication group between scientists and politicians.

government determinedly introduced austerity measures concerning science funding. This deliberate policy decision was made in order to encourage a particular type of research: that which would contribute to economic growth[755].

Despite this, and all of the concern over biotechnology advances through the 1970s, by the late 1980s there was still relatively little legislation concerning tissue from living donors (which, in part, included embryos), since the 1961 Human Tissue Act did not cover these issues[756]. Faced with this, entrepreneurial doctors were advised to get signed consent forms from their patients prior to removing tissue which may later be cultured in the laboratory[757]. In 1990, professor of law, Douglas J Cusine, claimed that researchers had been working almost in 'legal darkness' for several years, with a potential for several more; although the Warnock Committee's advice regarding licensing was deemed appropriate, Cusine highlighted that it not only needed to be in place, but it must be clearly shown to be operating. Time and interest in such matters for parliament was also thought to be a problem, which again reduced the probability of providing up-to-date legislation concerning recent developments in biotechnology.

It has been argued that the Thatcher government policies of the 1980s had greater impact on the biomedical sciences than those of the Reagan administration. It is possible that this was due to Thatcher's attempt to re-distribute research funding, making less available generally. This, the government would argue, would increase competitiveness, but therefore also increase selectivity, encouraging funds to be distributed more into applied than pure research. This change was not so profound in the USA, where the move towards support for applied research was more subtle. Essentially, the UK biomedical researcher had to adapt to an entirely different funding approach, whereas American researchers only needed to work with fewer resources[758].

In contrast to the significant amount of funding US universities obtained from the NIH (see below), the British equivalent, the MRC, provided around 12% of funding in 1985/6. Another 15% was provided by the Universities Funding Council

[755] Braun, 1993 p 272.
[756] Brahams, 1988.
[757] Wilson, 2011.
[758] Braun, 1993 p 272.

(UFC), which provided block grants to universities to administer how they wished[759]. The UFC faced stagnation of its funds through the 1980s. The MRC was less affected, however was unable to make up for the UFC shortfall. In addition, no indirect costs could be paid for out of MRC grants, placing more strain on a university's UFC income. Research facilities became insufficient (based on the swiftly expanding technologies and techniques available to biomedical researchers), and the MRC was therefore rejecting funding applications based on sub-par facilities.

The MRC was under pressure concerning what grants to approve from two external groups – the government providing the money, and the researchers applying for it. The highlighted government policy on scientific research strongly encouraged grants which would result in practical outcomes for economic growth. Not all researchers however would apply for funding based on this principle. Researchers and Research Councils were not autonomous enough to resist the political pressure. Unable to avoid government policy, UK Research Councils concentrated their resources into selected groups and laboratories. This resulted in a significant competitiveness that was introduced into British research science[760].

In Britain, the results of austerity measures taken through the 1980s resulted in researchers working more in larger teams (rather than individually), which often meant researchers working on projects not of their own design or interest, and becoming more interdisciplinary[761].

3.2 USA science: 1982-1989

It was not only in the UK where there were concerns over research funding; the recession that had hit the UK in the late 1970s and early 1980s was also apparent in the USA. Initially, the USA appeared to suffer less from funding reduction than the UK. Basic research, particularly anything related to defence, was still relatively well-funded until the mid-1980s. Reagan made an attempt to reduce spending in biomedical research, but, being a favourite of Congress, funding remained. In fact, federal funding for biomedical science increased by, on average, 3% per annum between 1981 and 1987[762]. As previously mentioned however, the demands of

[759] *ibid* p 273.
[760] *ibid*.
[761] *ibid*.
[762] *ibid* p 271.

biomedical science were increasing, and federal funding was still insufficient. Reagan took similar views to Thatcher concerning promoting economic growth, and also encouraged marketisation of professions with a look towards impressing the end user. Federal scientific funding was significantly less than researchers would have wanted. This had the effect of providing private funders with greater influence, as well as giving researchers somewhere to obtain funding from if they wished to work in an area of research not funded by federal money. Biomedical sciences did not tend to be in this group however; the NIH funded a significant amount of biomedical research in the 1980s – 75% of federal grants for biomedical research were made through the NIH[763]. This was very important, since the NIH allowed indirect costs to be built into grant applications (for example, building maintenance, heating costs). Post-World War II, many US universities were reliant on such grants for their indirect costs; indirect costs came to 21% of total costs in 1970; by 1988 this had increased to 31%[764]. The average size of research grants (at constant prices) between 1980 and 1989 increased from $97800 to $113900[765].

Despite some restraints on the amount of funding the NIH was able to provide, it did manage to retain funding for a significant number of projects designed out of intellectual curiosity – in fact the number of such projects funded from the NIH's total was 44% in 1970, increasing to more than 60% in 1987[766]. The NIH reduced the amount of funding it made available elsewhere (such as for training) to remain able to fund such basic research. This is a stark contrast to British research that particularly struggled to obtain funding for anything that did not immediately appear to have a practical outcome.

Through the 1980s, the Department of Health and Human Services continued to withhold approval for any therapeutic research making use of foetal tissue or cells, even though the NIH had proposals submitted that suggested carrying out such work. In response, the NIH established the Human Fetal Tissue Transplantation Research Panel in 1988, which voted to allow federal funding of therapeutic research 18 votes to 3. The Secretary for the Department of Health and Human Services, Louis Wade Sullivan (1933-), however agreed with the three panel members that believed such a

[763] *ibid* p 273.
[764] *ibid* p 278.
[765] *ibid* p 278.
[766] *ibid* p 279.

move would encourage abortion. With this decision, Sullivan extended the moratorium on federal funding for embryo research[767].

In April 1991, the American Association for the Advancement of Science (AAAS) held a conference to determine what had happened to funding during the previous decade, and to discuss what the Bush administration could do in response. It appeared that a significant amount of the discussion highlighted mistrust between scientists and the government, and *vice versa*. Scientists believed they did not have the opportunity to apply for appropriate funding for their research, whilst politicians believed that the scientific community were incapable of spending limited federal funds responsibly. Increasing numbers of fraud stories had been highlighted in the press, and in particular the misconduct of David Baltimore and Thereza Imanishi-Kari were considered damaging[768]. This was not to say that there were no advancements made in 1980s America; for example, the Human Genome Project was funded by Congress in 1986, initially with James Watson (1928-) as Director.

Concerns regarding animal welfare were also apparent in the USA. The first Animal Welfare Act was enacted in 1966, with amendments in the 1970s; in 1985 however, the Act included the Improved Standards for Laboratory Animals Act. Prior to this, Congress had discussed animal testing, and highlighted that: 1) some animal experimentation was necessary for research and education advancing knowledge and treatment for human and animal disease; 2) that alternative methods were continuing to be developed; 3) that measures should be taken to eliminate or minimise the unnecessary duplication of animal experiments (resulting in more productive use of federal funds); and 4) that measures should be taken to help meet the public concern for the welfare of laboratory animals whilst allowing research to progress[769]. Such an Act had implications for biomedical research and pushed new technologies towards finding alternatives to animal testing; this was particularly the case regarding federal

[767] Wertz, 2002.
[768] Abelson, 1991. Specifically on the case of Baltimore and Imanishi-Kari, see Kevles, 2000. Imanishi-Kari had been accused of misconduct when another researcher, Margot O'Toole, was unable to replicate her results. Baltimore was Imanishi-Kari's superior and co-author, and it was suggested that Baltimore should have supervised Imanishi-Kari's work more closely, particularly if he was listed as an author on her publications. Since Imanishi-Kari's work had been funded by the NIH, the case was discussed by the United States Congress, gaining worldwide attention.
[769] United States Department of Agriculture, n.d.

funding, such as through the NIH; the NIH provided $3423 million in grants in 1980, which had increased to $7145 million by 1989[770].

The American biomedical scientist, like their British counterpart in the 1980s, had to deal with increased competitiveness for funding. Many secured funding by opting to propose topics known to be favoured, and those where results could be produced in a relatively short amount of time[771].

The continuous struggle to obtain grant moneys for research had a significant effect in the UK and USA through the 1980s. Not only were researchers forced into proposing research that fit into government policy frameworks, they were also forced to compete on a much higher level than before. Such competition led to rivalry, jealousy, and mistrust between research groups, peer reviewers, assessors, and referees. This inevitably led to a reduction in range and quality of scientific endeavor[772]. Reduction of grant money availability occurred across all research sectors, including developmental biology. This therefore would have affected the type of grant proposals submitted for ESC research, and increased the rivalry between competing researchers.

4. UK: Using embryonic cells to create genetically engineered mouse models of disease (GEMMD)

After establishing the germline potential of his EK cells, Evans considered options for their mutagenesis. In 1983, Richard Mann, Richard Mulligan and David Baltimore at the Whitehead Institute for Biomedical Research (Massachusetts) and the Center for Cancer Research at MIT, published a paper describing the use of retroviral vectors for such work[773]. Inspired by this, Evans organised a visit to Mulligan's laboratory for a month in October 1985[774]. Despite several attempts by other scientists to tempt him away from the bench, Evans made a concession for only one: Oliver Smithies[775]. Smithies had just published work describing gene targeting by homologous recombination *in vitro*; interested, Evans took samples of his EK cell

[770] National Institutes of Health, Office of Budget, n.d.
[771] Braun, 1993.
[772] *ibid*.
[773] Mann, Mulligan and Baltimore, 1983.
[774] Evans, 2001.
[775] Nobel Foundation, 2007.

cultures to Smithies in Wisconsin for a weekend. Smithies introduced Evans to PCR (polymerase chain reaction) and his thermal cycling machine[776]. Mario Capecchi, then at the University of Utah, visited Evans for a week at his laboratory in Cambridge, with the aim of collecting cells and learning the associated techniques[777]. Evans conceded that PCR often now only warranted a few lines in experimental genetics papers, but it allowed almost any mouse mutation to be specifically targetable, leading to work in experimental genetics "illuminating our understanding of the mammalian genome physiology and human function in health and disease"[778].

In 1989, Evans published a review describing the use of ECCs and chimaeric animals in the context of rising technologies of genetic manipulation. Evans highlighted that "new genetic technology is likely to have a major impact both in genetic studies and, especially if it can be extended to larger mammalian species, in practical applications"[779]. These 'larger species' appeared to extend rapidly to pigs, as pluripotent ECCs were isolated and cultured, described in a paper published in 1990 by Evans' group at Cambridge. Such methods appear to have been at the forefront of Evans' work, since he also published new methods and 'technical tips'[780]; only a year later Evans' group described isolation and culture of sheep ECC lines[781]. In the 1990s then, ECCs (i.e. tumourigenic cells) were considered useful enough to warrant the research into larger mammals – apparently parallels between tumourigenic and normal cells was still in place.

It was later in 1991 that Evans moved department, after helping to secure funding and set up the Wellcome/CRC Institute for Cancer and Developmental Biology at the University of Cambridge[782]. The Institute had been set up in 1989 to 'promote research in the areas of developmental biology and cancer biology', allowing researchers to integrate and share information and expertise[783]. Such potential appears to have been realised: later in 1991 a group of researchers from the MRC National Institute for Medical Research, Imperial College, and the University of

[776] This interest eventually even led Evans into becoming a co-founder of the biotechnology company Animal Biotechnology Cambridge Ltd.. Nobel Foundation, 2007.
[777] Evans, 2001.
[778] Evans, 2001 p 1083.
[779] Evans, 1989 p 557.
[780] Chan and Evans, 1991.
[781] Notarianni *et al*., 1991.
[782] Nobel Foundation, 2007.
[783] Gurdon Institute, 2015.

Cambridge (including Evans) published work describing activation of specific genes in mice during ECC differentiation *in vivo* and *in vitro*[784].

Importantly to Evans, such work could have practical uses; the first hints of such research for medical application began to appear. Kuehn *et al*. (1989) (a group including Evans at Cambridge) published a paper in *Nature* describing the development of a mouse model for the human neurological disorder Lesch-Nyhan syndrome[785]. Using genetic manipulation, ESCs were modified and chimeric mice produced from these cells. Some gave rise to germ cell chimeras, allowing mutant mice to be bred, and a murine model of Lesch-Nyhan syndrome to be developed. Later, in collaboration with the Department of Biochemistry and Molecular Genetics at St Mary's Hospital Medical School, a group led by Evans at the Wellcome/CRC Institute established that the cystic fibrosis transmembrane conductance regulator gene (*cftr*) could be disrupted in ESCs using gene targeting. At the time, no animal model of cystic fibrosis was available, despite it being the most common autosomal recessive genetic disorder (affecting 1 in 2000 of the Caucasian population)[786]. The group were able to disrupt the gene in ESCs, and produce chimeras from these cells. In summary, the group announced their intention to continue their work in order to produce a cystic fibrosis mouse model, to enable further research into the pathophysiology of the condition, as well as make use of the model as a further way of testing potential new therapeutic drugs. This research was a collaboration between

[784] Poirier *et al*., 1991.

[785] Although Martin has referred to her isolated calls as embryonic stem cells (or ESCs) from her initial publication in 1981, Evans appears to have resisted this. Papers published by Evans through the early and mid-1980s still refer to EK cells – the term coined in Evans and Kaufman 1981. The 'EK cells' term can be seen in, for example, *Influence of injected pluripotential (EK) cells on haploid and diploid parthenogenetic development* (1984), and *The ability of EK cells to form chimeras after selection of clones in G418 and some observations on the integration of retroviral vector proviral DNA into EK cells* (1985). By the late 1980s, Evans had started to refer to these cells as ESCs. We can identify that in these later publications, Evans is referring to the same cells despite the change in terminology. For example, in *Expression of v-src induces aberrant development and twinning in chimaeric mice* (1989)**,** the following phrase is included: "In order for this approach to be successful, the *v-src* gene must be introduced and expressed *in vivo* both efficiently and reproducibly, and consequently we have chosen to use embryonic stem (ES) cells (Evans and Kaufman, 1981; Martin, 1981) as a means of effecting ectopic expression in the embryo. ES cells provide a powerful tool for analyzing mouse development, since they are developmentally pluripotent, capable of contributing to many cell lineages, including the germ cells, on their introduction into the embryo…and are amenable to genetic manipulation in culture" (Boulter *et al*., 1991, p 358.). This then demonstrates that the EK cells referred to in the earlier 1980s, were the same as the ESCs referred to later on.

[786] Ratcliff *et al*., 1992.

biological and clinical researchers, showing that whilst individuals tended to remain either as biologists or clinicians, collaborative research enabled applied biology to be directly applicable to medicine. In 1995, alongside the research group leader from St Mary's Hospital Medical School, WH Colledge, Evans published a review of gene therapy for cystic fibrosis. The publication reviewed the current thoughts and possibilities for suitable vectors and gene delivery mechanisms, and the first results from clinical trials[787].

In 1980s UK then, there was a definite move in research direction towards practical application; those involved in ESC research had to look towards potential medical application for their work by clinical collaboration. This affected the way ESC research was promoted and funded, with an emphasis on the practical, medical application, as opposed to pure biology ('fact-finding') research. Evans possibly ensured funding by collaborating with cinical researchers, clearly showing the practical applications of his work.

5. USA: Using embryonic cells for developmental biology

An examination of the papers published by Gail Martin and her laboratory at the UCSF between 1981 and 1989 reveals how Martin began utilising ECCs and ESCs for research into mammalian development. This selection of research direction can be seen soon after Martin returned to the USA.

In 1982, Martin and co-workers published a paper concerning a lethal mutation at the *t*-locus, which is known to cause death at approximately 9 days post-fertilisation (this is due to morphological problems in the ectoderm)[788]. The authors noted that previously, lethal *t*-mutations had been difficult to study due to the lack of available material; the method Martin described in her 1981 paper however allowed the authors to establish "pluripotent stem cell cultures" from murine blastocysts[789]. Using careful breeding, the authors were then able to use Martin's method to create cell lines from mice that were homozygous and heterozygous for the lethal mutation (t^{w5}). That the authors were able to establish a cell line immediately indicated that t^{w5}/t^{w5} alone did not result in general lethality. The creation of the t^{w5}/t^{w5} cell line using Martin's method demonstrated the importance of the technique; it allowed new

[787] Colledge and Evans, 1995.
[788] Magnuson *et al*., 1982.
[789] *ibid* p 750.

approaches to be investigated concerning the role of lethal and mutant alleles on development.

This did not mean that Martin abandoned her use of teratocarcinomas altogether however, and still made use of them throughout the 1980s. Observing ECC aggregate differentiation, Martin and her co-authors examined the expression of factors known to be associated with embryonic development. Applying inhibitors to the cell aggregates caused changes in the differentiation process, enabling the authors to understand the role of a particular factor (*N*-asparagine-linked glycoproteins) in the formation of endoderm layers in the developing embryo[790]. This is another clear example of results observed from pathological cells being applied to normal development.

Furthermore, Martin would make use of her teratocarcinoma stem cells to create chimeras - mouse embryos that would develop from a mixture of 'normal' embryonal cells and ECCs. Martin's preliminary work on chimeras established that they could be created and live births achieved, but a significant number of normal cells needed to be present for this to occur. Larger numbers of embryonal carcinoma cells present in the chimera were unable to support normal development. Martin hoped that such work would eventually lead to better understanding of the differences between ECCs and ESCs[791], although this work again also clearly showed that there were some recognised similarities between normal and abnormal cells.

Martin also shrewdly used her cells and techniques to examine the similarities between murine and human development. In 1985, Martin's laboratory published a paper comparing the human homeo-box (*Hu1*) and the mouse homeo-box (*Mu1*) genes (these sections of DNA had previously been demonstrated to have a 4kb 90% homologous region)[792]. Martin's laboratory differentiated human teratocarcinoma cells *in vitro*, and observed that levels of *Hu1* expressed increased. Using the same experimental conditions for the murine teratocarcinoma cells did not result in a similar increase however. Instead *Mu1* expression was observed to increase in embryos, between 10 and 17 days post-fertilisation. This type of research, Martin claimed, was useful in helping to understand the role of homeo-box genes during embryogenesis. Martin's laboratory also carried out similar work observing

[790] Grabel and Martin, 1983.
[791] Fujii and Martin, 1983.
[792] Hauser *et al*., 1985.

expression of two similar genes, called *engrailed*. Again, *engrailed* was observed to have a role during mouse embryogenesis, which had been previously shown to be the case in *Drosophila*[793]. As with *Mu1* and *engrailed*, Martin's laboratory examined the role of another gene during embryogenesis, utilising teratocarcinoma cells. N-*myc* had been shown to be expressed in neuroblastomas, however Martin's laboratory also demonstrated that N-*myc* was expressed in teratocarcinoma cells and normal embryo cells during development[794].

Returning to her work on the *t*-locus, the Martin laboratory carried out more research on other *t*-mutations, establishing that some alleles were important for survival of the early mesoderm, whilst others were lethal. Martin commented that "The results of this study are useful…in providing a unique source of experimental material…The technique of ESC isolation from mutant homozygous embryos provides a means of circumventing the difficulties inherent in accumulating sufficient numbers of mutant embryos for experimental studies. The cell lines obtained provide an essentially unlimited source of mutant homozygous DNA, as well as an abundant source of proteins and mRNAs"[795].

Although Martin (and Kaufman and Evans) had provided techniques for creating embryonic cell lines in 1981, the extract above from the last paragraph in a 1987 paper has a tone indicating Martin is still having to sell the idea of using such cell lines to other researchers. In the midst of 1980s pressure to reduce the number of laboratory animals in the UK and USA, Martin's demonstration that cell lines were particularly useful where animal models were difficult to obtain (or, in the case of a lethal genetic mutation, would not exist) seems particularly valid. In particular, many of Martin's publications through the 1980s indicate that her laboratory was funded in part by the NIH; such funding may not have been as forthcoming if Martin had decided to rely only on animals for her developmental research; likewise, Martin would not have made the knowledge advances (such as those highlighted here) if she had not made use of cell lines and relied on mouse embryos.

6. Influences on stem cell research: UK and USA, 1970-1989

Historian of biology Jane Maienschien has argued that cell biology and

[793] Joyner *et al*., 1985.
[794] Jakobovitis *et al*., 1985.
[795] Martin *et al*., 1987 p 27.

embryology in 1970s and 1980s America had some emphasis on 'engineering'; a focus that had been in place since the early twentieth century, particularly following the work of physiologist Jacques Loeb (1859-1924). Experimental work, claimed Maienschein, "focussed on research first, and applications later"[796]. I agree that research directions that formed in the earlier decades of the twentieth century in the USA had an effect on research carried out towards the end of the century.

As mentioned in Chapter 1, there was a clear goal in early twentieth-century American cell biology in the elucidation of cell lineage. Prominent biologists such as Edmund Beecher Wilson and Edwin Grant Conklin believed that examination of cell lineage during embryogenesis would help with establishing understanding of fundamental biological processes. Conklin in particular began his studies at the early stages of embryo development, studying cleavage patterns of blastomeres. As well as being technically complex, there were difficulties disseminating the data produced (since many diagrams and plates needed to be included to show what was happening)[797]. Similarly, Wilson played his part in cell lineage studies by looking at cell structure (Chapter 1). The middle decades of the twentieth century saw significant improvements in methods to identify and differentiate between cell types (such as improved microscopy and techniques in genetics), and new tools for use in cell biology[798]. Both embryology and cell aging were important areas of research in the 1960s, following on from the cell lineage studies established decades earlier. "The major difference", Maienschein argued, "between 1981 [isolation of murine embryonic stem cells] and 1998 [isolation of human embryonic stem cells] was not the extension of the techniques to human cells, but the advances in understanding the underlying genetic basis for development"[799]. From the work carried out in the USA by Martin, it is clear that she is part of this tradition. Similarly, Michael Morange has noted that human embryonic stem cells and murine embryonic stem cells are not equivalent considering the "motivations and goals attendant on their creation"[800]. (This will be examined in detail in Chapter 5.)

It is also important to consider the financial support available. As noted on Martin's publications, much of her work in the 1970s and 1980s was funded by the

[796] Maienschein, 2014 p 176.
[797] Laublicher and Maienschein, 2003.
[798] Maienschein, 2014.
[799] *ibid* p 208.
[800] Morange, 2006 p 540.

NIH and the American Cancer Society. As historian of biology Christina Brandt has highlighted, there was significant funding available in the 1970s for cancer research, although the mouse model that was used for much of this also had uses in developmental biology, genetics, embryology, and cell differentiation studies through the 1970s as well. The earliest ESC work that began in the 1970s was a result of improved techniques in embryo and cell culture and manipulation, and cancer research[801]. Teratocarcinomas were the cells that would bridge the gap between cancer and embryology; Martin was aware of this as early as the mid-1970s, when she wrote that embryonal carcinoma cells could be used for comparison with embryonic cells, and in the creation of chimaeras[802]; Ralph Brinster was also starting to create chimeras with teratocarcinoma cells at this time[803].

