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Regenerative medicine's historical roots in regeneration, transplantation, and translation

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ABSTRACT

Regenerative medicine is not new; it has not sprung anew out of stem cell science as has often been suggested. There is a rich history of study of regeneration, of development, and of the ways in which understanding regeneration advances study of development and also has practical and medical applications. This paper explores the history of regenerative medicine, starting especially with T.H. Morgan in 1901 and carrying through the history of transplantation research in the 20th century, to an emphasis on translational medicine in the late 20th century.

Regenerative medicine, as it has been labeled, typically calls for regeneration of lost function to address clinical medical problems. A widely adopted description recorded by the NIH captures several different aspects of the research, noting the several goals to replace lost structures, to regenerate failed functions, and to solve problems in new ways. Although these goals are not identical and may not even be compatible in some cases, the ideal involves them all: "Regenerative medicine is the process of creating living, functional tissues to repair or replace tissue or organ function lost due to age, disease, damage, or congenital defects. This field holds the promise of regenerating damaged tissues and organs in the body by stimulating previously irreparable organs to heal themselves. Regenerative medicine also empowers scientists to grow tissues and organs in the laboratory and safely implant them when the body cannot heal itself" (NIH, 2006).

Like most who have commented on the history of regenerative medicine, the NIH description points to the background of mid-20th century organ and tissue transplantation and then to more recent efforts to engineer tissue and grow tissues and organs "on demand." Imagine, the NIH authors suggest, "a world where there is no donor organ shortage. Where victims of spinal cord injuries can walk, where weakened hearts are replaced. This is the long-term promise of regenerative medicine" (NIH, 2006). The image is alluring indeed, and it is not surprising that the NIH roadmap to translational medicine has embraced regenerative goals. The campaign to regenerate lost structure and functions has obviously provided a compelling program to rally public interest as well.

Yet, current research draws on several different lines of historical study that have been grounded in different underlying assumptions and have benefitted from different techniques and methods. At root are studies of regeneration and transplantation, and it is worth looking more closely at those rich research traditions of the first half of the 20th century and at the ways they came together in productive ways in the second half century. A closer look at the background of empirical research and interpretation reveals underlying assumptions that have shaped the research.

Reflecting on history is not just a matter of learning about the past to avoid being condemned to repeat it, as George Santayana urged. More importantly sometimes is understanding the past to be able to repeat it, by returning to once-productive paths of research later set aside. This is the case with regenerative medicine. This research obviously did not begin out of nothing with the first published human embryonic stem cell experiments reported in 1998, as the popular story might suggest, but has instead had a long rich history of study within developmental biology focused on regeneration, transplantation, and translation. Therefore, the emphasis here is on the internal workings of the research rather than the larger contingent context in which the research took place.

T.H. Morgan's *Regeneration*

As so often happens in the history of biology, the story takes us back to Aristotle. This eager empiricist described aspects of regeneration, as of lizard and snake tails (in commentaries variously referred to as salamander tails and occasionally even limbs), in his discussions of animal generation (Aristotle). The 18th century brought much more study of regenerative capacities in hydra especially, in the

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context of debates about how development occurs (Lenhoff and Lenhoff, 1991). As historian Shirley Roe has explained, discussions ranged over the details of empirical observations as well as philosophical issues.

The most heated of those debates centered on the extent to which an individual organism was guided by some internal predetermined instructions or even involved the growing larger of what was already there perhaps in miniature but somehow preformed. In contrast to this preformationist view, others agreed with Aristotlean epigenesis as the mode of development and argued for a gradual emergence of form from the unformed. Shirley Roe and others have laid out the nature and substance of these lively debates, which included occasional physical duels and hinged on fundamental metaphysical assumptions about such issues as whether life is nothing but material or also involves a vital force or entity of some sort (issues of materialism vs. vitalism) as well as core epistemological assumptions about the extent to which empirical observation can reveal the fundamental facts of living organisms (Bowler, 1971; Roe, 1981; Maienschein, 2005).

At the turn of a new century, in 1901, Thomas Hunt Morgan provided a synthesis of what was known about studies of regeneration and the various available observations, experiments, and interpretations. Morgan had been invited to deliver a series of lectures at Columbia, and he expanded and published those lectures in his synthetic book *Regeneration* (Morgan, 1901; Sunderland, 2008). There, Morgan pointed to central questions that the phenomena of regeneration raise for biologists. He also revealed the extent to which he was committed to an epigenetic view of development as a gradual process responding to a mix of internal and external factors, with no hint of the importance that genetics would come to play in his work just a decade later.

In particular, Morgan focused on regenerative capacities of hydra, earthworms, planarians, and plants, and he also included regeneration of eggs and embryos, after they were experimentally cut or wounded. He explicitly noted that while older researchers had pointed to replacement of lost parts, physiological regeneration of function was surely as important. Morgan also saw it as important to determine the ways in which the injured part regenerated. In some cases, the remaining part of an injured organism undergoes a proliferation of cells, which then form the missing part though a process of "epimorphosis." In other cases, the remaining part does not proliferate cells immediately but rather is itself transformed into the missing part. This latter case involves a process Morgan called "morphallaxis." The existence of two different paths to regeneration suggested a complex internal set of responses to changing conditions including injury. It is worth reviewing the structure, reasoning, and empirical grounding for Morgan's important work, which served as summary and as stimulus to new research.

Regeneration can be affected by external conditions, such as temperature, pressure, food, light, gravity, or chemical change, or by internal factors including polarity, orientation, or the material constitution and structure. Morgan's discussion of internal factors included consideration of the influence of parts immediately proximate to the regenerating part and the nature of cell divisions and role of the nucleus in influencing how and even whether a cell would differentiate. While his focus remained on animals, he included a chapter on plant regeneration and asked about the extent to which plant processes could inform understanding of animal regeneration. His three empirical chapters 2–4 lay out the range of observations he had made as well as those published by others.

In the fifth chapter, Morgan took up the hypothesis that parts most liable to being injured and therefore in most likely need of regeneration are in fact those with the greatest regenerative powers (p. 92). August Weismann had argued that this resulted from the action of natural selection, which favored organisms capable of regenerating the parts most likely to be injured (Weismann, 1892; Churchill, 1968). Morgan rejected the argument and rejected Weismann's reasoning quite generally. Instead, he found through experimentation that other parts not likely to be injured are also capable of regenerating (p. 103). Furthermore, he noted many examples of organisms that are not often injured but have extensive regenerative powers. Yes, he concluded, the ability to regenerate is of adaptive value, but the ability is much more generalized than could be explained by Weismann's hypothesis (p. 107). In fact, Morgan questioned the adaptive powers of natural selection generally (p. 110) and suggested that it would be better to remain focused on the capacity to regenerate itself and not to be distracted by unsubstantiated and presumably unsubstantiable explanations based in presumed natural selection.