It can also be argued that there was a shift in stem cell studies between the early and late twentieth century. Late nineteenth and early twentieth century studies (as described in Chapter 1) were preoccupied with establishing the 'natural history' of cells and organisms, looking for the cells from which organisms could arise, and how this would support (or not) evolutionary theory, ontogeny and phylogeny. By the latter decades of the twentieth century however, less concern was given to the origins of stem cells, but their potential. There appears to be a shift in research focus, looking at what stem cells were capable of moving forwards in time (i.e., development, differentiation potential, etc.), as opposed to using stem cells to look backwards.

This chapter has not only demonstrated that the 1970s and 1980s were a changing era for biomedical science; it also shows the outcome of a debate occurring in the philosophy of science from the same time. In the 1970s and 1980s, some philosophers of science were split into two camps: the internalists and the externalists. By the mid-1980s, it has been argued that most philosophers of science were more convinced by the externalist argument[804]. Whilst internalists agreed that science existed within frameworks of social convention and political institutions, what was found was only that which could be seen in nature. The externalists showed that far from only existing in social and political frameworks, science and its constructions were actively influenced by it. Researchers learned to describe their research in

[801] Brandt, 2012.
[802] Martin, 1975.
[803] Brinster, 1974.
[804] Jacob, 1992.

particular ways, and refer to outcomes (or expected outcomes) using terminology that would explicitly hint at potential economic or therapeutic application. This chapter has shown that the work by Martin and Evans (and others) was directly affected by, for example, governmental and institutional policy, funding availability, and discipline genealogies. What was reported as outcomes (or potential outcomes) had clear, practical uses in knowledge creation in medical research (Evans) and biological research (Martin).

7. Conclusions

This chapter has examined the direction of life sciences research in the UK and USA through the 1970s and 1980s via the work of two prominent stem cell researchers: Martin J Evans and Gail R Martin. In particular, it has asked what encouraged and discouraged the work of Martin Evans (in his creation of GEMMD) and Gail Martin (research on development). The chapter has argued that research traditions, as well as global and local politics and economics has had a role.

Evans remained in the UK throughout the period covered by this chapter, concerning himself firstly with how newly-isolated teratocarcinoma cells could be used, developing an ESC line, then utilising this to create mouse models of disease, and later collaborations with clinical researchers. In contrast, Martin began her postdoctoral career in the 1970s alongside Evans in the UK, before moving back to the USA to develop her own laboratory. By the late 1970s, it was becoming clear that although useful, it was unlikely that ECCs could be considered 'normal' enough for them to be a good equivalent for embryonic cells, and that isolation and culture of embryonic cells would be especially useful for future research. Clearly, this is a further example highlighting the parallels between ESC and cancer research, as previously demonstrated in Chapter 3. Independently, both Martin and Evans developed a method of isolating embryonic stem cells.

In the mid-1970s, Evans claimed he described a goal to use mouse teratocarcinoma cells as a vector for studying mouse genetics (this was in his interview for a post at Cambridge). This appears to be an updated version of his 1972 suggestion that cultured pluripotent teratocarcinoma cells could be experimentally manipulated to study control of cell differentiation and determination. In the early 1970s then, Evans had a clear interest in developmental biology and interpreted his

results in response to the possibility of furthering knowledge and understanding in this field. By the later 1970s however, possibly in part due to newer techniques becoming available, possibly in response to funding changes, and / or possibly to further his chances of getting the post at Cambridge, Evans proposed the study of genetic diseases. Evans worked with clinical researchers to advance his work into medical applications.

This is in contrast to the work of Gail Martin. In 1981, Martin also noted the possibility for genetic manipulation of embryonic stem cells. She thought this was important to mammalian biologists, in particular those interested in development. Martin appears to have completely focused on development, from her early 1970s work in the UK to the early 1980s work in the USA. There was no hint at the creation of gene targeting as proposed by Evans.

In 2006, Davor Solter noted that ESC isolation was considered important in the 1980s for potential gene function analysis. He also stated that the application for ESCs in therapeutics and regenerative medicine had not been realised at this time. When interpreting such commentaries however, it is important to be aware of the values imposed by hindsight; Evans was to win a Nobel Prize for his contribution to gene targeting (and enabling the creation of GEMMD), easily creating the possibility for history to be reinterpreted based on this achievement. For example, Solter's claim that in the 1980s there was little consideration of ESC use for regenerative medicine is unsurprising; the term did not come into use until at least the 1990s, with few biomedical scientists beginning to use the term until the 2000s.

To consider why Martin and Evans may have taken different research directions, it was useful to consider legislation and politics in the USA and UK that may have affected their work. For example, both US and UK governments aimed at reducing the number of laboratory animals used, and in the USA this was put into practice via the Improved Standards for Laboratory Animals Act (1985) – with the NIH (and continuous funder of Martin's work) encouraged to move away from animal experimentation, it could explain not only why Martin continued developing her ECC work to lead to ESC isolation (again showing the impact of cancer research on stem cell research), but why she promoted her work after this as circumventing the requirement for relatively large numbers of animal embryos.

In contrast, Evans did not promote his research applications in the same way. For example, when first introduced to PCR, Evans noted that such technology was

vital for making mouse mutations possible, which could improve the understanding of human health and disease. Evans did not note that PCR could be used to study genetics during development, or to reduce the amount of genetic sample required for analysis. Evans instead explicitly referred to the "practical applications" of studying the genetics of health and disease[805]. Why would Evans elect to use such terminology? Was this an example of UK science policy transforming the attitudes, perceptions, and expectations of UK researchers? In comparison to the terminology and language used by Martin to promote her work in the USA, it is quite feasible for this answer to be 'yes'.

This chapter has considered, in some detail, the work of Martin and Evans in isolating and culturing mESCs for the first time, and how these new tools were used. The next chapter compares the contexts Martin and Evans were labouring under in the 1980s, to the equivalent in the 1990s: Thomson and Gearhart's 1998 isolation and culture of hESCs.

[805] Evans, 1989 p 557.

CHAPTER 5:
"HUMAN ES CELLS ARE NOT THE EQUIVALENT OF MOUSE ES CELLS": A CONSIDERATION OF THE MOTIVATIONS AND GOALS OF GENERATING HUMAN EMBRYONIC STEM CELLS

1. Introduction

"The significance of the derivation of human embryonic stem cell lines lay in their ability to proliferate while remaining in a pluripotent state, without differentiating...The therapeutic potential of these cells was apparent from the start..."[806]

This chapter seeks to assess a comment made by historian of biology Michel Morange, who queried why there was a gap of seventeen years between the isolation of mESCs and hESCs. After briefly showing that this gap could not have been caused by either technical difficulties or ethical concerns, Morange considers what else may have resulted in this delay. Morange eventually concluded that when motivations and goals of their creation are considered, mouse and human ESCs are not equivalent[807]. This, claimed Morange, is because of the different scientific contexts through which mESCs and hESCs were created. This scientific context refers to the (initial) difficulty in manipulating ECCs and ESCs, and their capacity for tumourigenesis. Since Morange has therefore already highlighted the influence of scientific context on research, this chapter will add a complementary view, highlighting that the scientific climate – i.e. the social, political, ethical, legal, and economical context - must also be considered to produce a comprehensive history. This will support Morange's claim that the motivations and goals were different.

Close attention was paid to the history of murine embryonic stem cell isolation in Chapter 4 demonstrating how political and economic factors, for example, affect how researchers work. This chapter will focus on the history of human embryonic stem cell isolation, before comparing the motivations and goals of mouse and human ESC generation in order to examine Morange's claim.

James Thomson was interested in developmental biology, in particular human embryogenesis. In the 1990s, Thomson was still restricted to using mouse models for his research, which were known to have various differences when compared to early human development. Thomson attempted to switch his research focus to non-human primates, which would develop in a more similar way to humans (than mice); primate research however was expensive, and did not provide Thomson with material on a regular basis. Thomson decided that if he could develop non-human primate

[806] Thompson 2013 p 70.
[807] Morange, 2006.

embryonic stem cell lines, he would have as much material as he wanted, at the low cost of cell culture; this led Thomson to develop a method of isolating and culturing cells from early embryos[808]. Thomson published his results in 1995, and waited for another laboratory to use the same techniques to isolate and culture human ESCs – an ideal tool for studying human development *ex vivo*. When this did not happen, Thomson decided to work on the project himself, and reported his success in 1998[809].

John Gearhart's background was different to that of Thomson's. In the 1970s and 1980s, Gearhart was researching developmental differences in Down syndrome. In the 1980s, mouse models began to emerge, including mouse models for Down syndrome. Eventually however, Gearhart felt that he had exhausted the use of mouse models, and would learn more about development in those with Down syndrome by working with human cells – in particular, early embryos cultured *in vitro* from hESCs[810].

This chapter offers a complementary argument, which fits alongside Morange's claim that the motivations and goals behind the isolation of mESCs and hESCs were different. This chapter shows that when contextual considerations of stem cell research are accounted for, motivations and goals are different; this, in part, is related to the political and economic contexts (such as those highlighted in Chapter 4), and the legal and ethical contexts that become relevant when producing hESCs in the laboratory. This is in addition to the different scientific contexts Morange refers to in his 2006 paper. Although Thomson's intellectual interest in development (his motivation for hESC isolation) was similar to that of Gail Martin's motivations (i.e. to learn more about development) in the late 1970s and early 1980s, it was shown in Chapter 4 that Martin was able to pursue this line of research only because in the US 'pure' research was still funded. Evans' work in the field of development resulted in his creating GEMMD. As demonstrated in Chapter 4, Evans was essentially coerced into this research direction by restraints in funding for pure research. This chapter will show that for Gearhart, the creation of better models of disease was also a result of his initial interest in development, however the scientific climate of the 1990s was different to that of the 1980s, resulting again in different approaches to the study of development. As Morange has previously highlighted, Gearhart was also working in

[808] Thomson *et al*., 1995.
[809] Thomson, Marshall, and Trojanowski, 1998; Thomson *et al*., 1998.
[810] Shamblott *et al*., 1998.

a different scientific context to Evans. Lastly, this chapter will also demonstrate the versatility possible in stem cell research, allowing researchers to switch between fundamental and applied research; this has also previously been shown in Chapters 3 and 4.

1.1 Morange's claim

Michel Morange (1950-) is a French biological sciences researcher with expertise in the history and philosophy of science. He obtained a in PhD in biochemistry and molecular biology from the Pasteur Institute in Paris, in 1978. Simultaneously, Morange was supervised by Jacques Merleau-Ponty (1916-2002) in the history of molecular biology. Morange then went to work with François Jacob (1920-2013) in his cell biology laboratory, also at the Pasteur Institute. Morange is currently Professor in biology at the University Paris 6 and Ecole Normale Supérieure, and Director of the Centre Cavaillès for History and Philosophy of the Sciences. Morange's work in history and philosophy focus predominantly on the history and philosophy of twentieth century life science.

In 2005, Morange began an ongoing series of articles in the *Journal of Bioscience*, named "What history tells us". In December 2006, Morange published the seventh article of the series, discussing the anniversary of Gail Martin's and Martin Evans' and Matthew Kaufman's isolation of mESCs: "Twenty-five years ago: The production of mouse embryonic stem cells".

In his 2006 paper, Morange queries why there was a gap of almost two decades between the isolation and culture of mESCs in 1981, and hESCs in 1998. Morange first questions whether there were technical differences that took time to figure out and overcome; however Morange swiftly disposes of this argument by highlighting that there were few technical differences between mESC and hESC isolation and culture – the requirement for certain factors in the growth media and the use of feeder layers are very similar. Secondly, Morange questions whether ethical concerns were a reason for the delay. Again however, Morange dismisses this argument – if there were ethical obstacles, these had still not been satisfactorily resolved, and so could hardly have been the cause of such a long delay in the 1980s or 1990s. Jane Maienschein has commented on this too, noting that there were few ethical concerns raised after the isolation and culture of mESCs; Maienschein claimed that bioethics in general was far more concerned with clinical medicine than

laboratory research, and it was only in 2007 (after Evans won the Nobel prize for work on creating genetic chimeras) that such research was noticed by the public, and therefore required more consideration by the bioethicists[811]. This is a different view to Morange, however the result is the same: "ethical obstacles" were not a significant problem for the isolation and culture of hESCs.

Morange believed that he saw the beginning of an answer to his question in a *Nature* piece following the paper published by Evans and Kaufman in 1981. In her commentary, Brigid Hogan makes no mention of the extension of the method to human cells, or of any medical use for the mESCs. Instead, Hogan identified that the mESCs were an ideal *in vitro* model to study cell fate in mammals. Hogan also mentioned that there was a possibility of generating mosaic animals by injecting isolated mESCs into blastocysts, as a way of studying development. The mESCs were seen as an improvement on the ECCs already available. This point is further emphasised by Hogan later in 2007, where again she makes no reference to hESC isolation following 'naturally' on from mESC isolation, or the potential for therapeutics[812]. It appeared then when scientists thought about mESCs, their immediate respeonse was not to extend this to hESCs, since their application is different. This is the context that Morange is referring to.

Following this introduction to his paper, Morange then described the link between the study of teratomas and eventually the isolation of ECCs and ESCs (as covered in greater detail in Chapter 4 of this thesis). The usefulness of cells that could be injected into a developing blastocyst was seen to be a particular triumph of this work; the study of gene regulation in microorganisms had been possible for some time, with biochemical and genetic studies focusing on *Escherichia coli*. However similar study of mammalian development had, up until this point, been impossible, as there was almost no (if any) experimental access to the post-implantation embryo. The development of ECC (and then ESC) lines had enabled different stages of development to be studied. In the 1970s, various researchers experimented with mECCs by inserting them into developing blastocysts, and studying cell-cell interactions and embryogenesis, up to the fate of injected ECCs in the adult mouse (see Chapter 4).

[811] Maienschein, 2014 p 187.
[812] Hogan, 2007.

As highlighted in Chapter 3 of this thesis, Morange also noted that such experiments demonstrated confirmation of the similarities between ECCs and ESCs, although there were limits to the mosaicism created by injection of ECCs into the blastocyst. For example, there was difficulty in obtaining transmission of the ECCs through the germ line of the mosaic mice. The development of an ESC line however would, in theory, overcome such problems.

In the fourth section of his paper, Morange specifically stated that the scientific context is relevant to the way in which ESCs were used to study development. Initially, Morange claimed, ESCs were thought to be a way of creating transgenic animals. The further possibility of examining gene function in development also swiftly emerged, as genes similar to those affecting development in *Drosophila* were identified (the homeobox-containing genes)[813]. ESCs could be used to test the effect these genes had on development, as they could be rendered non-functional, or different alleles could be specifically selected for. From this beginning, Morange argues, the production of knock-out and knock-in transgenic animals emerged as an important application of ESCs[814]. The clear follow-up to this then was the possibility of gene therapy, such as the replacement of a non-functioning gene with a functioning copy; this, suggested Morange, attracted the attention of biotech companies in the 1990s. If the creation of transgenic animals and elucidation of gene function were the aims of mESC isolation and culture, this shows that they were developed with a different motivation to hESCs, which would not be isolated and cultured with the aim of generating transgenic individuals.

In his concluding statement, Morange justified the study of history of biology by identifying that the discipline has two functions: to "discern the permanent transformation in science of 'objects'" and "to learn lessons and retrieve them from the past"[815]. With the context of this in mind, Morange's claim that the motivations and goals for creation of mouse and human ESCs identify that both types of ESC were created in different scientific contexts, and have perhaps developed different values since their isolation. The different scientific context in which human and

[813] See Chapter 4, section 5 of this thesis: Martin worked on homeo-box genes in the early 1980s.
[814] See Chapter 4, section 4 of this thesis: Evans created GEMMD in the 1980s, and this led to models of larger mammals in the 1990s.
[815] Morange, 2006 p 540.

mouse ESCs were isolated, Morange argued, tells us why there was such a gap of seventeen years between the isolation and culture of mESCs and hESCs.

1.2 Other reflections

Unsurprisingly, Morange is not the only scholar to have considered the leap from mouse to human ESCs. For example, Maienschein has also commented on the move from mouse to human ESC isolation in her 2014 book *Embryos under the microscope*, where she claimed that "in 1981, with mouse studies, the lines of research that lead to human pluripotent stem cell culturing began"[817], clearly linking the isolation and culture of mESCs with the isolation and culture of hESCs, which Morange suggests is not as clear-cut. Maienschein goes as far to claim that "the story of stem cell research from mice to men is well-known"[818]. She conceded however that there is a difference between the culture of mouse and human ESCs (i.e. the time between 1981 and 1998); this was not due to the extension of the technique (as also claimed by Morange), but caused by advances made in understanding the genetic basis of development[819]. This then is partly in agreement with Morange: Morange refers to the journey from mECCs to hESCs being "far from straight", which would imply that, as Maienschein says, the difference between mESCs and hESCs was not as simple as extending the methods used. Maienschein instead proposed that between 1981 and 1998, much had been learned about the genetics of development which was relevant to isolation and culture of hESCs. Morange's slightly more detailed extrapolation of this is that it was specifically the parallels between cancer and development that was an obstacle for later researchers.

Davor Solter has commented on the relationship between murine and human stem cells in his review looking at the history of stem cell research[820]. Solter claimed that whilst research making use of human and mouse tissue ran in parallel, advances using human cells "usually lagged behind by a decade or so", benefiting from the experience of mouse research[821]. In particular, Solter noted the lag between isolation and culture of mESCs and hESCs, like Morange and Maienschein, claiming that since the isolation techniques were comparable, and the markers (of pluripotency) were

[817] Maienschein, 2014 p 207.
[818] *ibid* p 207.
[819] *ibid* p 207.
[820] Solter, 2006.
[821] *ibid* p 319.

already available, there must be another reason for the seventeen year gap between mESC and hESC isolation and culture. Solter suggested that "the reasons for this delay are probably the difficulties involved in obtaining suitable embryonic tissue and an understandable reluctance of most investigators to work in a field that is fraught with potential legal problems and political and moral dilemmas"[822]. Success was achieved however, with the foresight of those private companies providing funding, and useful previous experience with non-human primate (p) ESCs; this is explored below in the account of Thomson's and Gearhart's research leading to hESC isolation. Although Solter implied that some of the delay may be accounted for by social and ethical concerns, he does not refer to the potential delay caused by the differences in scientific context Morange specifically refers to.

This chapter will consider Morange's claim, however examination of the history of human ESC isolation is required (a history of mouse ESC isolation was provided in Chapter 4), before returning to assess Morange's argument.

2. History of human embryos in research

2.1 History of human embryonic stem cell isolation and culture

"Studies on cytodifferentiation in mammals might be greatly assisted if undifferentiated cells capable of profound differentiation were available in culture. A possible source of such cells is the earliest stages of mammalian embryogenesis..."[851]

2.1.1 1980s: Shifting focus from mouse to human

Following the isolation of mESCs in 1981, much was learned about mammalian development, including the property of pluripotency. For example, cultures were scrutinised for the expression of *Oct4*, an important transcription factor involved in pluripotency, without which cells begin to differentiate (see also Chapter 6). Such research became important to understand how mESCs would grow *in vitro*, and availability of mESC lines enabled much to be learned about optimum growth conditions *ex vivo* in the years that followed. Such research and results ranged from the relatively simple selection of appropriate culture media, to more complex theories about epigenetic and transcriptional 'resetting' of cells once they are *in vitro*, possibly

[822] *ibid* p 324.
[851] Cole, Edwards, and Paul, 1965 p 501.

creating an artificial state[859]. Eventually, even nuances of mESC culture from different mouse strains began to surface[860]. Learning such details was important for understanding more about mammalian development, since the correct culture conditions would enable similar development *in vitro* as had been identified *in vivo*[861]. Mouse development could therefore be studied using the mESC lines derived from Evans and Kaufman's and Martin's techniques (as described in 1981), including the creation of murine disease models (as discussed in Chapter 4). James Thomson claimed that "a bunch of people doing really cool developmental biology shifted [their focus to knockout mice] and a lot of basic stuff kind of got lost for a while"[862]. This statement provides some support for Morange, who also suggests that some later researchers had 'forgotten' the paradigms of ECC and ESC research of the late 1970s and early 1980s whilst carrying out their work in the 1990s.

In 1987, Christopher Graham, at Oxford University, submitted a license application titled 'Derivation of cell lines from the human conceptus to investigate the growth regulation of embryonic and tumour cells for the development of effective preimplantation diagnosis'[863]. This is a continuation of the parallel recognised in Chapter 3 of this thesis, between normal and pathogenic development – a parallel that Morange suggests was forgotten in the 1990s. Graham had been working towards understanding the factors involved in growth regulation of human embryonic cells[864]. In correspondence with Anne Parson, Graham commented that he thought there may have appeared to be more groups trying to derive hESCs than were actually working to do so, implying that the ethical requirements and considerations may have been a hurdle some were not willing to jump[865], perhaps waiting for others to do this work, and for cell lines to become commercially available. In further correspondence, Graham referred to motivations and goals for those who may have been pursuing isolation and culture of hESCs, suggesting that 'on paper' (i.e. in official funding applications, for example) no-one would be trying to isolate hESCs for therapeutic purposes. That said, "all of us would have been exceptionally stupid not to realise

[859] For example, see Buehr and Smith, 2003.
[860] For example, see Ying *et al*., 2003.
[861] For example, see Nichols *et al*., 1996; Batlle-Morera, Smith, and Nichols, 2008.
[862] Park, 2011 p 61.
[863] Parson, 2004 p 266.
[864] For example, Hopkins *et al*., 1987.
[865] Parson, 2004 p 266.

their therapeutic value"[866]. Graham's comments suggest that there was an underlying understanding that hESCs may be useful in the long term for therapeutic medicine, but in the short term, the isolation and culture of hESCs was required for various research projects, including basic and applied biology.

2.1.2 1990s: James Thomson's hESCs from embryos

One of those studying development in the 1990s was James A Thomson at the University of Wisconsin, Madison. Thomson had doctoral training in veterinary medicine and molecular biology at the University of Pennsylvania, carrying out his thesis research on the role of genetic imprinting in early mammalian development at the Wistar Institute, Philadelphia. Thomson then embarked on a two year post-doctoral fellowship at the Primate IVF and Experimental Embryology Laboratory, Oregon Regional Primate Center[867].

Thomson was amongst those developmental biologists using the mESCs first isolated and cultured in the 1980s to learn more about early embryonic development; mice were an ideal model, since they were small, easy to use[868], cheap, and easy to keep. This said, although there are some parallels between murine and human development, there are also many differences, particularly in the early stages of embryogenesis. For example, the mouse produces no equivalent of human gonadotrophin, and the products of the placenta are different. Thomson, as a developmental biologist, was frustrated with the model available, and decided to make use of the primate research going on at the University of Wisconsin, Madison, to further his research into early human development. Thomson's attempts to obtain non-human primate embryos juxtaposed the cheapness and ease of using mice - a figure of around $2000 was put on obtaining each *in vivo*-created rhesus monkey embryo[869]. "…[T]he primate material was limited", claimed Thomson: "I thought stem cells made a lot of sense, because you could grow as many as you want"[870]. Thomson would use those techniques learned from the creation of mESCs, combined with other knowledge and understanding of keeping non-human primate cells in culture, to develop a line of pESCs.

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[866] *ibid* p 156.
[867] Thompson, 2013 p 69-70.
[868] Park, 2011.
[869] *ibid* p 60.
[870] *ibid* p 60.

Thomson had previously worked with Peter Andrews at the Wistar Institute in the 1980s, working with mECCs. Thomson claimed that he therefore knew the procedures and techniques used for culturing mECCs first-hand, and would combine this with the methods presented by Evans and Kaufman, and Martin in 1981 to start his work with the rhesus monkey embryos[871]. Thomson used trophectoderm-specific antibodies to identify and lyse the trophectoderm cells that surround the ICM of the developing blastocyst. The ICM cells were then transferred to a mouse fibroblast feeder layer (as Martin had used). Thomson was able to culture the cells for six months, and would carry out a series of tests to demonstrate that the cells were embryonic stem cells, and had not begun to differentiate, and that no genetic mutations had occurred. Thomson's results were published in 1995. In the last two paragraphs of this paper, despite Thomson's background in developmental biology and his claims that he wanted to further developmental biology research by generating cells more closely related to human cells than mESCs, Thomson wrote that hESCs could one day be isolated using similar methods, and could have many medical applications for conditions like Alzheimer's or Parkinson's[872].

Thomson said that he was "surprised" that few people seemed to be interested in generating hESCs after his 'proof of principle' pESC work was published[873]. "[S]everal people tried, but didn't succeed for whatever reason…we thought somebody else would do it, but as the months ticked by, and nobody else had done the human work, we decided to pursue that"[874].

Before Thomson could do any work using human embryos however, there were a lot of ethical and moral issues to consider[875], in addition to legal issues to be addressed. For example, the Dickey-Wicker amendment made federal funding unavailable for any research that involved the use of human embryos[876]. Alice Park's narrative of Thomson's efforts in the late 1990s suggest that funding was the main sticking-point for research into generating a hESC line. For example, Thomson could not continue working at the University of Wisconsin Primate Center, as this was a

[871] Parson, 2004 p 143.
[872] Thomson *et al*., 1995; Parson, 2004; Park, 2011.
[873] Park, 2011 p 65.
[874] *ibid*; Parson, 2004 p 145.
[875] Park, 2011.
[876] The Dickey-Wicker amendment was passed in 1995, signed by Bill Clinton. It prohibited the Department of Health and Human Services from using funds for research where a human embryo would be destroyed, or for the creation of human embryos for research purposes.

federally funded facility. Thomson applied for internal university funding to work on creating hESCs, but was refused (Thomson believed that this may have been because the university was concerned that funding his human embryo work may cause enough controversy to lose the university millions of dollars in NIH funding)[877].

The funding vacuum gave biotechnology company Geron their foot-in-the-door. Geron was founded in 1990, and publicly traded (NASDAQ) since 1996. One of many early 1990s biotechnology start-up companies, Geron was funded by venture capital, hoping to secure future revenue from patents. Cooper claimed that Geron successfully monopolised the emerging discipline of regenerative medicine[878]. Stem cells would be a key area of interest for Geron's founder Michael West; West had completed a PhD in cell biology, and followed-up this interest with Geron. West's other interest was aging (hence "Geron" - Greek for 'old man'), and the first research Geron invested in was telomere research[879]. Geron had shown an interest in ESC research in the early 1990s, after Howard Cook had demonstrated that the telomeres of eggs and sperm did not shorten with age like those in somatic cells. West's logic was that if germ cells were like stem cells, then stem cells might be the key to aging and regenerative medicine[880]. Roger Pederson, a biologist at University of California at San Francisco, claimed that West had approached him before the Dickey-Wicker amendment, offering to fund his research. Pederson had refused, believing that the government would continue to support stem cell research, that federal funding would be available, and that any stem cell lines derived would be freely available for sharing between laboratories. After Dickey-Wicker however, Pederson's views changed. Pederson got back in touch with West, and agreed to Geron's funding terms. West seized the opportunity, and realised that if Pederson would return to Geron for funding, others in the field must be desperate for funding as well. West asked Pederson for names of those who he thought might also be suffering from lack of federal funding, and the names of James Thomson and John Gearhart were offered[881]. The following day, West flew to Madison to talk to Thomson. It was good timing -

[877] Park, 2011.
[878] Cooper, 2008 p 142-3.
[879] Telomeres are repetitive sequences of nucleotides found at each end of the chromosome, which are believed to protect the chromosome ends from damage or fusion with other chromosomes. These shorten with age, and are thought to have an effect on the aging process.
[880] Parson, 2004 p 147.
[881] Park, 2011.

Thomson's application for internal funding had been turned down a few days prior. Apparently reluctant, but without any viable alternative, Thomson signed-up for Geron funding, and it's intellectual property implications[882].

Although Thomson had obtained financial backing for his project, there were other decisions to be made, which were also affected by the Dickey-Wicker amendment. For example, Thomson had to move to a laboratory that was not funded by federal money, and equip it (eventually Thomson found space near the university's IVF clinic, and apparently cobbled together a laboratory from discarded equipment from other laboratories)[883]. Thomson was also concerned by the ethical implications of his work, and got in touch with another University of Wisconsin colleague, Alta Charo (an expert in law pertaining to scientific research), for advice. When interviewed by Park, Charo claimed that Thomson's aim was not to make any political point, but was solely interested in researching human embryology[884]. Thomson also met with the founder and director of the University of Wisconsin's program in medical ethics, Norman Fost. Fost has said that Thomson was aware of the controversy of the work he was proposing, and cared about doing the right thing. Thomson knew others (including at the university) might have been upset about the work he was proposing. Fost would later head the university review board discussing approval for Thomson's project[885]. Having discussed the ethical and legal implications of Thomson's proposed work, Charo also asked Thomson whether he was personally comfortable with the project he was planning. Years after Thomson had successfully isolated and cultured hESCs, an interviewer asked Thomson a similar question; Thomson said that if hESC research did not make you a little uncomfortable, you had not given it enough thought[886]. Parson wrote that after the announcement of Dolly's birth in 1998, Thomson almost halted his work following some public controversy[887] - perhaps a hint of what would fall on the shoulders of Thomson if he succeeded in his endevours.

Thomson's ethical and legal discussions with Charo and Fost were not unwarranted - it apparently took the University of Wisconsin two years to approve

[882] Parson, 2004 p 148.
[883] Park, 2011.
[884] *ibid* p 69.
[885] Parson, 2004 p 149.
[886] Park, 2011 p 69.
[887] Parson, 2004 p 173-4.

Thomson's project, in July 1995. Concerns were not only ethical, but legal - again, the university did not want to risk losing millions of dollars in federal funding if Thomson's project had not been given the upmost attention to detail. For example, there were concerns about federally-funded utility supplies (water and electricity for example) to Thomson's make-shift laboratory. Basic equipment, such as computers, would also have been previously purchased using federal funds. Once the university had agreed to Thomson's project and his laboratory space, to avoid further complications, Thomson carried out all of the practical work himself[888]. Fost reported that the institutional review board spent significant time looking at three previous reports to help them make their decision: the Warnock Report (UK, 1984), the Canadian Royal Commission on New Reproductive Technologies in Canada (1993), and the NIH Human Embryo Research Panel (US, 1994). All of these reports had reached the conclusion that although the preimplantation (human) embryo (day 1-7) warranted serious moral consideration, it did not have the same moral status as an infant. The institutional review board at the University of Wisconsin concluded that it was unlikely to reach a different conclusion to these three reports, and Thomson's project was permitted to go ahead[889].

Again reluctantly however, Thomson found that he needed another collaborator. Although Thomson had arranged with the university's IVF clinic that he could use donated embryos, these were few and far between (since the clinic was very small). Thomson needed more material to work with. Just as Geron had filled a funding vacuum, another collaborator filled the materials vacuum. Joseph Itzkovitz-Eldor, at the Rambam Medical Center (Haifa, Israel), had been following Thomson's work after the publication of his pESC paper in 1995, and was keen to collaborate[890], presumably imagining that a hESC line could be useful for IVF research. Itzkovitz-Eldor's IVF clinic in Haifa was much larger than that in Wisconsin, and could provide many more donated embryos. Thomson, in need of Itzkovitz-Eldor's embryos for his work, agreed to the collaboration, and Itzkovitz-Eldor sent Thomson embryos and a student, who would learn more about Thomson's cell culturing techniques.

Thomson initially struggled with culturing the embryos he was sent. For IVF, these embryos would have been frozen soon after fertilisation (day 1 post-

[888] Park, 2011.
[889] Parson, 2004 p 151-3.
[890] Park, 2011.

fertilisation), and if they were going to be used, defrosted and cultured for 2-3 days prior to transfer. However, Thomson needed the embryos to live *in vitro* for 5 or 6 days, and develop normally. To help achieve this, Thomson sought the expertise of the university's IVF laboratory director, Jeffrey Jones. At first, the pair were unsuccessful. Jones attended a conference in May 1996, and heard a presentation from David Gardner, an embryologist at Monash University (Australia). Gardner spoke about a culture system he had used to keep embryos developing normally *in vitro* for 5 days. Jones contacted Gardner for help, but American-Australian logistics made collaboration difficult. In 1997, a member of Gardner's laboratory was offered a postdoctoral fellowship at Wisconsin, providing Thomson with a contact with whom he could work regarding accessing Gardner's culture formula. Gardner's approach had been to mimic the mother's environment *in vitro*; in practice, this had doubled the number of IVF embryos that would survive up to birth. Thomson and Jones' collaboration with Gardner finally came to fruition when the legalities between the US and Australian groups had been worked out. In January 1998, Thomson and Jones could begin culturing embryos in Gardner's media, and, as expected, healthy blastocysts developed. After this stage, Thomson could use the culturing techniques learned from pESCs to isolate and culture the hESCs[891].

Within a few weeks of Itzkovitz-Eldor's embryos arriving, and alongside an additional few embryos from the university clinic, Thomson had successfully replicated the rhesus monkey technique and was culturing human embryonic stem cells. The method Thomson used to isolate the hESCs was to culture 36 donated cleavage-stage embryos to the blastocyst stage (20 embryos developed to this stage); this process took 4-5 days. From these blastocysts, 14 inner cell masses were isolated (named H1 to H14), five of which developed into cell lines *in vitro*. In the 1995 pESC paper (which Thomson referred to regarding the method for isolating and culturing hESCs in 1998), the method noted that a blastocyst was flushed from the uterus of a rhesus monkey, the trophectoderm lysed, and the inner cell mass cells separated. The cells were then cultured on a feeder layer of irradiated mouse embryonic fibroblasts. The first outgrowths could usually be seen after 9-15 days. After five weeks, the cells morphologically resembling human ECCs were isolated,

[891] Parson, 2004 p 170-1, 174.

and clonal colonies established[892]. This, according to the account in Park, appeared to be the most difficult work. It would take only a few days for the growing cell colonies to begin stratifying (i.e. growing on top of one another), or for a few outlying cells to begin differentiating. Every few days, Thomson would need to change the nutrient media the cells were growing in, and remove any differentiating cells. When there were too many cells present in a single flask, Thomson would have needed to 'split' the cells. Small clusters of hESCs would be collected and re-plated into a fresh flask. Thomson needed to do this approximately once per week. The hESC lines developed by Thomson were not created from isolated cells, but from small colonies (of between approximately 50 and 100 cells); i.e. the hESCs described in 1998 by Thomson were not clonal colonies. Thomson defended this by claiming that the cells shared a uniform morphology (similar to hECCs and pESCs), making it "extremely unlikely" that a mixed population of cells was expanded; Thomson conceded however that they "cannot rule out the possibility" that there may be some variation in the differentiation potential amongst the cells of each line[893]. One reason Thomson may have had to generate heterogeneous hESC colonies was that he learned that the human cells preferred to be cultured at higher densities than the pESCs. When re-plating his cells, Thomson would have needed to make sure that there were enough hESCs present for them to be in contact with each other; if there were too few hESCs in each flask, then those present would die.

Park's narrative suggested that the isolation and culture of the cells was not the most testing part of Thomson's project however: this would be demonstrating that the cells growing were unequivocally hESCs. Thomson's continuing publications detailed the work carried out to present evidence that the cell lines developed were ESCs; this was more difficult than the 'standard test' for other laboratory based animals - which was the ability to contribute to all tissue types in chimaeras - since it was not legally possible to do this with human cells. Thomson's previous work on pESCs was again useful, as chimeras could not be generated to demonstrate pluripotency of these cells either. Based on this experience, Thomson *et al*. proposed that the essential characteristics required to demonstrate that the cells were ESCs were:

1) derivation from the pre-implantation embryo

[892] Thomson *et al*., 1995.
[893] Thomson *et al*., 1998 p 1146.

2) prolonged undifferentiated proliferation
3) stable developmental potential to form derivatives of all three embryonic germ layers[894].

Thomson *et al*. used a significant part of the paper to describe how they had demonstrated the three criteria identified, and potential hESC uses.

Thomson applied for patents for his work on hESCs, being no stranger to patent applications. The Wisconsin Alumni Research Foundation (WARF) filed Thomson's first patent on 20 January 1995, which referred to Thomson's pESCs (seven marmoset pESC lines, and two rhesus pESC lines), and the method for their derivation. This was added to a year later, when Thomson applied for the 1995 patent to be abandoned in favour of new matter not included in the original application; this new matter was the characterisation of two pESC lines, and isolation of another, developed from rhesus monkeys. A continuation of this patent was applied for in December 1998, following criticism that the work was not sufficiently different from those of other patent holders. Eventually, WARF obtained patent 5843780 for the pESCs cultured. A year later, WARF filed a separate application, for the human cell lines Thomson had developed. The application was almost identical to the primate cell patent[895]. US Patent 6200806 was issued in 2001, and covers the method for isolation and the five original hESC lines generated (i.e. both the process and the product). This patent gave WARF control over who may work with the cell lines, who may use Thomson's method, and therefore the purpose of future research using hESCs. WARF established an exclusive licensing agreement with Geron. Geron had rights to three modified cell lines (and WARF retained the rights to the five unmodified cell lines). It is not possible to patent hESCs in the UK; hESCs can not be patented since they have the potential to develop into an entire human body, and human beings / persons can not be patented[896].

2.1.3 1990s: John Gearhart's isolation and culture of hEGCs

It is possible that the work of Leroy Stevens on teratocarcinomas (see Chapter 3)[897] inspired the theory that pluripotent cells may be derived from PGCs. When this

[894] Thomson *et al*., 1998 p 1145.
[895] Rohrbaugh, 2003.
[896] Cooper, 2008 p 144, 146.
[897] For example, see Stevens, 1962.

research was carried out in the early 1990s, it was possible to culture early embryos *in vitro* with a relatively high success rate, and, with the echos of Stevens' work behind them, researchers began to isolate the PGCs and culture them. Just as with mESCs, the correct culture conditions for isolated embryonic germ cells (EGCs) were honed.

John Gearhart grew up in Philadelphia, attending school whilst his mother and older brother worked on the family farm[898]. With ambitions of becoming a fruit-tree grower, Gearhart studied plant genetics and the University of New Hampshire. Gearhart would remain in academia however, moving to Cornell for a PhD in *D. melanogaster* genetics. Anne Parson claimed that Gearhart was working in the newly emerging, more interdisciplinary field of developmental biology that had evolved from embryology (see also Chapter 2)[899]. Although Gearhart's PhD project focused on fruit fly genetics, he was still able to follow his previous interest in plant genetics and development; close to Gearhart's laboratory space at Cornell was Frederick Steward's bench; Steward had recently cloned a carrot plant from a single, mature cell. Parson noted that Gearhart did not collaborate with Steward, but that his work was influential; after completing his PhD, Gearhart was set to go and work in France with Jean Paul Nitsch, who had recently grown a tobacco plant from a pollen grain. Unfortunately, as Gearhart was about to leave for France, Nitsch was killed in a car accident. Finding alternative employment, Gearhart would move to the Institute for Cancer Research in Philadelphia, to the laboratory of Beatrice Mintz (see Chapter 3), in autumn 1970. Under the direction of Mintz, Gearhart would spend his time learning about the culture of mECCs - in particular, Stevens' teratomas and strain 129 mice (see Chapter 3). Gearhart had the opportunity to briefly meet Stevens at a conference in Venice, in 1972[900].

Mintz had previously worked with Tibby Russell at JAX, where they had determined the migratory route of PGCs in mouse embryos. This had helped Stevens to demonstrate that these cells were involved in later teratoma formation. Mintz, having already successfully created murine chimeras, was looking to use mECCs to generate new chimeras. Gearhart's role was to culture the mECCs, however he

[898] Wu, 2011.
[899] Parson, 2004 p 157-9.
[900] However, Stevens went missing one evening after becoming inebriated; a group searched for him, but to no avail. Eventually Stevens re-established contact with his colleagues from Istanbul, having decided to leave the conference, take a vaporetti along the Grand Canal to the train station, and board the Orient Express. Parson, 2004 p 162-3.

struggled to prevent the cells from differentiating in culture. Eventually the group succeeded, and in 1975, Mintz' laboratory published their results, showing that mECCs could contribute to normal development of a mouse chimera[901]. Gearhart left Mintz' laboratory the same year, to move to the University of Maryland. Whilst there, Gearhart continued researching mouse development. He worked closely with the anatomist Gladys Wadsworth, and the pair would spend time together dissecting (human) stillbirths. Wadsworth would narrate her dissections aloud, and here Gearhart learned about foetal anatomy. Parson claimed that Wadsworth would ask Gearhart questions about embryogenesis during these dissections, in particular how each region of the foetus would develop from cells in the embryo.

In 1979, Gearhart moved to Johns Hopkins University to teach human embryology and conduct research into mammalian embryogenesis. Gearhart followed the trend in transgenics, attempting to use the methods to learn more about the genes involved in embryogenesis, and the role of trisomy 21 in development of Down syndrome symptoms (this included the creation of a Down syndrome mouse model).

Gearhart's work utilising the mouse model of Down syndrome (during the late 1980s) was a 'peak' time for mouse models of disease. Historically, researchers used mice in the laboratory as they were easy and cheap to care for, and would reproduce relatively easily and frequently (every nine weeks). Genetically and physiologically, mice are also relatively similar to humans. Initially, mouse models of disease were identified as those conditions present in both mice and humans, and selectively bred for; strains were developed that had increased incidence of certain diseases (such as strain 129 mouse teratomas, noted in Chapter 3). Once mutagens had been found, these were used as a relatively crude way of causing genetic mutations. Later, newer techniques were developed for affecting specific genes or chromosome regions, referred to as transgenics. As noted in Chapter 4, Martin Evans would share a Nobel Prize for his research that, in part, led to development of very specific mouse models of disease in the 1980s[902].

In 1986, Gearhart and colleagues wrote about a mouse model for Down syndrome; although the condition is caused by trisomy 21 in humans, this was not the case in mice. However, many of the genes on human chromosome 21 were found to

[901] Mintz and Illmensee, 1975.
[902] For a detailed discussion on the history of transgenic mice, see Myelnikov, 2015.

have murine analogs on mouse chromosome 16. The trisomy 16 mouse, Gearhart argued, would be useful for examining the development of the Down syndrome phenotype, and an increased understanding of the molecular mechanisms of the syndrome. In particular, the mouse model of Down syndrome would provide "the opportunity for detailed anatomical, biochemical, and physiological studies throughout development, as well as after experimental manipulations *which cannot be performed in humans*" (my emphasis)[903]. Gearhart is here showing his hand: that the mouse model would allow greater understanding of the syndrome as a phenotype (as opposed to cell cultures, samples from patients etc.), and could be subject to experimentation. Mouse models would allow Gearhart to study the embryonic development of mice with 'Down syndrome'. These aims would shape his later motivations in generating hEGCs for further study.

By the early 1990s, Gearhart was a leading expert in mouse models of Down syndrome, but was keen to expand his research to learn more about the syndrome in humans. Gearhart believed that if he could retrieve stem cells from human embryos with and without trisomy 21, he could compare the two, particularly with a focus on differentiation and embryogenesis. To this end, Gearhart approached David Blake, the assistant dean of the medical school at Johns Hopkins, for advice. When asked about derivation of hESCs, Blake belived that obtaining hESCs from surplus IVF embryos may be too controversial, and asked if Gearhart could think of another source of stem cells for his research. Reaching back to his experiences and knowledge of mice, Gearhart recalled the work carried out by a contemporary, Peter Donovan. At the National Cancer Institute, Donovan had isolated and cultured mPGCs. In the foetus, these cells would only exist for a few days before differentiating. In culture however, Donovan had transformed the cells into embryonic germ cells (EGCs), capable of differentiating into a range of cell types. Gearhart reasoned that if he were to isolate and culture the primordial germ cells of human foetuses, he would be able to culture hEGCs. Blake agreed that this method could be less ethically troublesome[904].

[903] Reeves, Gearhart, and Littlefield, 1986 p 810-811.
[904] Parson, 2004 p 164-6.

Discussing this potential line of research with his postdoctoral fellow, Michael Shamblott, and colleague John Littlefield[905], the group decided that they would obtain their hPGCs from therapeutically aborted foetuses. This would prevent any controversy from obtaining foetal material from elective abortions, and would also benefit their work by arriving at their laboratory quickly. The next discussion considered funding; although Gearhart's group were eligible for federal funding (since they would be using tissue from therapeutic abortions), Gearhart, Shamblott, and Littlefield opted to try and obtain private funding; this way, they would not have to justify their spending of taxpayers money[906]. Johns Hopkins review board, like their counterparts at the University of Wisconsin, took time to discuss Gearhart's proposal; Gearhart submitted his project to the review board in 1993, and it was given the go-ahead in 1996. Again, enter Geron's Michael West. Initially, Gearhart's project was running on internal funding granted by the university, however West convinced Gearhart to accept his funding offer, made just a few months after funding Thomson's work. West decided that funding Gearhart was a hedge – West did not know whether Thomson's project would be successful, and saw Gearhart's work as a back-up[907].

Gearhart was able to obtain fresh, therapeutically aborted fetuses from a Johns Hopkins-affiliated hospital, the Bayview Medical Center. Once the foetus was in Gearhart's laboratory, Shamblott would carry out much of the work dissecting out the genital ridge, and culturing the tissue. After a short amount of time, Shamblott had begun the culture of the removed genital ridges, however the initial culturing attempts failed. The cells stopped proliferating after a very short amount of time (as had previously been observed *in vivo*). Gearhart returned to the work he knew had been carried out previously – that by Donovan (Brigid Hogan had also carried out similar work to Donovan at the same time, however the narration provided to Parson by Gearhart suggests that it was Donovan's work that he was familiar with)[908]. Gearhart looked again at Donovan's culturing techniques, and adapted them for Shamblott's cultures in early 1997. The cultures began proliferating over longer periods of time,

[905] Shamblott is still an active researcher, making use of various types of embryonic stem cells. Littlefield was a pioneer of amniocentesis.
[906] Parson, 2004 p 166-7.
[907] *ibid* p 167-8. There is no reference to Thomson and Gearhart believing that they were in competition with each other; this may have been because they were unaware of each other's work.
[908] Parson, 2004, p 165, 169.

lasting for weeks. In July 1997, Gearhart announced at the International Congress of Developmental Biology in Utah that he and Shamblott had been culturing their cells continuously for months. However, the group still needed to demonstrate pluripotency, and publish their results[909].

Ultimately, both Gearhart and Thomson would publish their work within days of each other – Thomson in 6 November 1998 issue of *Science*, and Gearhart in 10 November 1998 issue of *Proceedings of the National Academy of Science* (*PNAS*). Both groups had demonstrated that the cells would proliferate without differentiating for months, and were capable of differentiating into cells of all three germ layers[910]. Gearhart demonstrated this potential by publishing images of immunohistochemical analysis of embryoid bodies cultured from the cells isolated. Using this method, cells would stain depending on their type. Gearhart provided evidence of cells expressing various proteins of all three germ layers in embryoid bodies developed from six different cell lines isolated[911]. Thomson demonstrated the pluripotency potential of his hESCs by comparing the surface markers expressed with those from pESCs, mESCs, and hECCs. Thomson also demonstrated that his cells could create cells from all three germ layers by creating teratomas; Thomson injected the cells into mice, where the cells expanded and developed into teratomas, which included a variety of cell types (such as epithelium, bone, muscle, and embryonic ganglia)[912].

3.“Motivations and goals”: a comparison of mouse and human embryonic stem cell isolation

3.1 Morange re-visited

In 2006, Morange’s historical reflections on the isolation of mESCs in 1981 led him to claim that there were no “ethical obstacles” or “technical difficulties” that would have resulted in significant time passing between the isolation and culture of mESCs and hESCs[913]. Since technical nor ethical difficulties could have been resulted in the seventeen-year gap, Morange stated that hESCs are not the equivalent

[909] *ibid* p 171-2.
[910] *ibid* p 174-5.
[911] Shamblott *et al*., 1998.
[912] Thomson *et al*., 1998.
[913] Morange, 2006 p 537.

of mESCs if the motivations and goals of their creation are considered[914]; it was this that resulted in the long period of time between mESC and hESC isolation. The motivations and goals differed, argued Morange, because of the change in scientific context between the 1980s and 1990s. In Chapter 4, I detailed the history of Martin's and Evans' work isolating and culturing mESCs in 1981. In the current chapter, I similarly recorded the history of Thomson's and Gearhart's work towards the isolation and culture of hESCs in 1998. These narratives will now be utilised to examine Morange's claim further.

3.2 Reflections on motivations and goals for isolation of mESCs

It has been suggested that the work of Gail Martin and Martin Evans (with Matthew Kaufman) was part of an initiative to derive pluripotent cells from embryos; the techniques established using teratocarcinomas and ECCs enabled the move to ESCs (as established in Chapter 4)[915]. Martin, an expert in cell culture, observed the embryoid bodies that form in mECC culture, and extrapolated this observation: ECCs (and therefore ESCs) could be used as laboratory models for early development[916]. It was this idea, Brigid Hogan argued, that led mESC isolation and culture to follow-on from mECC isolation and culture[917]. However, the research into ESCs (following from ECC research) was not only motivated by intellectual curiosity; as highlighted in Chapter 4, Martin was able to develop mESCs to further her research on development, due to the political and economic situation in the USA (where funding was available for such 'pure' research. Evans meanwhile utilised the isolated mESCs to create GEMMD, since funding in the UK was available primarily to those conducting applied research. The routes and decisions taken by Martin and Evans appear to have been dictated by several elements, including social, political, legal, and economic factors in the USA and UK respectively (as considered in detail in Chapter 4), as well as intellectual curiosity (see Figure 12).

[914] *ibid* p 540.
[915] Nichols and Smith, 2011.
[916] Hogan, 2007; Martin, 1980.
[917] Hogan, 2007.

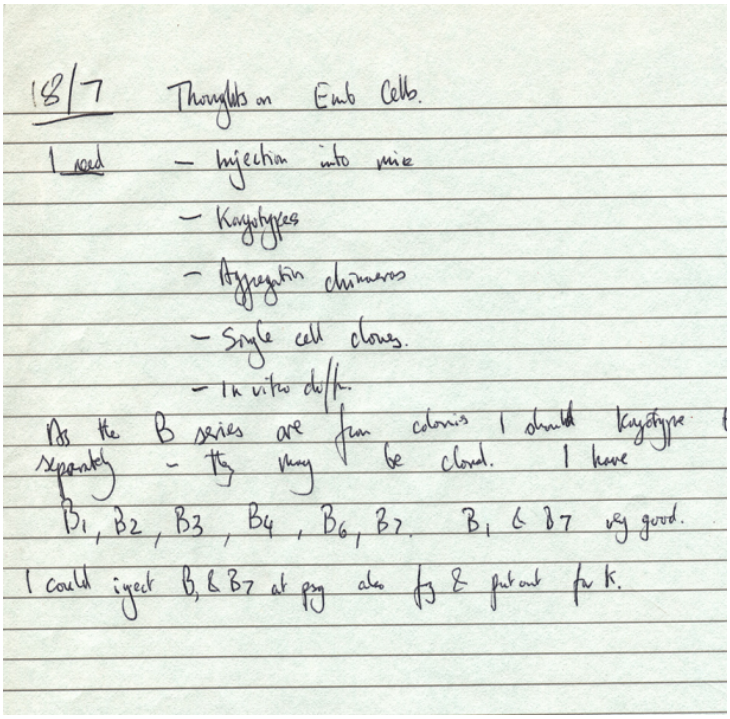

Figure 12: Notes from Evans' notebook about his expectations for isolated EK cells. From Evans' Nobel Lecture, 2007.

Morange also commented on the uses of mESCs after their isolation in 1981 in his 2006 discussion. Morange claimed that "ES cells were first seen as another way to generate transgenic animals"[918]; as noted in Chapter 4, this was the work initiated by Evans. In the mid-1980s, Evans visited the USA (in particular to see Richard Mulligan at MIT, and Oliver Smithies at Wisconsin) to look into methods of gene targeting and recombination via PCR (polymerase chain reaction). So successful was this line of work, that in 1989, Evans was able to publish a review of methods for mECCs and chimeric animals for genetic manipulation, which he claimed was "likely to have a major impact both in genetics studies and, especially if it can be extended to larger mammalian species, in practical applications"[919]. Manipulation of cell fate then was an important consideration in Evans' work.

Although Morange does not refer specifically to Martin's work on development, his argument is still valid: the scientific environment of the 1980s was different to that of the 1990s, and therefore motivations and goals had to be different. As discussed in Chapter 4, the improved ability to study development was a goal for the isolation and culture of mESCs by Martin (and Evans) – interest in development

[918] Morange, 2006 p 539.
[919] Evans, 1989 p 557.

would initially preclude any other motivations, however, Martin's 'pure' research was enabled by the political and economic situation of biomedical and biological research in the USA. One reason for this is that this identifies a need to move away from ECCs for research. When ECCs were initially isolated in the 1970s, they were praised as a viable, useful alternative to actual embryonic cells since they were accessible and could be practically used in the laboratory; further research would show that they were no more tumourigenic than other embryonic cells, and had a stable karyotype[920]. ECCs could also be used to generate chimeras, which would not develop any tissue abnormalities despite their oncogenic history.

Morange's suggestion that "a biochemical study of the differentiation process *ex vivo*"[921] may have been a motivating factor in the isolation of mESCs demonstrates that Morange implicitly considers developmental research (such as that carried out by Martin), however phrases it as research into *differentiation* as opposed to *development* (this again perhaps a reference to the value placed on cell fate research through the late twentieth century). Examination of Martin's published work, I argue, clearly shows that Martin was concerned with development (and particularly mammalian embryogenesis). For example, in 1985, Martin's laboratory published a paper which compared human and murine homeo-box genes, which have a role during development[922]. (Morange also highlighted the relevance of homeo-box genes in development research[923].) Martin has recently stated that the work she carried out in the 1970s and 1980s showed that mECC and mESC cells could be used to study embryogenesis, which led her to study mammalian *development* in the 1980s[924]. This shows that Martin was interested in cell fate research, in the context of embryogenesis.

Morange claimed that in the early 1980s, interest in mESCs was at "the fundamental level", and that there was no mention of extention to humans or medical research[925]. This, Morange argued, was because mESCs were isolated under a specific scientific context that would not apply to hESCs. For example, Evans was motivated by political, social, and economic factors to create murine disease models

[920] For example, see Mintz and Illmensee, 1975; see also Chapter 4.
[921] Morange, 2006 p 539.
[922] Hauser *et al*., 1985; also see Chapter 4.
[923] Morange, 2006.
[924] Martin, 2015; also see Chapter 4.
[925] Morange, 2006 p 537.

(see Chapter 4); no legitimate researcher would attempt to create human models of disease using the same method, so hESCs were of no use in this context. Morange is therefore correct – Evans' mESCs were sufficient for any research he wanted to carry out.

This leads us closer to answering why it took seventeen years to isolate hESCs after mESCs; Morange has argued that it was because there was a gap after mESC isolation before the realisation came that hESCs may be useful for medicine. This argument supposes that biological research is not carried out to establish 'fundamentals', but rather to ultimately provide more sophisticated medicines. As argued in Chapter 4 however, this is often encouraged in certain political and social climates.

3.3 Reflections on motivations and goals for isolation of hESCs

In 2006, Morange claimed that mESCs were used for fundamental research post-isolation, with little thought given to isolation of hESCs or potential medical benefits for several years. For some researchers, this appears to have been true; Evans could not create human models of disease (as he did with mice), and Martin could not culture large numbers of human embryos in her laboratory to study their development.

In this chapter, I have shown that just as Martin and Evans each had initial similar intellectual curiosity for development, leading to the isolation and culture of mESCs in the 1980s, Thomson and Gearhart had the same precluding motivation for the isolation and culture of hESCs in the 1990s. What differed however was the scientific context (as argued by Morange), and the social, political, ethical, economic, and legal contexts (as shown in Chapter 4 and this chapter). Martin, Evans, Gearhart, and Thomson were interested intellectually in development; the scientific, social, political, economic and legal context of the 1980s and 1990s however resulted in changing how each researcher was motivated to achieve their intellectual goals.

Thomson's work benefitted from the research carried out into culturing blastocysts for successful uterine transfer after IVF. (Ironically, whereas this research focused on attempting to keep the blastocyst alive and healthy in culture in order for more successful IVF, Thomson made use of this to allow blastocysts to mature prior to splitting open and extracting the ICM cells.) This IVF research was concerned with establishing normal development *in vitro* prior to uterine transfer – such research

then was heavily focused on establishing normal development under laboratory conditions. After Thomson had isolated and cultured hESCs, then demonstrated their ability to proliferate for extended periods of time and differentiate into cells from all three germ layers (i.e. the isolated and cultured cells were capable of both self-renewal and differentiation), Thomson made use of his cells for learning even more about development and cell fate – for example, after isolation and culture of pESCs, Thomson published a paper detailing his investigation of neural differentiation of pESCs, with a view to examining neural tube formation[926]. Thomson would work with hESCs to establish something similar regarding human development; in 2001, Thomson published a paper explaining that hESCs would differentiate and form neural tube-like structures[927].

Gearhart's work appears to have approached human embryonic cells from an entirely different angle. From the late 1970s, Gearhart had been studying Down syndrome, and in particular how the effects of human trisomy 21 affected embryogenesis. By the 1990s, Gearhart was a leading expert in mouse models of Down syndrome, however was becoming frustrated at the limitations of the murine models for studying a human condition. Discussions with colleagues suggested that using surplus IVF embryos for his research may be too controversial, leading Gearhart to return to his previous experiences with Wadsworth and dissections of both embryos and foetuses, and expertise in murine biology. Following Donovan's work isolating PGCs from mice, Gearhart attempted to carry out the same work in therapeutically aborted foetuses. Using these cells would, Gearhart believed, help him to more accurately model what was occurring during development in those with Down syndrome.

4. Conclusions

In 2006, philosopher of biology Michel Morange reflected on what had occurred in stem cell research on the twenty-fifth anniversary of the isolation and culture of mESCs in 1981. In the concluding remarks of the paper, Morange mooted

[926] Thomson, Marshall, and Trojanowski, 1998.
[927] Zhang *et al*., 2001. In this paper, it is noted that there is a potential for hESCs to be useful in transplant therapy. However, I do not believe that this is necessarily because this was a key motivation or goal for Thomson's work. As noted by Graham, it was clear that transplantation therapy was always a potential for isolated hESCs.

that the reason that there was a relatively long time between isolation of mESCs and hESCs (seventeen years), was because the "motivations and goals attendant upon their creation" were so different[928]. This chapter has been used to examine Morange's claim in detail, by looking in particular at the work by James Thomson and John Gearhart (since Chapter 4 has previously considered the work by Martin and Evans). Thomson and Gearhart both isolated human embryonic cells in the late 1990s, publishing their papers within days of each other in 1998.

The developmental biologist Thomson was working throughout this era, carrying out research focused on the role of genetics in embryogenesis – in particular, Thomson was interested in primate development. Frustrated with the mouse models available (where, for example, there are key differences between mouse and human in products of the placenta), Thomson tried to make use of non-human primate models. These however proved expensive and impractical; Thomson's solution was to culture the non-human primate embryo cells himself. This resulted in the isolation and culture of pESCs in 1995. Although Thomson waited to see whether another laboratory would be successful in generating hESCs using the same technique, no such results were forthcoming. Thomson applied to do the work himself, and, after agreement from his university, funding from Geron, and enough fertilised eggs, he began his work. Thomson reported the successful isolation and long-term culture of hESCs in 1998.

John Gearhart's approach was somewhat different. Gearhart's expertise in embryology were being utilised in Down syndrome research; by the 1990s, Gearhart was a leading expert in the field, where he was making use of mouse models to try and understand the effects of human trisomy 21 (trisomy 16 in the mouse) in development. Like Thomson, Gearhart found himself frustrated with the mouse model available, and believed that more could be learned from human embryonic cells. After discussion with colleagues, Gearhart decided that isolating stem cells from blastocysts would be controversial, and elected to obtain stem cells from a different source – PGCs. Recalling his previous experience in mouse research and anatomy of human embryos, Gearhart would take inspiration from Donovan's work isolating mPGCs. Gearhart's postdoctoral fellow, Shamblott, would work on the

[928] Morange, 2006 p 540.

therapeutically aborted foetuses, carefully dissecting out the hPGCs and culturing them, generating hEGCs.

Following the review of Thomson and Gearhart's work, a closer examination of the isolation and culture of mESCs and hESCs with regard to their motivations and goals was required. Morange argued that the motivations and goals between mESC and hESC isolation occurred because of the different scientific contexts of the 1980s and the 1990s. Although all four researchers (Martin, Evans, Gearhart, and Thomson) had similar intellectual goals (i.e. the study of development), they were motivated to achieve these in different ways. In addition, I have shown in this chapter and Chapter 4, that the researchers were particularly motivated to apply their research down particular routes based on the political, economic, ethical, and legal contexts they were working in.

In order to fully examine Morange's claim, I have revisited the motivations of Martin and Evans; Martin and Evans both had an intellectual focus on development. Although in the USA Martin was able to attract funding to carry out fundamental research without significantly changing her research programme, Evans, in the UK, where there was political pressure to fund applied research, had to re-direct his goal and study development in a way that would have practical outcomes. Evans' work resulted in the creation of GEMMD (for which he won a Nobel Prize). *Nature* writer Gretchen Vogel added that most research post-1981 focused on creating transgenic mice rather than culturing tissues, with only a few working on cell fate research. This work gained prominence post-1998 however when hESCs were isolated and cultured, and the work on mouse cells could be applied to human cells[929]. It can be seen from Vogel's commentary that cell fate research was considered to be a prominent feature of stem cell research up until at least the 1980s; a theory supported by this thesis. Although this thesis will also argue that cell fate research continues into the twenty-first century (see Chapter 6), it is clear from Vogel's comments that consideration of cell fate is a prominent theme of stem cell research.

Whilst the scientific context that Morange refers to is the same for both Evans and Martin, in Chapter 4 I demonstrated that other factors could influence the direction of intellectual curiosity. The history of hESC isolation provided in this chapter suggests that there were further different motivators for the isolation of

[929] Vogel, 2000.

hESCs in the 1990s. In his 2006 review, Morange already identified the different scientific contexts between the 1980s and 1990s, in particular the capacity of various cells to generate tumours, and how difficult the cells are to manage. Both Thomson and Gearhart had an intellectual interest in development – just like Martin and Evans. Thomson was keen to use hESCs to better understand human embryogenesis. Gearhart's isolation of hEGCs was motivated by the need to find a more useful model of Down syndrome during development. Morange's article highlights that the scientific context was different – particularly concerning the relationship between undifferentiated cells and tumourigenicity. Morange claims that after the early 1980s, this characteristic was forgotten, only to be rediscovered later. The political, social, economic, legal, and, in addition, ethical context of the work Thomson and Gearhart wanted to carry out were again different to the scientific climate of the 1980s, resulting in the motivations for their research (such as obtaining funding, working ethically) being different for Thomson and Gearhart as compared to Martin and Evans. This has been shown to be the case in this chapter.

Here, therefore, I offer a complementary addition to Morange's claim, and demonstrate that context is important for assessing the motivations and results of research. Although this chapter has shown that there are some parallels – all of the researchers had a fundamental interest in development and cell fate – these are outweighed by the different scientific, social, political, legal, ethical, and ecomonic contexts of science in the 1980s and the 1990s. The overall interest in development clearly precluded the differences in research direction taken by each individual. Evidence from Thomson and Gearhart suggest that the relatively long period of time between isolation and culture of mESCs and hESCs was significantly affected by ethical, legal, and ecomonical concerns (such as the influence of the Dickey-Wicker amendment); their narratives suggest that the research would have been carried out sooner if, for example, their research had been allowed immediately following application to their university boards. This chapter has shown that these contexts then influenced how the researchers were motivated to achieve their aims, and therefore supports Morange's view that the motivations and goals for the isolation and culture of mESCs and hESCs were different. This chapter has shown that motivations of researchers need to be considered in order to provide an accurate history that takes into account the 'before, during, and after' of research – i.e. the initial intellectual

curiosity, the motivations pushing research in a particular direction, and interpretation of results – to create an accurate, comprehensive historical account. The following chapter will consider how the interpretation of these results is also needed to appreciate a scientists understanding, and how this is used to develop theories and influence future experimental design. The following chapter will consider this in the context of whether stem cells are entities, and how cell fate can help to unpack this theory.

CHAPTER 6:
ARE STEM CELLS ENTITIES?
A VIEW FROM HISTORY

1. Introduction

The aim of this last chapter is to examine whether stem cells are entities, or whether 'stemness' is a state, or phase, that cells move through during their lifetime. It has been assumed, since the idea of a stem cell first arose (Chapter 1), that the stem cell was a physical entity. Initially, Haeckel suggested that this was the first unicellular organism from which all multicellular organisms developed. A little later, Haeckel also used the term *Stammzelle* to refer to the fertilised egg – a single cell with the potential to create an entire organism. Since the mid-nineteenth century, theoretical, then later experimental work (such as those experiments described earlier in this thesis), relied on or were designed on the basis that stem cells were entities – there was a specific population of cells which that had properties of self-renewal and differentiation, and that could be isolated and cultured in the laboratory.

However, at the beginning of the twenty-first century, a new theory regarding stem cells emerged: the idea that stem cells are not entities. Instead, the theory proposed that all cells have the ability to become stem cells. This required cells to change their state in order to become a cell type capable of both self-renewal and differentiation. This theory proposed that there is not a single 'pool' of stem cells that remain in the adult body for repair and maintenance, but that any cell can move into a stem cell state as required; this acquiring of stem cell properties (i.e. self-renewal and differentiation) has been referred to as 'stemness'.

The stemness view has been advocated particularly by Lucie Laplane as a tool through which CSCs could be targeted for treatment. In her 2014 chapter *Stem cell epistemological issues*, Laplane set-out her philosophical arguments supporting the stemness theory, and noted some recent experimental work also potentially supporting the need for a different way to think about stem cells. In this chapter, I expand on the ideas and experimental work highlighted by Laplane. Laplane began her chapter by considering whether it is possible to distinguish stem cells from non-stem cells by looking at the properties of self-renewal and differentiation. In particular, to establish this, Laplane considered whether there are molecular markers of stem cells, referring to the findings of three research groups, led by Lemischka at Princeton, Melton at Harvard, and Lim at Singapore. I also examine the results of these groups in this chapter, to determine whether any molecular marker of stemness has been identified. Laplane then asked whether a stem cell could be a natural kind, making use of

Richard Boyd's 'homeostatic property cluster' and the defense provided by Wilson, Barker and Brigandt that stem cells are natural kinds (if the homeostatic property cluster method is used). Devloping on Laplane's work, I conclude that the property cluster provided by Wilson, Barker, and Brigandt is not specific enough to separate non-stem cells from stem cells, and therefore does not support the stem cells as natural kinds argument. Considering her account of the lack of molecular markers of stem cells, and the arguments against stem cells being a natural kind, Laplane then debated whether "the concept of stem cell refers to "*entities*" or it refers to a particular cell "*state*""[931]. In order to explore this concept further, in addition to looking at the molecular marker and natural kind arguments covered by Laplane, I also consider the role of the niche, and what we can learn from ESCs and plasticity[932]. In order to add to Laplane's work, I consider whether this new approach to stem cells can be applied to historical research, and whether the results of historical ESC research in particular can be re-interpreted in light of this potentially paradigm-shifting theory.

2. Definitions of stem cells

"Descending, as cells thus do, from an original mother-cell, and this by cleavage of the nucleus of that mother-cell, and all subsequent nuclei being propagated in the same way, by fissiparous generation…every nucleus…is a sort of *centre*, inheriting more or less the properties of the original nucleus…and exercising an assimilative power".[933]

"The name 'stem cell' seems to me the most simple and appropriate one, because all other cells stem from it and because it is in its most literal sense the stem father as well as the stem mother of all the countless generations of cells of which the multicellular organism is later composed".[934]

"This stem-cell maintains its own existence by uninterrupted multiplication, on the one hand, and on the other it differentiates…".[935]

The definition of a stem cell as typically used in late twentieth and early twenty-first century biology was written by Russian biologist Vera Danchakoff in

[931] Laplane 2014 p 700.
[932] Laplane also considers SCNT and iPSCs in her 2015 paper, *Reprogramming and Stemness*.
[933] Barry, 1847 p 213.
[934] Haeckel, 1877 p 144 (transl. Maehle, 2011).
[935] Danchakoff, 1916 p 401.

1916. As demonstrated in the selected quotations above however, Danchakoff was not the first to identify that there appeared a requirement for a cell population to exist which was capable of both self-renewal and differentiation. In the 1840s, considering early embryonic development and the role of the nucleus, English physician Martin Barry suggested that all cells descended from the fertilised egg, and all of the information needed to create the adult was contained within the nucleus (the product of mixing the parental germ cells)[936]. The first to use the term *Stammzelle*, or 'stem cell', was Ernst Haeckel, in his lectures on *Natürliche Schöpfungsgeschichte*, published in 1868; Haeckel used the term to refer to unicellular organisms, which he believed to be the phylogenetic ancestors of multicellular organisms. The quotation given above is from a later text by Haeckel, *Anthropogenie* (1877), where he identified a stem cell as the fertilised egg (in order to differentiate this from the pre-fertilised egg)[937]. The last quotation above is from a paper on the origin of blood, by Danchakoff, written soon after her move to the USA from Russia. Danchakoff also used the term 'mother-cell' in her work; Danchakoff was not the only academic to do this. For example, Harvey E Jordan (1878-1963) would use the term mother cell to refer to those cells at the base of the epidermis that would give rise to other cells of the skin[938]. During the early years of the twentieth century then, 'stem cell' referred to the fertilised egg, or embryonic cells, whilst those which we now call adult stem cells, were referred to as mother cells.

Danchakoff's succinct definition, considering both the potential for asymmetric division and differentiation into several cell types, is typical of definitions and descriptions still in use in the twenty-first century; for example:

"What is a stem cell? The traditional answer to this question is that a stem cell has two properties: the ability to self-renew and the potential of differentiation".[939]

"Stem cells are defined as cells that can give rise to more cells like themselves, as well as more specialized, or differentiated, cells".[940]

[936] See Chapter 1.
[937] See Chapter 1, and Maehle, 2011.
[938] For example, "A densely packed mother cell of [the basal] layer gives rise to two daughter cells of very similar constitution which are only slightly altered as they pass to the upper layers". Jordan, 1911 p 463.
[939] Laplane, 2014 p 693.
[940] Fagan, 2013a p 1147.

"HSCs [haematopoietic stem cells] make a choice of either self-renewal or committing to differentiation. The balance between self-renewal and differentiation is considered to be critical to the maintenance of stem cell numbers".[941]

In both philosophy of biology and biological science then, the potential for self-renewal and differentiation are typical properties of those cells referred to as stem cells.

2.1 The potential for self-renewal

Self-renewal is understood as the creation of one or two daughter cells that share the same properties as their parent cell. Philosopher of biology Melinda Fagan has referred to this as *sameness*[942] (which implies comparison against a set of characteristics). Fagan noted initially that no two cells can ever be the same in all respects (for example, cells may differ in position or in their interaction with the cells around them). In this instance however, sameness refers to a set of characteristics that Fagan calls *traits*. For Fagan, "Self-renewal occurs within cell lineage L relative to a set of characters C for duration τ, if and only if offspring cells have the same values for those characteristics as the parent cell(s)"[943].

2.2 The potential for differentiation

As the original parent cell divides, one or two daughter cells created may not share all of the same properties as their parent; Fagan referred to this as *difference*, again comparing a set of characters (C), which vary between two time points (t_1-t_2), including $0 \leq \tau \leq \infty$ cell divisions[944]. The other *difference* Fagan noted was the process of differentiation, which caused cells to become increasingly different from their parent, whist becoming increasingly similar to specialised, or mature, cell types[945]. As highlighted by Laplane, such *difference* is not sufficient to claim that *differentiation* has occurred however; instead, in addition to being different to its parent, the daughter cell must have also changed in a particular direction (on its way

[941] Mosaad, 2014 p 68.
[942] Fagan, 2013b.
[943] *ibid* p 22.
[944] *ibid* p 23.
[945] *ibid*.

to developing into a specialised cell). Differentiation therefore implies a directional change *and* diversification[946].

2.3 Is the current working definition enough to differentiate stem cells from non-stem cells?

Neither the processes of self-renewal or differentiation are specific to stem cells. Some cells that are not currently classed as stem cells have the potential to self-renew (such as macrophages[947]), whilst others have the potential to differentiate (neural progenitor cells, for instance[948]). However, Seaberg and van der Kooy (for example) have argued that every cell that have the properties of *both* self-renewal and differentiation are stem cells, relying on these properties as practical indicators of stem cells (i.e. a useful concept to identify, isolate, and utilise stem cells in the laboratory)[949]. There is a problem with this however, as highlighted by Fagan. Cells reproduce by division; when this occurs the parent cell ceases to exist and only two daughter cells remain. These daughter cells may either be copies of its parent (i.e. demonstrating self-renewal), or be differentiated. Assymmetric division cannot be achieved under experimental conditions because of the different environments required for self-renewal and differentiation. At the single cell level then, both properties cannot be demonstrated simultaneously in the laboratory[950]. Other scientists have argued that there is too much heterogeneity amongst 'stem cell' populations to support the position of self-renewal and differentiation as being finite enough properties to isolate stem cells (in the laboratory)[951]. The following sections of this chapter will consider various examples to demonstrate that whilst the self-renewal and differentiation definition may have been useful at the beginning of the twentieth century, it may be beneficial to consider 'stemness' as a state, or function, at the beginning of the twenty-first.

[946] Laplane, 2014 p 695.
[947] Soucie *et al*., 2016.
[948] Varga and Nagy, 2017.
[949] Seaberg and van der Kooy, 2003.
[950] Fagan 2013a p 1152. Single cell transplants achieving organ regeneration in living organisms may be a counter-example to this, but *in vivo* self-renewal can only be inferred (not measured) and differentiation potential cannot be determined (Fagan, 2013a p 1157).
[951] Blau, Brazelton, and Weimann, 2001.

3. Natural kinds

In her 2014 chapter *Stem cell epistemological issues*, Laplane sought to clarify the stem cell concept, whilst emphasising its ambiguities[952]. Laplane began by discussing the properties of self-renewal and differentiation, then assessing whether this meant that they would belong to a natural kind. In particular, Laplane assesses the validity of the claim made by Wilson, Barker, and Brigandt that, using the homeostatic property cluster approach (typically utilised to argue for natural kinds in biology), stem cells are a natural kind. Fagan has also commented on Wilson, Barker, and Brigandt's assessment; since this has therefore demonstrated itself to be a key issue in stem cell philosophy, it deserves consideration here. In this section, I expand on Laplane's and Fagan's work with further exploration of the homeostatic property cluster approach to designating natural kinds, and exploring the application of this to stem cells.

Laplane and Fagan are not the only philosophers who have asked whether cell types can be a natural kind. In his 2013 paper, *Cell types as natural kinds*, Matthew H Slater considered various arguments to establish whether different cell types, such as blood cells, or muscle cells, could be natural kinds. Slater asked on which grounds cell types could be separated, suggesting, for example, developmental history (similar to separating species based on their evolutionary history), whether the cells have particular structural features, or their function. This latter may be problematic since cell function is not fixed, but is temporally and environmentally dependent (for instance, the stage in a cell's cell cycle will affect its function and structure)[953]. Even considering genetic homogeneity (particularly since the genetic paradigm is so influential in current biological science) fails to enable us to separate species or cell types (for example) enough to be used as a categorising tool. Highlighting the issue that arises with categorising or separating many things in biology, Slater noted that biological diversity is very different from, for instance, chemical diversity, where each element is clearly distinct from each other. In biological organisms, or cell types, there is no "continuum of similarity", and perhaps then suggesting that there is no biological 'essence'[954]. Slater highlighted that there are few examples of

[952] Laplane 2014 p 693.
[953] Slater, 2013.
[954] Slater, 2013 p 170.

biological essences that have been suggested, in comparison to chemistry, which appears to be littered with natural kinds.

The claim that stem cells are natural kinds implies that

a) stem cells exist in nature, and
b) stem cells belong to a kind - all stem cells share common properties that make them similar to each other, and different from other cell types.

As previously noted, the properties of self-renewal and differentiation are not enough to support the above, since other cells are capable of these properties; i.e., stem cells cannot be a natural kind.

There are those who disagree with this logic however, based on other agreed natural kinds in biology. In particular, discussions concerning natural kinds in biology have previously focused on the 'species problem' (i.e. are species definitions natural divisions, or are they constructed?). The theory of evolutionary development has also suggested that a species cannot be a natural kind, since, by definition, species are constantly changing. If this is the case, species cannot have a static set of intrinsic properties which make them the same as each other but different from other natural kinds. As philosopher David Hull (1935-2010) observed, taxonomy was suffering because of the difficulty in identifying properties of species that were both necessary and sufficient[955]. Similar arguments can be used to argue against cell types being natural kinds. In response to this, Hull instead discussed the use of 'clusters' (as opposed to the previously dichotomous phylogenetic taxonomies)[956]. Ian Hacking (1936-) proposed that a natural kind is:

a) defined by a set of necessary and sufficient properties (relations etc.) such that
b) the possession of these properties is, as a matter of fact rather than logic, indicative of a very large number of other methodologically interesting properties, such that
c) these defining properties are natural rather than social properties[957].

The theory of property cluster kinds do not appeal to Hacking since the properties in the cluster are not natural, but rather defined by human social decisions[958]. In response, Richard Boyd (1942-) developed the idea of the

[955] Hull, 1965.
[956] *ibid*.
[957] Boyd, 1991 p 127; Hacking, 1991.
[958] Boyd, 1991 p 140-141.

"homeostatic property cluster" to define a set of properties that often co-exist in nature[959]. Boyd argued that there are "scientifically important" (i.e. natural) properties, which are not clustered by social decision, since the determination of the cluster is "causal rather than conceptual" (i.e., are not accidental and homeostatic)[960]. Boyd argued that the properties he promoted for his approach needed to be "causal homeostatic mechanisms"[961]; only kinds elucidated from such properties would "cut the world at its joints"[962]. To test his theory, Boyd considered the species problem, arguing that his homeostatic property clusters support the claim that biological species are natural kinds, since they share "homeostatically related morphological, physiological and behavioral features which characterize [their] members"[963]. The homeostatic property cluster then also allows for evolutionary development and speciation, which requires the existence of populations intermediate between the parent species and emerging species[964]. The homeostatic property cluster, argued Slater, continued what he refered to as a "bottom-up" approach, since it was developed around causal mechanisms; although not perhaps traditional essentialism, Boyd's homeostatic property cluster account used essentialism as a starting point[965].

The homeostatic property cluster approach appears then to support the view that cells are natural kinds, with a population of parent cells, a population of intermediate cells, and a population of specialised, differentiated cells. I argue that this supports the view that *stem* cells are not natural kinds, but that stemness is a property that falls into the *cell* homeostatic property cluster.

As highlighted by Laplane, Robert Wilson, Matthew Barker and Ingo Brigandt however have utilised Boyd's homeostatic property cluster as support for stem cells as a natural kind. Wilson, Barker, and Brigandt identify stem cells as heterogeneous, however believe that a certain combination of properties, which are specific to stem cells, can define them (as a natural kind):

[959] Boyd, 1999.
[960] Boyd, 1991 p 141.
[961] Slater, 2013 p 175.
[962] Boyd, 1991 p 139.
[963] Boyd, 1999 p 142.
[964] *ibid* p 142.
[965] Slater, 2013.

- morphologically undifferentiated
- ability to self-renew (cell division with at least one daughter cell of the same type) over an extended period of time
- ability to give rise to various differentiated cell types (pluripotency, or at least multipotency)
- developmentally derived from certain cells or tissues
- located in specific parts of tissues
- particular complex profile of gene expression and presence of transcription factors
- found in a certain cellular-molecular microenvironment ("niche"), which influences the stem cell's behaviour
- low rate of cell division[966]

As stated in the definition of the homeostatic property cluster theory, none of these properties are necessary, whilst different subsets are sufficient, for a cell to be defined as a stem cell, and a natural kind[967]. Slater tested ways of categorising cell types in his paper to examine whether the homeostatic property cluster method might enable us to view cell types as natural kinds. In one example, Slater tested whether genetic properties may be used to separate out biological things, particularly, as he suggested, genetic properties are the "obvious candidate for essences"[968]. Perhaps subconsciously, Wilson, Barker, and Brigandt have been influenced by the strong genetic paradigm influencing current biology when generating their property cluster. For example, they refer sepecifically in one property to the complex gene expression profile; a lack of genetic homogenetity has already been identified as an issue with enabling the classification of different biological species as natural kinds. A further problem with the property cluster provided by Wilson, Barker, and Brigandt is that it refers to the developmental history of stem cells. As highlighted by Slater, at the moment, we do not know enough about the development of individual cell types in the body for this to be an appropriate property. A single cell type may arise from a variety of regions in the body – dedifferentiation and redifferentiation being possible (as demonstrated by iPSCs – see below) suggests that many differentiated cell types may be capable of reverting back to a stem cell-like state – dedifferentiating – and

[966] Wilson, Barker, and Brigandt, 2007 p 208.
[967] Laplane, 2014.
[968] Slater, 2013 p 172.

becoming a different cell type – redifferentiating – afterwards. Morphology could also be a problematic property to usefully identify stem cells.

Not only are there some problems with the properties identified, but a further difficulty arises when other cell types, not currently considered to be stem cells, also have properties that are subsets of the above definition. Laplane has argued that Wilson, Barker, and Brigandt's definition then is not specific enough to "provide any positive demonstration that stem cells do belong to a natural kind"[969]. Furthermore, Fagan has argued that Wilson, Barker, and Brigandt's list is unsatisfactory since it offers no explanatory or predictive power, ignores the differences between *in vivo* stem cells and those artificially created in the laboratory (such as iPSCs), and lastly gives no guidance to diversity (i.e. the properties on the list are too ambiguous)[970]. It requires more than having a set of propeties in common (more or less) for something to be a natural kind; the somewhat essentialist homeostatic property cluster approach should explain why natural kinds are projectable[971]. The arguments presented above suggest that this is not the case. Slater concluded that the homeostatic property cluster approach was "not well suited…to accommodate our classificatory practices regarding cellular kinds"[972]. Slater offered an alternative: the stable property cluster account of natural kinds, which "avoids the vagueness and theoretical difficulties involving causal mechanism" attributed to the homeostatic property cluster approach[973]. The aim of Slater's stable property cluster approach is to enable philosophers to focus on the stability of natural kinds, as opposed to dwelling on the way in which stability may be achieved[974]. Slater conceded however that even his new approach may not enable all cell types to be defined as a natural kind. Although he believed that there is a "common phenomenon" behind our conceptualisation of erythrocytes being a kind of item, just as one would conclude an electron to be[975] (and this may be explained by the stable property cluster account), Slater does not appear convinced that a cell type may neatly be defined as a natural kind.

If we can not use the homeostatic property cluster (or the stable property cluster) to establish that stem cells are natural kinds, this, claimed Laplane, leaves

[969] Laplane, 2014 p 700.
[970] Fagan, 2013a p 1149.
[971] Slater, 2013.
[972] *ibid* p 176.
[973] *ibid*.
[974] Slater, 2013 p 177.
[975] Slater, 2013 p 178.

biology in an uncertain state concerning the ontology of stem cells: is stemness the essence required for a stem cell natural kind, or is stemness a property that can be transiently expressed by many cells[976]? Based on their critiques, Slater, Fagan, and Laplane appear to agree that stem cells are not a natural kind, also unconvinced by Wilson, Barker, and Brigandt, or the general approach of the homeostatic property cluster to identify cell types as natural kinds.

4. The niche

The 'niche' was a term used initially in the context of stem cells by Schofield, specificially referring to the nature of haematopoietic stem cells. In his 1978 paper, the first to use the term 'niche' in this context, Schofield assessed whether the colony-forming units identified by Till and McCulloch were stem cells[978]. Schofield hypothesised that the haematopoietic stem cell becomes "essentially a fixed tissue cell" when it is in close proximity to other cells in the bone marrow[979]. "The cellular environment which retains the stem cell", Schofield continued, "I shall call a stem cell 'niche'"[980]. When the stem cell is in this 'fixed' state, it can only produce copies of itself (i.e. self-renewal), and not differentiate. Where there is no niche, a resulting daughter cell will differentiate. Schofield implied that the size of the niche dictates how many stem cells there are in the pool. By Schofield's initial definition then, and the way that 'niche' has been interpreted since, it is an active participant in the functionality of stem cells. Further evidence supporting the niche theory would be found in invertebrates, specifically *D. melanogaster* and *Caenorhabditis elegans*. In these species, germ cells were observed to reside in a very particular distal end of a tapered structure in the gonads, where they were able to communicate with other somatic cells[981] (although it is currently understood that similar contact with a

[976] Laplane, 2014 p 700.
[978] Schofield concluded from his findings that CFU-S (colony-forming units – spleen) cells are not stem cells, since their ability to self-renew appears to be finite. Schofield, 1978. For a review of Till and McCulloch's work identifying CFU-S cells in the mouse, see Lancaster, 2009.
[979] Schofield, 1978 p 13.
[980] Schofield, 1978 p 13.
[981] For example, see Xie and Sprading, 2000; Crittenden *et al*., 2002.

basement membrane may also be an active participant of the microenvironment[982], and the extracellular matrix is likely to have a role too[983]). If, as suggested previously, stem cells are to be defined by their function, our current understanding is that it is often the niche that controls such functions (i.e. self-renewal and differentiation). For example, in adult mammals, haematopoietic stem cells circulate freely, but can only self-renew and differentiate in specific locations, in response to biological cues[984]. Generally, the role of the stem cell niche is to provide a microenvironment to enable the cells that reside within to maintain their stem cell properties. Stem cells are also required to react to changes in their system or surrounding tissue; the niche also has a role to play here.

Many niches have now also been identified in mammalian tissues, including the bone marrow, subventricular zone (in the brain), and hair follicle bulge, for example. Just as the expression of particular genes or proteins have been linked with maintaining 'stemness' in stem cells (see section 6), several extracellular factors have been purported to have a role in maintaining the microenvironment of various stem cell niches, such as fibroblast growth factor in the bone marrow[989], tenascin C in the brain[990], and β1 integrin in the skin[991]. Varying numbers of such factors are important in controlling proliferation and differentiation of cells in the niche. For example, the protein Sonic hedgehog[992] is required to distribute different subsets of skin cells to either the interfollicular epidermis or the hair follicle bulge[993]. The factors affecting cells in the niche (or directing cells towards the niche) are not limited to proteins however. For example, at the point where the two cell types responsible for bone remodelling (osteoblasts and osteoclasts) meet, there is a high concentration of calcium ions (Ca^{2+}). Bone marrow haematopoieteic stem cells are particularly sensitive to surrounding calcium levels, since they have calcium-sensing receptors in their membranes. Genetically engineered mice, created to lack these calcium-sensing

[982] For example, there are cells in the gut of *Drosophila* that have the ability to self-renew and differentiate, and are primarily in contact with a basement membrane (which separates the stem cells from surrounding muscle cells). Ohlstein and Spradling, 2006.
[983] For example, see Jenson, Lowell, and Watt, 1999.
[984] Scadden, 2006.
[989] For example, Li and Li, 2006.
[990] For example, Garcion *et al*., 2004.
[991] For example, see Tumbar *et al*., 2004.
[992] A protein involved in vertebrate embryo organogenesis, cell division in the adult, and possibly cancer development. For a review, see Varjosalo and Taipale, 2008.
[993] For example, see Levy *et al*., 2005.

receptors, demonstrated that haematopoietic stem cells were unable to populate the bone marrow[994]. The niche appears to have a further function in the haematopoietic system: stem cell trafficking[995]. Whilst cell entry and exit to and from the niche is important biologically, it has also provided a useful model for experimentation. For example, blood cell production in the early foetus begins in the liver (possibly from cells which migrate from the placenta and / or yolk sac [aorto-gonadomesonephros]), before moving to the bone marrow later on in development. It is thought that calcium signalling is an integral part of this process, since mice genetically modified to lack calcium-sensing receptors do not have the movement of haematopoietic stem cells away from the foetal liver to the bone marrow[996]. The above example shows that migration and trafficking are essential to haematopoietic development and function, which are only possible due to a functional niche.

In addition to maintaining stemness then, can the niche induce it? Experiments carried out in fruit flies suggest that this may be a possibility. For example, experimentally removing the stem cells from the ovarian germ cell niche resulted in re-occupation of the niche by somatic cells, which began proliferating[997]. Furthermore, alteration of the niche's microenvironment induced mature progenitor cells to revert to stem cells[998]. Similar research has been carried out investigating the niche of mouse testes, where similar dedifferentiation has been observed[999].

It is also possible that a misplaced niche may result in cancer, where a vacancy may result in occupancy by a somatic cell, or where altered niche signalling may increase proliferation or induce dedifferentiation, resulting in significant disruption in the tissue. Despite some suggesting that the cancer stem cell concept is only a few decades old[1000], Robert Remak proposed in 1854 that cancer may arise from "misplaced somatic cells comprising embryonic "rests" or residues" which had not

[994] Adams *et al*., 2004.
[995] Trafficking of cells in the body is a process whereby signals or 'honing mechanisms' cause cells to deliberately move from one region to another. In other instances, cells may migrate; this movement is passive, as cells are not provided with any honing signal. For example, the haematopoietic cells which populate the liver in the foetus migrate to the liver; the haematopoietic cells which move from the liver to the bone marrow later in development are trafficked. For example, see Moore, 2001.
[996] Adams *et al*., 2004.
[997] Kai and Spradling, 2003.
[998] Kai and Spradling, 2004.
[999] Nakagawa, Nabeshima, and Yoshida, 2007.
[1000] For example, Scadden, 2006.

migrated to the appropriate region of the body[1001]; a theory popularised by Julius Cohnheim. Another German pathologist, Hugo Ribbert, added to the embryonic rest theory, suggesting that cancer could arise from cells which "had a disturbed relationship with their neighbours", causing abnormal proliferation[1002]. (For more on the embryonic rest theory of cancer, see Chapter 3.) The niche then, initially understood as a place of stem cell residence, has more recently been shown to have an active role in proliferation, differentiation, and trafficking. Cells moving into the niche can adopt the ability to redifferentiate and self-renew, supporting the state view of stemness.

5. Embryonic cells

A particular type of embryonic cell, the totipotent cell, has been the victim of "surprising confusion" particularly with regards to the ethics of research[1003]. Much of this confusion arises given the conflicting definitions given of totipotency, in comparison to what experimental procedures have indicated their properties to be. For the purposes of this section, totipotency is taken to be the capability of differentiating into any cell type of the adult organism, in addition to extra-embryonic cells (such as the placenta). These are the cells that arise from the first few divisions of the fertilised egg (zygote) (and maybe only up to the four-cell stage); by five days post-fertilisation, there are cells in the blastocyst that are no longer capable of developing into extra-embryonic tissue, nor other components of the blastocyst itself. These cells, the cells of the inner cell mass (ICM), are now pluripotent, capable of producing those cell types observed in the adult[1004] (Figure 13). I argue that the totipotent cells, those first few daughter cells of the zygote, cannot be considered stem cells if the traditional definition of self-renewal and differentiation is used.

[1001] Krebs, 1947 p 270.
[1002] Witkowski, 1983 p 271; see also Maehle, 2011.
[1003] Condic, 2014 p 796.
[1004] For a brief overview, see Condic, 2014.

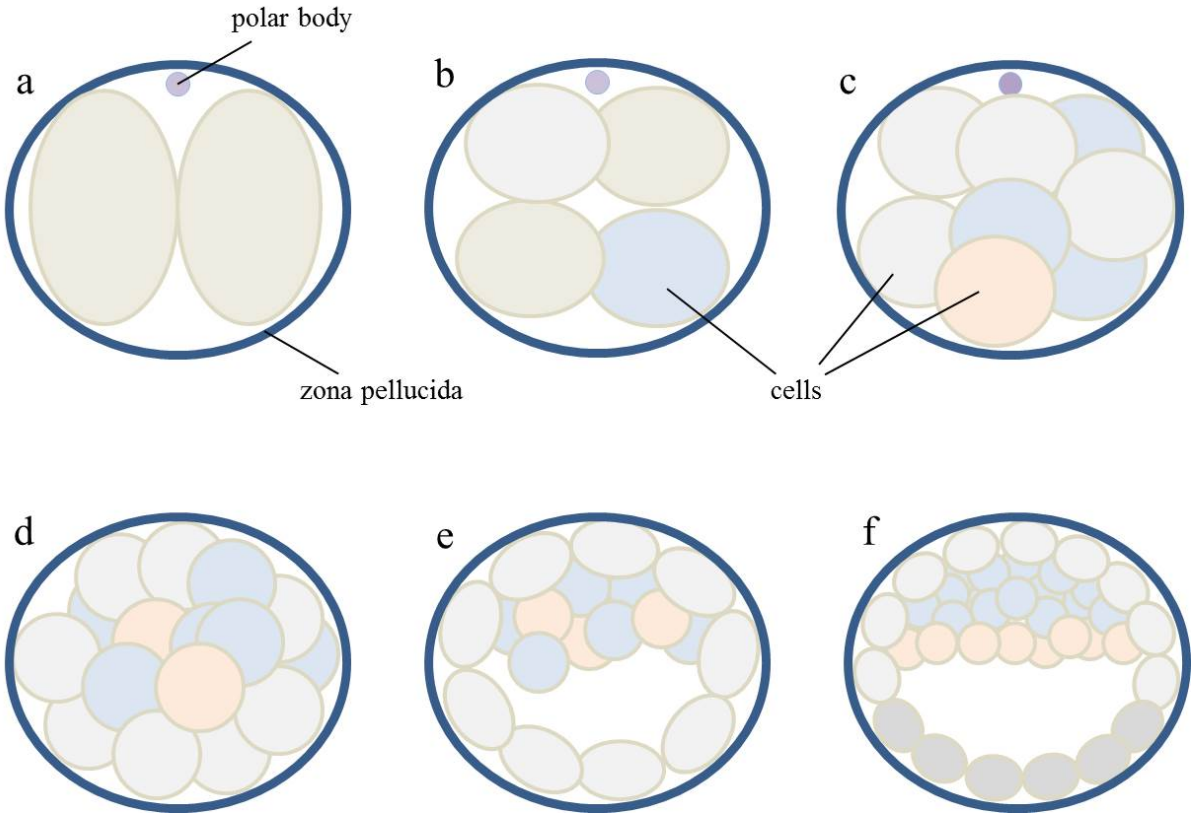

Figure 13: A diagrammatic representation of development of the early embryo, from the two-cell stage, to development of the blastocyst. (a) shows the embryo at the two-cell stage, surrounded by the zona pellucida. (b) and (c) shows the four-cell stage and eight-cell stage respectively, where there has been some differentiation of the cells. After three cell divisions (c), there are no totipotent cells remaining, since all cells at the eight-cell stage have started to differentiate. The polarity of the embryo is also established at this stage, and when the blastocyst begins to form (e), the inner cell mass compresses on one side of the embryo, whilst the trophectoderm cells move to the outside. Once organised (f), the blastocyst contains four cell types: the polar trophectoderm (light grey), the mural trophectoderm (dark grey), the primitive endoderm (pink), and the epiblast (blue).

It is true that totipotent cells have a greater ability to differentiate than any other cell type, since they can contribute to (and organise) all cells of the developing embryo and adult, plus extra-embryonic tissues. However, this totipotency is lost after the first two cell divisions, with polarity being established after the third round of cell division (in mice)[1005]. During the fourth and fifth cell divisions, asymmetrical division is again possible[1006], resulting in the formation of 'outside' and 'inside' cells,

[1005] See Takaoka and Hamada, 2012, for an overview. Establishing polarity early on in embryonic development is essential, as different cell types develop at each pole, and later this will orient head and tail of the mammalian embryo.
[1006] Tarkowski and Wroblewska, 1967; Johnson and Ziomek, 1981.

creating the morula[1007] (Figure 14). By the blastocyst stage (at five to six days post-fertilisation in humans), there are at least four cell types present: polar trophectoderm, mural trophectoderm[1008], primitive endoderm[1009], and epiblast[1010] (the latter two cell types forming the ICM). All of these cell types can be identified by the different genes expressed[1011].

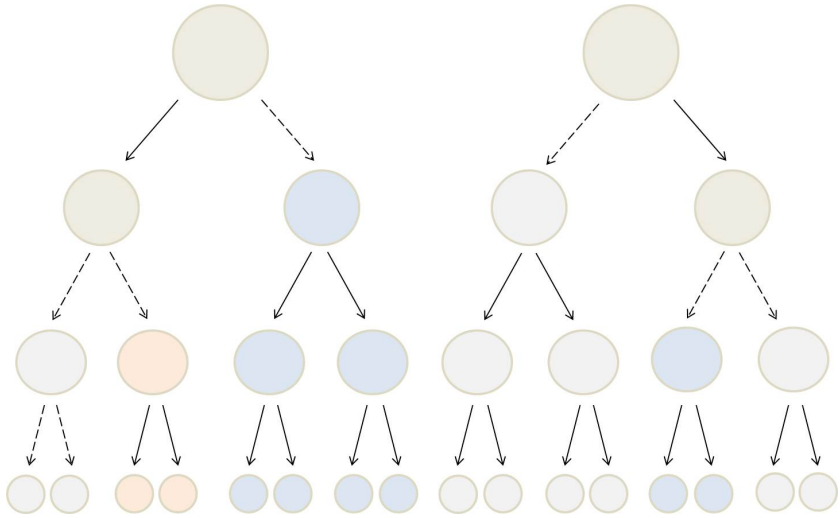

Figure 14: A diagrammatic representation of the possible lineage of cells of the early embryo up to the sixteen-cell stage (four cell divisions). Solid arrows show self-renewal, and dashed arrows show differentiation. No totipotent cells (beige) remain after the four-cell stage.

What is clear from our current understanding of mammalian development is that the ability of the totipotent cells to divide asymmetrically, creating a copy of itself and a more differentiated daughter cell, does not occur. Instead, changes in gene expression as early as the eight-cell stage result in different cell types with different differentiation potentials. By this stage of development then there are no totipotent cells remaining; if stem cells are capable of self-renewal as well as differentiation, there should be a remaining totipotent cell population in the developing embryo, and the adult. However, this is not the case, and no totipotent cells remain by the third cell division post-fertilisation. Totipotent cells are therefore

[1007] Nishioka *et al*., 2009.
[1008] The trophectoderm is the epithelial outer layer of the blastocyst; the polar trophectoderm lines the ICM, whilst the mural trophectoderm lines the blastocoel, the fluid-filled space adjacent to the ICM.
[1009] This is a layer of epithelial cells which contributes to further development in the post-implantation embryo.
[1010] These cells will give rise to the three germ layers (ectoderm, endoderm, and mesoderm).
[1011] For an overview, see Tarkowski and Wroblewska, 1967.

not stem cells if one adopts a definition for stem cells that require self-renewal and differentiation as properties.

This appears to be something of a contradiction however; how can the first cells created as the zygote divides not be stem cells? After all, the fertilised egg was considered to be the ultimate *Stammzelle* as Haeckel developed his concept: the cell all other cells stem from. Our current understanding however causes totipotency to support the state view of stemness.

Why is it then that cells in the embryo appear to lose stemness (as in the totipotent example given above) when cells of the adult are quite capable of gaining stemness (see sections 4, 7)? Although there are one or two examples of commitment reversal reported in the embryo[1012], this is at a very low frequency in comparison to the occurrence in the adult. Biologist Dov Zipori suggested that this may be a safety mechanism: that reversibility during embryogenesis would "hamper the process, and introduce a huge and intolerable number of mistakes"[1013]. The program of embryogenesis is supposed to be unidirectional, and too much potential for commitment reversal could be harmful. Instead, it is kept to a minimum, particularly during very early development (as described above). Alternatively, Zipori claimed, such plasticity is a useful property. The adult is more defined in size and structure, where mistakes from commitment reversal could be less detrimental. However, as highlighted in Chapter 3, studies into the likelihood of a cancer stem cell (CSC) propose that not only may an adult stem cell develop cancer properties (uncontrolled differentiation, for example), any cell may dedifferentiate enough to become a CSC, and generate a tumour (or tumours). Cancer has been described as "a derangement of normal tissue homeostasis", demonstrating unregulated proliferation and abnormal differentiation[1014]. Tumours have often been described historically as having an undifferentiated appearance, which supports the notion that perhaps dedifferentiation is occurring. The pathways that control mechanisms of differentiation and self-renewal are the same in both normal and pathological tissue growth, as highlighted in Chapter 3. In some cancer theories of the latter half of the twentieth century, cancer was caused by uncontrolled self-renewal of progenitor cells; more recently, the

[1012] Such as epithelial cells dedifferentiating, or the epiblast cells being less potent than cells of the later, gastrulating embryo (Zipori, 2009 p 201). See Blanpain *et al*., 2004 and Rossant, 2008.
[1013] Zipori, 2009 p 201.
[1014] Daley, 2008 p 171.

possibility of dedifferentiation and the presence of a CSC have gained support, and the seach for this cell began.

Summarising the evidence provided for the potential of somatic cells to dedifferentiate (and become CSCs), Daley provided an overview of those tissues where the CSC was clearly implicated in cancer development. In particular, epithelial tissues (such as the gut and skin) and blood tissues have well-known cancers that may arise from mutations in a single stem cell, or from a progenitor cell. These progenitor cells would normally have a limited capacity for self-renewal and differentiation, however "alterations in the physiology" of these cells may make them candidates for the CSC[1015]. Studies of some types of leukaemias suggest that there is a genetic change in progenitor cells that change them into cells with stem cell properties; for example, granulocyte-macrophage progenitor cells may acquire a mutation in the oncogene MLL-AF9. This oncogene is associated with human acute myeloid leukaemia; those granulocyte-macrophage progenitor cells that begin expressing this gene begin self-renewing in a pattern typical of haematopoietic stem cells[1016]. Since these cells do not normally self-renew at such a rate or so indefinitiely, this event is pathological.

There is a slight questionmark over the theory that differentiated cells may become dedifferentiated in cancer, primarily since stem cells remain in many adult tissues and can become the CSC themselves. For example, there are stem cells that have mutated and begin expressing features of embryonic development; these cancers arise from the processes of EMT that are normal (essential!) for embryonic development, however are pathological in the adult (see Chapter 3 for detail on this physiological event)[1017].

Zipori's observation gives sound reasoning for the apparent loss of stemness in the cells of the embryo, and the potential for gaining stemness in the cells of the adult. This hypothesis supports the stemness as a state claim.

[1015] Daley, 2008 p 171.
[1016] Krivtsov *et al*. 2006; Daley, 2008.
[1017] Daley, 2008 also reviews a few studies demonstrating EMT in cancer.

6. Genetic and molecular markers

"Life is a one-way trip. Plants and animals start as embryos and progress through irreversible developmental stages to eventual death".[1018]

Until the late twentieth century, differentiation was considered to be a unidirectional, irreversible process. Definitions of development often suggest such irreversibility, since through development, cells progressively differentiate whilst their potential to become other cell types diminishes. Such differentiation and loss of potential can be observed early in development, with the cells of the ICM eventually developing into the foetus, whilst the trophectoderm cells manage attachment and placenta formation (see above). As cells of the ICM differentiate into endoderm, ectoderm, or mesoderm, they lose the potential to become cells of the other two types. Eventually, in the adult organism, specialised pools of stem cells remain for maintenance of tissues. As noted by Maienschein, the concept of irreversibility through development has persisted since the beginning of cell biology[1019].

6.1 The epigenetic landscape: Waddington's model

In 1957, developmental biologist Conrad Hal Waddington (1905-1975) proposed a model of development, depicting cells as rolling down different bifurcating channels, acquiring irreversible changes on the way[1020]; the well-known diagrammatic representation of this appeared in Waddington's 1966 book, *Principles of development and differentiation* (Figure 15).

[1018] Vogel and Normile, 2012 p 178.
[1019] Maienschein, 2014.
[1020] Waddington's aim was not only to pictorially describe the process of cellular differentiation and cell fate, but to include a range of biological phenomena, including evolutionary theory. Allen, 2015.

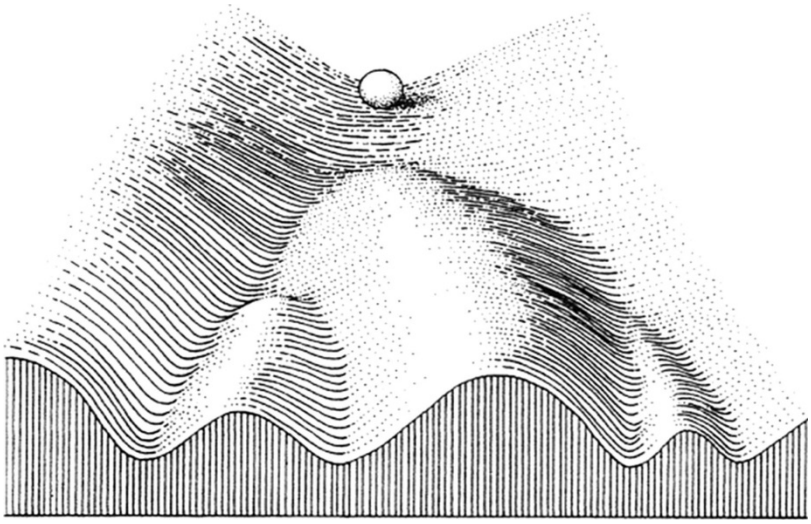

Figure 15: Waddington's illustration of the epigenetic landscape, from *The strategy of the genes: A discussion of some aspects of theoretical biology*, (1957), p 29.

Waddington first used the term "epigenetic landscape" in 1940; he would assert that the channelled, sloped landscape would act as a mediator between genes and the adult organism. The term 'epigenetic' would refer to the environmental (or external) influences that would have an effect during development[1021]. In this particular work, Waddington was attempting to understand the mechanisms involved in development, and in particular embryological development, from the single cell zygote to development of different cell types, tissues, and organs. Drawing on the continuing popularity of the tree metaphor in describing changes in animals and cells[1022], Waddington explained that cell differentiation occurred as a "series of branching decisions, taken under the control of genes"[1023]. Alongside a photograph of a complex arrangement of railway lines, Waddingdon wrote that "You are looking at an incline called the Hump. The wagons are pushed over the Hump and go running downhill and are sorted out by the systems of points into the various sidings. Now an embryo is in some ways analogous to a set of trucks sliding down the Hump"[1024]; for Waddington, cell fate was a one-way journey, with cells becoming more and more differentiated as they rolled down the hill, the path taken determined by the expression of a particular set of genes.

[1021] Allen, 2015.
[1022] Dröscher, 2014.
[1023] Waddington, 1940 p 12.
[1024] Waddington, 1935 p 96.

Waddington's epigenetic landscape would support the entity view of stem cells. Here, we begin with the zygote, the ultimate stem cell, capable of forming an entire organism. With each cell division, cells become more and more differentiated, until they end up at the bottom of the hill or end of the track, in a final, differentiated, specialised cell state. Waddington did not suggest that there was any opportunity for cells to move back up the hill or reverse back along the tracks.

6.2 'Stem cell genes'

The use of genetic and molecular markers to identify stem cells was thought to be a potentially useful, practical way to isolate stem cells from non-stem cells in the laboratory. The suggestion that stem cells could be identified based on molecular markers is not new; for example, when reviewing haematopoeitic stem cells in 1980, Till and McCulloch suggested that identification of cell surface markers would be a particularly useful way of isolating stem cells and purifying stem cell populations[1025]. Why would we not believe that stem cell markers exist? After all, it's how we have learned to classify differentiated cells[1026]. More recently, using techniques such as DNA microarrays and oligonucleotide arrays, it is possible to separate cells based on the expression of particular surface proteins, or by the expression of particular genes. If a stem cell molecular fingerprint was identified, it would mean that stem cells from any tissue could be swiftly and easily isolated *in vitro*: a potentially important tool for research and medicine. Therefore, many researchers have spent considerable time and effort attempting to identify those genes or proteins that are expressed only by stem cells - myself included[1027]. In Chapter 5 for example, I have highlighted those genes identified by James Thomson when he submitted patents for methods to isolate pESCs. I will now discuss three such studies in detail.

In one study, carried out by a group under Harvard geneticist Douglas Melton, 216 unique genes were identified that were expressed in mouse embryonic stem cells, neural stem cells, and haematopoietic stem cells[1028]. Whilst 100 of these genes had no known function, the function of the others are understood to be involved predominately in cell signalling (35), transcriptional regulation (14), and cell cycle

[1025] Till and McCulloch, 1980.
[1026] Zipori, 2009.
[1027] Lancaster, 2012.
[1028] Ramalho-Santos *et. al.,* 2002.

regulation (13). Another group, under Princeton molecular biologist Ihor Lemischka, carried out a similar study to Melton's group, also published in 2002[1029]. In this study, murine and human haematopoietic stem cells were examined alongside embryonic stem cells, allowing the group to identify those genes that were specific for foetal and adult murine haematopoietic stem cells, genes specific for murine and human haematopoietic stem cells, and genes in cultured embryonic stem cells. In total, 283 genes were identified as being expressed in all murine stem cell populations tested. Thirdly, a group under Bing Lim at the Genome Institute of Singapore commented on Melton and Lemischka's studies (both published in *Science*) and included additional analysis. Lim's group identified 385 genes expressed in embryonic, neural, and haematopoietic stem cells.

However, in their paper considering 'stemness', Yan Leychkis, Stephen Munzer and Jessica Richardson note that only six of these genes (less than 2%) were identified as potential stem cell markers by both Melton and Lemischka's groups[1030]. Lim's group themselves identified that only one gene was commonly identified by all three studies: α6 integrin. This is part of a small group of proteins that act at the cell surface, affecting adhesion and signalling. Integrin proteins have also been implicated in promoting tumourigenesis. The integrin family of proteins are found in a large variety of cell types throughout the body (for example, epicardium[1031], dental pulp cells[1032], and more than thirty types of stem cell[1033]. The groups themselves identified potential reasons why their results were so different: the varying nature of cells cultured *in vitro* as opposed to *in vivo* cells, and the differences in microarray techniques used. As Leychkis, Munzer and Richardson note however, this is not enough to explain such significant discrepancies. Commenting on the Melton, Lemischka, and Lim results, *Science* news reporter Gretchen Vogel suggested that an additional problem may have been isolating pure populations of stem cells to examine initially. This is typical of sociologist of science Harry Collins' *experimenter's regress*[1034]; it is not possible to identify pure populations of stem cells without identifying their molecular markers, yet it is not possible to identify the molecular

[1029] Ivanova *et al*., 2002.
[1030] Leychkis, Munzer, and Richardson, 2009.
[1031] Ryzhov *et al*., 2017.
[1032] Shi *et al*., 2017.
[1033] Krebsbach and Villa-Diaz, 2017.
[1034] Collins, 1985; Franklin and Perovic, 2015.

markers without pure populations of stem cells to test. As the Melton, Lemischka, and Lim groups found out, the experimenter's regress raises concerns regarding the usefulness of their experimental evidence for evaluation of their hypothesis.

Further critiques of Melton, Lemischka, and Lim's results claimed that perhaps the research demonstrated that "there is no such thing as intrinsic stemness at the molecular level, such that perhaps stemness should be understood as a relational property between cells and their microenvironment generating the functionality of stem cells"[1035]. Here, Jason Robert is suggesting that stemness itself is a property that can be gained or lost depending on environmental cues; i.e., a stem cell is not a natural kind, or an entity, but that stemness can be a state, or phase of a cell's life. Zipori has also written on the potential reasons for a lack of stem cell molecular markers, suggesting that the "stem state" is equivalent to a "standby state", from which differentiation is able to occur[1036]; expression of many genes allows the stem state to retain its flexible character (see also section 7). Lack of specific molecular markers supports the state view.

6.3 Haematopoietic stem cells

One of the most well-characterised tissue-specific stem cell populations (of the adult) is the pluripotent haematopoietic stem cell[1037]. Haematopoietic stem cells are also considered important for repair and maintenance of tissues outside of the blood system; their ability to make use of the circulatory system to move around the body has shown that bone marrow-derived stem cells have functioned in the brain[1038], in oocyte generation[1039], gastric epithelium[1040], pancreas[1041], liver[1042], retina[1043], heart, and muscle[1044]. A traditional protocol for isolating haematopoietic stem cells has been to use fluorescence-activated cell sorting (FACS), which allows cells to be separated based on the expression of surface proteins. Presumptive haematopoietic stem cell populations isolated using this method however can contain up to 20% non-

[1035] Robert, 2004 p 1007.
[1036] Zipori, 2009 p 179.
[1037] Blau, Brazelton, and Weimann, 2001.
[1038] For example, Brazelton *et al*., 2000.
[1039] For example, Johnson *et al*., 2005.
[1040] For example, Houghton *et al*., 2004.
[1041] For example, Iskovich *et al*., 2007.
[1042] For example, Theise *et al*., 2000.
[1043] For example, Dorell *et al*., 2004.
[1044] For example, Bittner *et al*., 1999.

stem cell cells; "Even the most rigorous isolation protocols currently available result in heterogeneous populations that are enriched for HSC [haematopoietic stem cells] but in which some of the cells fail to demonstrate pluripotency and/or long-term reconstituting ability"[1045].

The examples selected in 6.2, and 6.3, concerning molecular and genetic markers of stemness indicate that it is, if not impossible, extremely difficult to isolate pure populations of stem cells[1046]. This then supports the state view of stemness.

7. Plasticity

Plasticity in cell biology refers to the capacity for cells "to alter their phenotype in response to changes in their environment"[1047]. The plasticity of cells is a relatively new finding; previously held views suggested that once specialised, cells were unable to dedifferentiate and re-specialise (as proposed by Waddington, for example [see section 6.1]). In his description of the niche in 1978, Schofield suggested that cells that were not necessarily stem cells could re-enter the niche to replenish the stem cell population[1048]; an indicator that there was some plasticity of cell fate. Although the exact molecular mechanisms underlying such plasticity are not completely understood, research has been able to produce two clear examples of cell plasticity: somatic cell nuclear transfer, and induced pluripotency. For the current discussion, plasticity is an important concept; if cells are capable of differentiating and dedifferentiating (to the point of being able to recreate an entire organism), is it possible that 'stemness' is therefore a state cells can pass through (perhaps more than once), as directed by intra- and extra-cellular signals? In order to examine this, somatic cell nuclear transfer and induced pluripotency are discussed in further detail.

7.1 Somatic cell nuclear transfer

Somatic cell nuclear transfer history and procedure was previously discussed in Chapter 2; in this method, a nucleus is transferred from a differentiated cell into an enucleated oocyte, which, if allowed to develop, can create a genetic clone of the

[1045] Blau, Brazelton, and Weimann, 2001 p 831. See also Morrison and Weissman, 1994.
[1046] This may be in part due to the many other factors believed to be involved in stemness, such as DNA methylation, intracellular protein distribution, and post-translational protein modifications (see Zipori, 2009 p 179-80 for a review).
[1047] Skipper, Weiss, and Gray, 2010.
[1048] Schofield, 1978.

nuclear donor. The success of this method has demonstrated not only that all genes required for formation of an entire new organism are retained in differentiated cell nuclei, but that these genes can be 'reactivated' when exposed to factors present in the enucleated oocyte. It is not a stem cell that enables the creation of a new organism in SCNT: it is a nucleus. Therefore, SCNT cannot demonstrate stemness alone – it only shows that the nucleus is capable of plasticity (i.e. that many genes can be 'switched on' or 'switched off' given the appropriate signals). Importantly for this discussion is the additional demonstration of plasticity; given the correct signals and cellular machinery, the nuclei of differentiated cells are still able to recreate an entire organism, including the appropriate organisation and multiple cell types (including extra-embryonic cells) such a task requires. This has also been referred to as 'reprogramming' (for example, by Zipori), as the nucleus is modified by the cytoplasm of the receiving cell. It is likely that such reprogramming or dedifferentiation reverses the processes of DNA methylation, enabling expression of a wider set of genes[1049]. Yamanaka's work has provided clear indications of what these signals are, however, as yet, we only know that this occurs *in vitro*. We have no evidence that this occurs *in vivo*.

7.2 Induced pluripotency

In 2006, Kazutoshi Takahashi and Shinya Yamanaka published a paper in *Cell* demonstrating that overexpressing four transcription factors (*Sox2*, *Klf4*, *cMyc* and *Oct4*) in somatic mouse cells was sufficient to induce pluripotency. This method worked for several types of somatic cells, and the resulting cells termed iPSCs (induced pluripotent stem cells). In a review of methods demonstrating cell plasticity published in 2010, Yamanaka and fellow stem cell biologist Helen Blau refer to iPSCs as "the strongest example so far of the plasticity of cells"[1050], presumably since the nucleus is not removed and transplanted (as in somatic cell nuclear transfer), but the entire cell can be induced to dedifferentiate by triggering overexpression of certain transcription factors. In 2012, Yamanaka (alongside John Gurdon - see Chapter 2) was awarded the Nobel Prize in Physiology or Medicine for his work

[1049] Zipori, 2009.
[1050] Yamanaka and Blau, 2010 p 709. Yamanaka and Blau's paper refers in the title to "a pluripotent *state*" (my emphasis), suggesting that pluripotency is a state cells may pass through and return to.

demonstrating that differentiated cells may re-acquire stem cell properties. Already (and, perhaps, prematurely), the demonstration of cell plasticity has been referred to as 'paradigm shifting'[1051].

It is interesting to note that *Sox2*, *Klf4*, *Myc*, and *Oct4* are also transcription factors associated with dedifferentiation of somatic cells *in vivo*, associated with cancer formation. Expression of either *Oct4* or *Sox2* is been associated with irregular homeostasis and tumourigenesis[1052]. Both *Oct4* and *Sox2* are expressed in tumours lacking differentiation[1053]. *Myc* is an oncogene that was established in the 1990s as having a role in 'immortalising' tumour cells, enabling them to continue replicating and perpetuating cancer[1054]. *Klf4* has been previously associated with colorectal cancers[1055]; it's close relative, *Lin28*, is also associated with liver cancer[1056]. The above examples demonstrate that the transcription factors identified by Takahashi and Yamanaka not only dedifferentiate somatic cells into a stem cell state *in vitro*, but cancer research has demonstrated that abnormal upregulation of these factors *in vivo* are significant in the development of tumours.

Both somatic cell nuclear transfer and induced pluripotency demonstrate the potential plasticity of somatic cells by altering the epigenome, 'forcing' the expression of pluripotency genes in otherwise stable cells. Zipori has argued that this is only possible with such relative ease in the laboratory because it is part of a program that also occurs *in vivo*, where cells revert back to a "standby" state[1057]. Takahashi and Yamanaka's 2006 work then is not definitive in our quest for stemness, since their creation of iPSCs was carried out *in vitro*. Yamanaka and Blau suggest that there must be mechanisms to regulate the pluripotency-inducing transcription factors noted above, and those cell type-specific transcription factors maintaining the differentiated state of somatic cells. As described above, and in further detail in Chapter 3, there are clear indications that dedifferentiation of somatic cells is not a phenomenon that only occurs through artificial means *in vitro*. Abnormal expression of *Sox2*, *Klf4*, *Myc*, and *Oct4* occur *in vivo* and are thought to be, at least in part, responsible for dedifferentiation of somatic cells to a stem cell

[1051] For example, see Cox and Rizzino 2010; Burns and Blau, 2014; Laplane, 2015.
[1052] Hochedlinger *et al.*, 2005; Chen *et al*., 2006.
[1053] Ben-Porath *et al*., 2008.
[1054] For example, Wang *et al*., 1998.
[1055] Wei *et al*., 2006.
[1056] Guo *et al*., 2006.
[1057] Zipori, 2009 p 199.

state. That this phenomenon can occur both *in vitro* and *in vivo* is strong evidence for the stem cell state argument, clearly showing how cells can acquire properties of self-renewal and differentiation long after previously being specialised.

8. Historical overview of the entity and state views

When the earliest stem cell research began in the nineteenth century, much hypothesising was based on microscopical observation combined with an effort to understand general biological systems (evolution, or development, for instance); a useful example of this is the work carried out by Haeckel (see Chapter 1). Haeckel, as previously noted, was the first to use the term *Stammzelle*, which came to mean the fertilised egg[1058]: the single cell with the potential to form an entire organism. Haeckel was influenced by the *Stammbaum* of Charles Darwin to explain the 'differentiation' of species from one another, which Haeckel adapted to apply to cells. The inference was then that the stem cell (fertilised egg) was an entity, and after its first cell division, the cells began to differentiate towards their own cell fates, now lacking the potential to recreate an entire organism that the original fertilised egg had. This can be particularly seen in Weismann's work in the late nineteenth century (Chapter 2); Weismann argued that 'determinants' were divided between daughter cells as each parent cell divided. This ongoing loss of the 'id' meant that cells would become less and less diverse after each cell division, eventually containing only the information needed to function as a single cell type. In Weismann's germ plasm model then, no cell could dedifferentiate - the information to do this was not available inside the cell. Hans Driesch would also ponder over vitalistic properties, following his own experiments separating sea urchin embryos (see Chapter 1); Driesch considered the impact of his results on "'mechanistic' causality", observing that one could "cut up a thousand-celled embryo *at will* and from the fragments obtain the whole organization as the result of development"[1059]. The epigenetic landscape model, which Waddington began to develop in the 1930s (see 6.1), like Weismann's germ plasm theory, suggested that cell fate was a linear process. Wherever development was considered to be this linear process, the stem cell entity view worked. These early hypotheses however were only that: hypotheses. It was not until

[1058] Haeckel, 1877.
[1059] Driesch, 1951 p 108. See also Baltzer, 1969 p 109-10.

the mid-twentieth century that stem cells were isolated and made available for use in the laboratory by Till and McCulloch. This turning-point was not only important for providing the tools for practical experimental work on stem cells, but also perhaps the beginning of a conceptual change.

Up until Till and McCulloch's work, no stem cell had been (knowingly) isolated and cultured[1060]. All work prior to this point then could only be hypothetical; experimental methods could not make use of stem cells to test any hypotheses. As highlighted by Fagan, without information from experiments, "claims about stem cells are massively ambiguous – we don't know what is being talked about"[1061]. After the work of Till and McCulloch, and many others who followed (such as Stevens, Martin, Evans, and Thomson), stem cells became available for experimentation in the laboratory. It is at this point, I argue, the shift concerning entity and state views of the stem cell began. Haeckel's view of the *Stammzelle* stuck because it appeared to fit the information gathered by observational work. For example, Weismann's germ plasm theory suited the original stem cell concept. Likewise, the cell lineage studies described (Chapter 1) also assumed that stem cells were larger, since they contained more chromatin than somatic cell types – this again fit with the early stem cell concepts. Moving into the twentieth century, and experimental embryology, experiments were designed and results were interpreted based on the theoretical stem cell concept – for example, Danchakoff's work was carried out on the understanding that stem cells were entities, and her results were interpreted using this model. As the stem cell moved from being theoretical, to being observed, to being an experimental agent, the underlying suppositions remained, and influenced stem cell research throughout the twentieth century, and into the twenty-first. I argue that the practical work that has been carried out in the latter half of the twentieth century and beyond has in fact demonstrated that stemness is a state, and that stem cells are not an entity.

Very soon after stem cells became available for experimental work their plasticity became clear. For example, Briggs and King's, and Gurdon's work on somatic cell nuclear transfer (Chapter 2), research demonstrating the role of the niche in creating and maintaining stemness, Martin and Evans' work on embryonal

[1060] It is possible that Ross G Harrison possibly had some experience culturing stem cells, however this was prior to experimental understanding of stem cell culture (for a review, see Maienschein, 2010). It is also possible that Alexis Carrel may also have cultured cells capable of self-renewal (see Wilson, 2011).
[1061] Fagan, 2013a p 1151.

carcinoma cells (Chapter 4), the groups led by Melton, Lemischka, and Lim showing lack of a stem cell molecular fingerprint, and Yamanaka's demonstration that pluripotency can be induced, all support a different concept: that stemness is a state.

It is not only the work of Yamanaka or Gurdon that is important to support the stemness view. The work studying cell plasticity only occurred *in vitro*. The historical experimental work highlighted included some examples of *in vivo* results that support the stemness view, such as the lack of molecular markers. It is also important to review historical observations and experiments and review these results; if these results did not support the stemness view, it would significantly weaken its claim.

9. Conclusions

This chapter has discussed several examples and considered whether they support the entity or state view of stem cells. Initial consideration of the traditional definitions of 'stem cell' or 'stemness' has shown that the traditional definition (of potential to self-renew and differentiate) was first proposed in the nineteenth century (for example by Martin Barry and Ernst Haeckel), and became the clear, concise definition in the early twentieth century, pioneered by developmental biologists such as Vera Danchakoff. Later examples show that this is a definition still in use by researchers in the twenty-first century. This definition however is now inadequate: non-stem cells also have the potential to self-renew or differentiate, leaving twenty-first century biology to require a different definition, considering stemness as a function, and as a state, or phase, cells move through.

In order to further clarify this argument, an examination of the possibility for stem cells to be natural kinds was carried out; if stem cells could be demonstrated to be a natural kind, it would support the entity view. The difficulty that self-renewal and differentiation potential are not specific enough to stem cell only populations damages the natural kind claim. However, Wilson, Barker, and Brigandt have argued that the requirements for natural kinds should be updated, adapting Boyd's proposal for homeostatic property clusters; regardless of the heterogeneity of stem cell populations, the homeostatic property cluster, covering other properties as well as self-renewal and differentiation potential, allows stem cells to be considered a natural

kind, supporting the entity view. Others disagree however, claiming that the homeostatic property cluster is still not specific enough to exclude non-stem cells.

The importance of the stem cell niche is also examined. The niche, a specific microenvironment in the adult organism where stem cells reside, is considered to be an active participant in the functionality of stem cells; this is because it provides signals to the residing cells, telling them to be quiescent, to divide, or to migrate. If this is the case, then the niche has an active role in maintaining stemness. Experimental work has demonstrated that removal of the cells from the niche induces previously differentiated cells to move into the niche; altering the protein expression pattern of the niche also causes somatic cells to dedifferentiate. Historically, embryonic cells that are away from their niche, were once thought to be a cause of cancer, and gave rise to the CSC theory (see Chapter 3).

As a further example to examine the state and entity views of stem cells, embryonic cells are discussed, and in particular those cells created early on in development, following the first few cell divisions. The first cells created by division of the zygote have been named totipotent stem cells. However, I argue that since these cells begin to differentiate after the second cell division, and there is no asymmetrical division at this stage, totipotent cells cease to exist at this point (i.e. the four-cell stage), with a different cell population existing by the eight-cell stage (third division). The upshot of this is that, given the traditional definition of stemness (i.e. the potential to self-renew and differentiate), the first cells of the developing embryo, the totipotent cells, cannot be considered stem cells. This then supports the state view.

Referring back to the work of Conrad Waddington in the mid-twentieth century, I then begin to examine the evidence provided by genetic and molecular markers of stem cells. As has been previously noted, up until the late twentieth century, differentiation was considered to be both unidirectional and irreversible. An example of such an understanding of development is given by Waddington's epigenetic landscape model, that proposes cells are like marbles, running down a slope (irreversible), navigating a series of channels (unidirectional). Waddington's model supports the entity view - cells may begin as a stem cell, however intra- and extracellular cues will cause the cell to differentiate along a specific pathway, until it is mature and completely specialised. I then move on to consider the search for genetic and molecular stem cell markers; research that has been contributed to by

many researchers worldwide in the late twentieth century and early twenty-first. In particular, I note the comparison made between the findings of three distinct research groups, all of which have compared different stem cell populations in order to identify similar gene expression patterns. The only gene all groups found to be expressed in all populations tested was α6 integrin, a gene expressed by many different cell types throughout mammalian organisms. This research then supports the state view, since there appears to be "no such thing as intrinsic stemness at the molecular level"[1062]. Haematopoietic stem cells, possibly the population that has been studied the most, also does not have a distinctive molecular signature; the use of FACS to separate cell populations is still unable to isolate a stem cell population from a non-stem cell population, as up to 20% of the cells isolated using FACS are non-stem cells (i.e. cannot both self-renew and differentiate). Again, this supports the state view.

Lastly, I examine our historical and current understanding of plasticity, considering the examples of somatic cell nuclear transfer (a mid-twentieth century technique), and induced pluripotency (a twenty-first century technique). Plasticity was demonstrated by developmental biologist John Gurdon in the 1960s, as he began somatic cell nuclear transfer work in amphibians (see Chapter 2), and the nucleus of a differentiated cell was shown to be capable of regenerating an entire organism if it was injected into an enucleated oocyte. Demonstration of such plasticity supports the state view. Further demonstration of plasticity is provided by Yamanaka's induced plurpotency technique, which involved inducing overexpression of a series of transcription factors in somatic cells. These differentiated cells then dedifferentiate, and become pluripotent cells. This then is particularly strong evidence for the state view; it demonstrates that differentiated cells can revert to a stem cell-state with relatively little interference. Such is the significance of this research, induced pluripotency has been described as paradigm shifting.

This thesis has identified several aspects of embryonic stem cell history which were relevant to their conceptualisation, isolation, and to our knowledge and understanding of stem cells. This sixth chapter has, to an extent, brought together the previous chapters to consider whether stem cells should be considered as states or entities.

[1062] Robert, 2004 p 1007.

What is highlighted by considering examples through the nineteenth, twentieth, and twenty-first centuries, is that initially, evidence supported the entity view of stem cells. Barry and Haeckel's theoretical stem cells, hypothesised based on their observations and understanding of development and evolution, were entities. These were cells that, in theory, were created from the first cell divisions of the fertilised egg, and could multiply and change, giving rise to all of the cells of the organism. In the mid-twentieth century, Waddington's ambition to find unifying laws in biology led him to his epigenetic landscape model, which dictated that cells would begin as immature and undifferentiated, follow a single path towards their fate, as they would become mature, specialised cells.

It was only research that began with somatic cell nuclear transfer, technical improvements (which allowed for more genetic and molecular research, such as FACS), continued learning about the properties of the stem cell niche, and lastly (and most recently) induced pluripotency that has suggested that a different concept of the stem cell is required. One suggestion for this is the state view: that cells may begin as stem cells, differentiate, then become stem cells again during their lifetime. The examples highlighted above suggest that, at the very least, an expansion of the 'traditional' definition of stem cells is needed; this was highlighted fifteen years ago by Blau, Brazelton, and Weimann in their short discussion in *Cell*. It is this change in the stem cell concept that is potentially paradigm shifting.

CONCLUSIONS

1. Thesis summary

As yet, there have been no larger scale contributions to the history of ESC research; as noted in the Introduction, research into the history and philosophy of stem cell research has so far been distinct (with little or no integration), and covering only sections of stem cell history at a time. The aim of this thesis was to provide a more extensive overview and discussion of stem cell research history, with a particular focus on embryonic stem cell history. This history has been somewhat neglected in favour of other stem cell histories (such as the work on haematopoietic stem cell history), or relegated to the after-thoughts of ethical or legislative discussions. This thesis, in part, aims to remedy this by analysing several facets of embryonic stem cell history that may already be more widely known (such as the work of Gail Martin, Martin Evans, John Gearhart and James Thomson), but from a previously little-considered view-point, or in order to examine some wider questions about the history and philosophy of biological science more generally.

The first chapter of this thesis explored how the term 'stem cell' arose. This began with *Stammzelle*, a concept developed by Ernst Haeckel. Initially, Haeckel referred to the first unicellular organism as the stem cell. Later, this definition also encompassed the fertilised egg, from which all other cells of the embryo could develop. The popularisation of the term 'stem cell' in English was likely to be significantly influenced by the publication of EB Wilson's *The Cell* in 1896 (and further editions thereafter); in *The Cell*, Wilson refered to the progenitor cells of gametes as stem cells, which arise in the first divisions of the fertilised egg. Wilson was part of a groups of researchers focused on cell lineage at the time, who observed embryos of various species in order to establish the role of each early cell of the embryo. Also of interest is the work of Vera Danchakoff, who used 'stem cell' in a slightly different way to Wilson in the early twentieth century; Danchakoff referred to the progenitor cells of the haematopoietic system as stem cells. This appears to have been influenced from German research, which also refered to ancestors of blood cells as *Stammzellen*.

The second chapter begins in a similar fashion to Chapter 1, with a focus on the cell nucleus. In order to examine how genetics became such an influential paradigm in stem cell research, the second chapter tested the claim of Garland Allen, who

argued that genetics initially developed under the embryology paradigm. The examples in Chapter 2 however suggest that there may be alternate explanations to the development of disciplines in biology. Both Meunier and Winther dismiss Allen's claim, since, they argue, genetics and embryology were always separate; Allen's account was too focused on Morgan, who actually separated development from heredity studies, not genetics from embryology, claimed Meunier. Winther argued that genetics followed a formal style of thought, whereas embryology followed the compositional style, and therefore the disciplines were not initially connected as Allen suggested. Chapter 2 made use of Radick's explanation of Weldonian genetics, early embryology experiments, and twentieth century work (such as SCNT) to argue that the disciplines of genetics and embryology were linked, and remain linked through into the twenty-first century.

Chapter 3 considered the parallels between ESC research and cancer, in particularly CSCs. Following a brief history of cancer, focusing on the nineteenth-century suggestion that cancer could arise form cells left over from embryonic development, twentieth-century research into teratomas becomes an important focal point. This chapter identified several ways in which cancer and embryonic development were studied in parallel, or direct comparisons were made. Lastly, this chapter considered the CSC concept, in a similar way to the ESC concept was explored in Chapter 1.

Continuing to examine the links between the normal and abnormal, Chapter 4 resumed chronologically where Chapter 3 concluded. Since ESCs were not available for research in the mid-twentieth century, teratoma cells were thought to be a viable alternative. Chapter 4 described how ECC lines were developed, and then how these techniques were used in the isolation and culture of mESCs by Martin and Evans, in the USA and UK respectively. The chapter goes on to elucidate what Martin and Evans used these new tools for, and what their motivations were. Exploration of the social, political, and economic situation that Martin and Evans were working in demonstrated that these contexts affected their research. Whilst in the USA, Martin was able to continue with fundamental research into development, Evans was motivated into pursuing more practical applications.

Chapter 5 examined a claim by Michel Morange that hESCs were not the equivalent of mESCs, since they were each produced in different scientific contexts. Since Chapter 4 had previously considered the motivations and goals of mESC

isolation and culture, Chapter 5 focused on the isolation and culture of hESCs; this was achieved independently by Thomson and Gearhart in 1998. Also demonstrating that political and economic factors affected stem cell research, the chapter explored the role of Geron (who funded the research), as well as the backgrounds of Gearhart and Thomson prior to their hESC research; this enabled testing of Morange's claim. The chapter concluded that Morange was correct in asserting that the scientific context of work in the 1980s and 1990s was different; Chapter 5 also offers a complementary view: that the political, social, legal, ethical, and economical contexts also affected the delay in isolating and culturing hESCs after the isolation and culture of mESCs.

Chapter 6 of this thesis explored the underlying paradigm of stem cell research since stem cells were conceptualised in the nineteenth century; the presumption that stem cells were entities. This chapter was influenced by Laplane's suggestion that we should instead think of stem cells as cells that are in a stem cell 'state'; this is called 'stemness'. Cells in this state have stem cell properties, however can move into and out of this state through their lives. In this chapter, several examples are provided from both philosophy and history to provide evidence for this new way of thinking about stem cells. This includes asking whether stem cells are natural kinds, what the role of the niche is, the fate of early embryonic cells, whether there are molecular markers of stem cells, and what plasticity studies have shown us. Although historically, stem cell research functioned under the stem cell entity paradigm, this chapter showed that as we learn more about stem cell, experimental results indicate that the stem cell state model may be more accurate than the stem cell entity model. Historical results could be re-interpreted under the stem cell state paradigm. This suggests that stem cell research may now be undergoing a paradigm shift, from treating stem cells as entities, towards needing to think about stemness as a state.

Through these chapters, this thesis aimed to answer three research questions. Firstly, what was the significance of social and political factors on (embryonic) stem cell research? Secondly, what was the role of cell fate, and cell fate research, on ESC research? And thirdly, which paradigms have been influential in (embryonic) stem cell research?

2. Answering the questions

Firstly, there was the query regarding the role of social and political context in stem cell research. Bensaude-Vincent has already claimed that the effect of society on scientific research has been made clear, however its effects have not been specifically considered in mESC research and its implications, as in Chapter 4 of this thesis. Chapter 4 shows very specifically the interacting roles of the public, politics, economics and science in the resulting research carried out. It was argued that by the mid-twentieth century, science could no longer be "value-free", and was dependent on social framing[1063]. This was particularly the case for biological sciences research dependent on animal experimentation. Fleck's suggestion that science could not be considered in isolation from the 1930s hints that social and political forces were influencing research in the early twentieth century, if not before. This is one of the conclusions in Chapter 1 of this thesis, where it is clearly shown that social conflict and political power influenced cell biology in nineteenth century Germany.

In the mid-nineteenth century, the Berlin School emerged under the leadership of Johannes Müller, encompassing figures such as Theodor Schwann and Jakob Henle. Across the country in Breslau, another school was developing, under Johann Evangelista Purkyně. Purkyně was Czech by birth; the Bohemian peoples east of Germany were becoming increasingly Germanised, but remained second-class citizens. In Chapter 1, evidence is presented to support the argument that the Czech-born Purkyně was given less far less credibility for his work than his German counterpart in Berlin (i.e. Müller). For example, it may be that Gabriel Gustav Valentin identified the cell as a 'fundamental unit' of both plants and animals in Breslau, years before Schwann's Cell Theory was published in 1839. Purkyně however continued to use two different terms for animal and plant cells, *Zellen* and *Körnchen* respectively. Arguably, *Zellen* became the favoured term through the popularity of Schwann's *Zellentheorie*. It is also likely that one of the reasons for the popularity of Schwann's work over Valentin's was the underlying racism that perceived the Breslau School as lower quality than the Berlin School. Clearly then, as early as the mid-nineteenth century, social and political factors were affecting scientific research.

[1063] Jacob, 1992 p 488; Fleck, 1979 (1935).

The impact of social and political conflict on research in the mid-nineteenth century was not an isolated incident. After the end of World War I, there was a reduction in research through Europe (as finances were diverted to significant re-building projects), and an increase in the USA. There are records of many researchers, particularly from Eastern Europe and Russia, migrating westwards to Britain and the USA. This resulted in a change of publication language from significantly German, to predominantly English. In Chapter 1, some detail is given of the Russian biologist Vera Danchakoff, who moved from Russia to the USA. Her shift to publishing in English is typical of other migrants. In particular, Danchakoff is selected as an example since she was researching embryogenesis, and made use of the term 'stem cell' in English in the early years of the twentieth century. Her use suggests that the term was familiar to an English audience by that time. Again then, Danchakoff's move shows how social, political, and economic factors resulted in a shift in the physical centre of biology from Europe (and particularly Germany) to the USA. This also affected a change in publication language.

Whilst animal experimentation was a particular issue in the late 1970s in the UK, it all-but disappeared from public and therefore political concern in light of the general economic downturn, and the imminent general election. Thatcher was elected Prime Minister based on her promise to improve the economy. Meanwhile, in the USA, public pressure resulted in a 1973 ban on federal funding availablility for any research on human embryos. In the 1980s, Thatcher reduced funding for scientific research in the UK, adding the caveat that any research that was carried out needed to contribute to the social and economic needs of the country. The public would not have generally been in a position to oppose this stance, concerned far more with general strikes, and the decline of some significant industries (such as coal mining and steelworking). The public during the 1980s would not have been primarily concerned with government spending significantly on 'pure' or fundamental research. In the USA, although there were also effects seen of the global economic downturn, there were less significant cuts to science funding in the early 1980s. Whilst President Reagan, like his counterpart Prime Minister Thatcher, was keen to emphasise practical applications of scientific research, this became catered for by increased marketisation and commercialisation of some sectors. This meant that federal funding could be available for fundamental research. The upshot of these economic policies in the UK and USA respectively meant that Martin Evans was

driven towards applied research, whilst Gail Martin, who has said herself that she didn't find funding particularly difficult to obtain during this period, was able to continue focusing on fundamental research.

The use of the newly-available mESCs then was clearly affected by social, political, and economic contexts, which varied between the UK and USA. Whereas instinctively one may believe that US capitalism would encourage the move towards applied research, Chapter 4 demonstrated that in fact this was more of a necessity in the UK.

The importance of the social, political, economic (and ethical and legal) contexts of scientific research are further highlighted in Chapter 5. This chapter set-out to further consider the evidence for Morange's claim that mESCs and hESCs were not equivalent, since they were isolated and cultured in different scientific contexts. Chapter 5 agreed with Morange's claim, and added further, complementary evidence: that the social, political, economic, ethical, and legal contexts also had a role, and help to explain the seventeen-year gap between the isolation and culture of mESCs and hESCs.

As previously mentioned, no federal funding was available in the 1990s for either Thomson's or Gearhart's projects; instead, this had to be provided by the private sector (and was, from Geron). In Chapter 5, it is argued that Thomson and Gearhart were motivated to apply their research skills to the isolation and culture of hESCs because of the political, economic, ethical, and legal framework they were working in. Politically, their research was affected by the Dickey-Wicker amendment, for example. Economically, only private sector funding enabled Thomson and Gearhart to continue with their individual projects. The account provided by Thomson in particular highlighted how long he waited for ethical approval before he could continue with his research. Again, Thomson's account provided a useful example of the significance of legal context, with his work having to take place without federally funded laboratories and equipment.

Throughout this thesis then, there have been specific examples provided to demonstrate the influence of the public, politics, economics, ethics, and legislation on stem cell research. This thesis does not claim that such influences were previously unknown – of course this is not the case. What the examples in this thesis have shown however is that throughout history, stem cell research has never been "value-free", and has been continually under these influences. Furthermore, it has been

demonstrated that these influences may result in unexpected conclusions, such as the availability of funding in the USA for pure research through the 1980s, that was less forthcoming in the UK. Any history of similar research then must be prepared to take such contexts into account. In addition, future changes to, for example, public opinion or funding bodies must take into consideration the effects of such changes on future research.

The second research question addressed by this thesis queried the importance of cell fate; both the significance of cell fate itself, and of research carried out on cell fate. In some ways, this follows on from the first research question, since it asks what influenced the decisions made to pursue certain research. Maienschein argued that cell fate has directed much of our research in embryology and stem cell biology; this thesis aimed to ascertain exactly how and where cell fate research was influencing ESC research, and, to an extent, whether this hundred-year old approach was still appropriate for stem cell research in the twenty-first century.

In Chapter 1, the conceptualisation of the stem cell already highlighted that there may be some future enquiry into cell fate. In 1877, when making use of the word *'Stammzelle*', to refer to the fertilised egg, Haeckel stated that the term "seems to me the most simple and appropriate one, because all other cells stem from it...all the countless generations of cells of which later on the multicellular organism is composed"[1064]. Haeckel recognised that all of the other specialised cells of the organism must, in some way, emerge from this single cell, with all of its potential.

At the end of Chapter 1, there is also a brief description of the cell lineage studies that became popular in the USA in the late nineteenth and early twentieth centuries (the significance of early twentieth-century USA for stem cell research is mentioned above). Of particular influence for example were EB Wilson and CO Whitman. Examination of the earliest stages of development could shed light on fundamental biological processes. This work was intimately linked with Haeckel's work regarding the belief that ontogeny was a record of phylogeny. Likewise, EG Conklin would examine the cleavage patterns of blastomeres in order to learn more about phylogenic differences; this work was technically tricky, and dissemination of the results somewhat problematic at the time. The cell fate of stem cells for Wilson

[1064] Haeckel, 1877 (transl. Maehle, 2011).

and those who followed his example in *The Cell* however was only germ cells. The German tradition however demonstrated that the ancestors of other systems (in particular haematopoietic cells) could also be stem cells; arguably, this was introduced to an English audience at the beginning of the twentieth century by Danchakoff.

Later, as explored in Chapter 2, Hans Spemann and others began work exploring how each of the early cells of the embryo contributed to later growth, organisation, differentiation, and development. The difference between this work and the cell lineage studies in the USA was that the latter relied more on observation, whereas the work of Spemann, Roux, and His for example all made greater use of experiment (Chapter 1). In late nineteenth- and early twentieth-century Europe, researchers such as Mangold, Roux, Driesch, and Delage would all be carefully studying and manipulating the early embryos of various species in order to figure out just how the single fertilised egg could manifest and organise all the specialised cells required of the embryo and the adult. This became entwined with the study of heredity and later, genetics (see below).

Chapter 3 also considered the importance of cell fate and cell fate research, although from a different perspective. Chapter 3 examined the parallels between CSCs and ESCs – or abnormal and normal, or pathological and non-pathological. Whilst Chapters 1 and 2 focused exclusively on the normal, Chapter 3 demonstrated not only that cancer studies were essential for furthering stem cell studies, but that specific teratoma studies began because of the continuing curiosity around cell fate. Teratomas have historically been found interesting because of the range of specialised cell types observed in the mature tumour: specialised cells descended from all three germ layers yet developed from a single cell. How this single CSC (as it is now understood) could be responsible for forming a tumour of all these cell types focused research on cell fate. Such was the initial confusion over the ability of cancer cells to vary their fate, that Barry Pierce was required to change the title of his 1959 paper, replacing the word 'differentiation' with 'metamorphosis'! It was also from this interest in cell fate that the realisation emerged that the ECCs from teratomas would make adequate substitutes for ESCs (then unavailable) for cell fate research. It was reasoned that what was occurring in teratomas must have some parallels with normal embryonic development. It was from such studies that Martin's and Evans' research developed, enabling isolation and culture of mESCs; following this, Martin's work

continued into the study of development – arguably an extension of earlier cell fate research (Chapter 4).

In Chapter 5, there was also a role for cell fate. Although the chapter focused on the motivations and practical steps taken by Thomson and Gearhart as they found ways to isolate and culture hESCs, the brief examination of these steps highlighted the importance of cell fate. This is clearest in the story of John Gearhart's isolation and culture of hESCs. In the 1970s, Gearhart worked alongside the anatomist Wadsworth, as she dissected stillbirths. Gearhart worked with Wadsworth in order to understand how each region of the foetus would develop from cells in the early embryo. When in the 1990s he wanted to produce a better laboratory model for Down syndrome from human cells, Gearhart recalled his 1970s exploration of cell fate in the embryo, in particular the migration of EGCs along the genital ridge, from which Shamblott could dissect out and culture the hESCs.

Lastly, in Chapter 6, the importance of differentiation ability as a property, and the potential to change cell fate becomes a key consideration. The ability to differentiate (i.e. decide cell fate) is one of the two properties that traditionally distinguished stem cells from any other cell type (the other property being the ability to self-renew). Historically, August Weismann implied that differentiation was unidirectional with his germ plasm theory, where 'determinants' would be split between cells, meaning that once a cell had only its specific set of 'determinants', it could only function as that cell type. Following this idea, Waddington developed a thesis of cell fate in the 1930s, producing his famous epigenetic landscape. Again, this depicted cells as rolling down a hill or rail track, on a one-way route to specialisation.

This notion of cell fate began to change following the results of Briggs and King's then Gurdon's research on SCNT. Briggs and King demonstrated that nuclear transfer was practically possible, whilst Gurdon's work showed that adults could develop and survive from SCNT. Gurdon's work was the first to suggest perhaps cell fate was not the one-way street it was previously envisaged to be. Just as Gurdon demonstrated this at the organism level, Yamanaka demonstrated this at the cellular level; that each cell, transcribing the correct genes, and given the correct environment, can dedifferentiate and redifferentiate. iPSCs are a clear indicator that cell fate is not unidirectional.

The plasticity of cells (alongside other properties described in Chapter 6) result in the conclusion drawn by Laplane and others that the properties of stem cells (the ability to self-renew and differentiate) are transient – i.e. 'stemness' is a state that cells can move into or out of, and stem cells as entities do not exist. This, potentially, may herald a change in the paradigm that stem cell research is currently carried out under.

This leads into the third and final question of this project: the importance of paradigms in stem cell research. As the concept of the stem cell was developing through the nineteenth century, queries regarding heredity and evolution were developing alongside. This arguably resulted in the studies becoming intertwined, especially through the research on embryology and evolution. This is explored through the second chapter of this thesis.

In 1985, Allen proposed that heredity research developed under the paradigm of embryology. After heredity became the discipline of genetics in the twentieth century, Allen claimed that the field was then able to distance itself. This occurred specifically following the work of TH Morgan. Chapter 2 concluded that Allen's assertion was correct - initially. Examples studied later in the chapter, including Meunier's critique (that Allen's work was too Morgan-centric), Radick's description and use of Weldonian genetics, and examples of nuclear transfer through the twentieth century, lead to the conclusion that the disciplines of genetics and embryology are still closely linked in the twenty-first century.

Experimental embryology arguably began with His' work, testing Weismann's germ plasm theory; this was the view taken by Spemann. Heredity studies were carried out through the new experimental embryology techniques that were developing in the late nineteenth and early twentieth centuries. Weismann and Haeckel each proposed that there was the initial 'transmission' of information from parents to offspring, then 'translation' into traits observed in the adult; Allen used this as evidence to suggest that during this period, there was no distinction between genotype and phenotype. The changing methods of science during this period however required that testable hypotheses were developed – Morgan was one of the younger generation keen to develop experiments through which such presumptions about embryology and genetics could be tested. Conklin was also part of this movement, claiming that more could be learned about heredity through

developmental studies. Allen argued that this was where the split occurred however; although theoretically it made sense to study transmission and translation together, experimentally it was difficult. Allen argued that the study of heredity then became separated from its embryology paradigm, enabling the development of 'genetics'.

Meunier however argued that this split never actually occurred, instead suggesting that genetics and embryology developed out of different research traditions. Meunier actually argued that Morgan's role in the early twentieth century was to separate development from heredity, not genetics from embryology. This is supported by Winther's view that there were two styles of thought in biology: formal, and compositional. Genetics followed the formal style, whilst development was compositional. In Chapter 2, this is explored further by looking at another example from the era: Weldon's studies of genetics.

Weldon criticised the re-discovered Mendelian genetics by claiming it was over-simplified. For example, it was not simple to separate peas into either green or yellow categories, as Mendel had. Weldon spent most of the first decade of the twentieth century formulating an alternative explanation to Mendelian genetics. Radick's studies of Weldon's work highlighted the use of experimental embryology; although Radick referred to this as surprising, from the previous examination of experimental embryology supplied in Chapter 2, it does not really seem surprising at all. Weldon's research led him to believe that gene expression was contextually dependent, and that the interaction of several genes in fact affected phenotypes. Radick's work comparing Mendelian and Weldonian genetics showed that without embryology, a clearer explanation of complex genetic mechanisms could not have been demonstrated. Weldonian genetics needed the embryological paradigm.

This effect continued after the early twentieth century work of Weldon. In Chapter 2, this is demonstrated by detailing the work of researchers in the mid- and late twentieth century, who carried out nuclear transfer experiments; experiments proposed by Yves Delage in the late nineteenth century, and called 'fantastical' by Spemann, for the new knowledge and understanding that could be gained if such a technique could be developed. Delage and Spemann proposed such experiments to establish a better understanding of two functions: the way in which information could be passed from parent to offspring (heredity), and how information that would provide for development could be held (genetics). These functions of the nucleus could only be better understood using techniques from embryology. Thus far then,

Allen's argument that genetics developed under the paradigm of embryology could be viewed as correct. Chapter 2 then goes on to describe the work of, for example, Briggs and King, Gurdon, and Illmensee in the development of nuclear transfer techniques. The over-riding requirement for these techniques was to enable the function of the nucleus to be better understood.

Briggs, King, Gurdon, and Illmensee all utilised nuclear transfer as an (eventually established) embryological technique to learn more about genetics. Therefore, Allen was incorrect to suggest that the field of genetics separated from embryology in the early twentieth century, following the work of Morgan. Instead, there are plenty of examples available to show that the disciplines of genetics and embryology remained closely linked throughout the twentieth century.

Further discussion of paradigms is particularly important for the arguments presented in Chapter 6. The aim of this chapter was to establish whether a case could be made for a new theory of stem cells: the stem cell state theory. This itself has already been suggested as potentially paradigm-shifting, so needed to be examined as part of the research question querying the role of paradigms in stem cell research. Chapter 1 described the manner in which the concept of the stem cell was developed; this assumed that the stem cell was an entity. Haeckel proposed that the stem cell was the fertilised egg, for example. More recently, a theory emerged suggesting that stem cells were not entities, but that cells could acquire stem cell properties (i.e. the ability to both self-renew and differentiate) at various points throughout their life. This is referred to as 'stemness'. This new view has been advocated by several researchers, most clearly and most recently by Laplane, who suggested that this new approach to stem cell research could be useful in developing treatments for cancer. Chapter 6 of this thesis examined whether the new proposed view of stemness is philosophically, historically, and practically sound.

Through Chapter 6, it emerged that the theoretical work on stem cells, produced up to the mid-twentieth century, assumed that stem cells were entities. Stem cells became available for experiments after the mid-twentieth century, and experiments were designed based on the assumption that stem cells were entities; this continued right into the twenty-first century. For example, research groups headed by Lemischka, Melton, and Lim all based their search for molecular markers of stem cells on the assumption that stem cells were entities. This work arguably also demonstrated the continuing influence of the genetics paradigm over stem cell

research; the potential discovery of a genetic marker that would identify all stem cells (as distinct from non-stem cells) was considered to be a pinnacle of achievement – if genetics could be used to separate stem cells, then this would be a definitive identification tool. Instead, none of these groups were able to find either a genetic marker, or a specific genetic profile that could be used to separate stem cells from non-stem cells.

Through Chapter 6, further evidence is provided that suggested historical experimental results could be re-interpreted under the new stemness view, as could more recent experimental work. The role of the niche, the differentiation of early embryonic cells, and lack of molecular markers (as above) all point towards the stemness view being the most accurate. Also in Chapter 6, cell plasticity is explored, again in order to test which view of stem cells is likely to be the most accurate. This is arguably the clearest evidence available for demonstrating the stemness view: the nucleus of any cell can be placed in an enucleated fertilised egg, which contains and can activate the information required for an entire organism to be created. The SCNT, or 'cloning' technique demonstrated not only that the potential to develop into another entire organism is present in each nucleus, but again highlighted our reliance on genetics to provide knowledge and understanding in biology. The emphasis on SCNT is that all that is needed to grow, organise, and develop a new organism is contained within the nucleus, and, more specifically, with the genetic information contained within. This clearly showed that when trying to define new knowledge and understanding in biology, that knowledge and understanding produced under the genetics paradigm is considered to be the most convincing.

Lastly, regarding stem cell research specifically, it may now be that in the twenty-first century, stem cell research becomes increasingly influenced by the stemness view, instead of relying on the stem cell state view. Laplane showed how useful this new view of stem cells may be in the practical search for cancer treatments. When reviewing her book for *Nature*, stem cell scientist Hans Cleavers referred to the stemness view as a new way for researchers to design their experiments, and to interpret results. Although this is an approach still strongly linked with the importance of genetics, it may also be accurate to suggest that this is the beginning of a paradigm shift in stem cell research, away from the old paradigm that assumed stem cells were entities, and towards the view that any cell, given the appropriate environment and genetic signals, can become a stem cell.

3. Outlook

Previous work into the research directions taken by scientists has been carried out on some scale (such as the identification of American work of the late nineteenth and early twentieth centuries being concerned with cell lineage studies), although the evidence provided in this thesis suggests that there is a rich seam of research to be mined on the shifts in research directions (based on experimental design, theoretical to experimental objects of research, social, political, cultural effects etc.), particularly in the twentieth century. This could include, for example, closer analysis of the interaction between the national politics of science and research, especially focusing on specific examples (previous studies have tended to generalise to an extent). It might also include the effects of global politics, such as the effects on research caused by the influx of migrants to America from Eastern Europe, Russia, and Germany before and during WWII.

It will also be possible to continue examining the influence of the potential paradigm shift in stem cell research, from the entitiy view to the state view. Future interdisciplinary work alongside biological sciences researchers using ESCs, would enable the historical and philosophical perspectives suggested in this thesis to be tested. It would also be potentially beneficial to scientists, as they would have a different stem cell concept as the basis on which to design their experiments, and interpret results. This may not only have uses in cancer therapies (as suggested by Laplane's ongoing work), but for other areas of research, including developmental and genetics studies; scientists appear to be open to this new way of thinking about stem cells (as suggested by Cleavers' review of Laplane's work in *Nature*), and seeing how this emerges in the near future will be of particular interest.

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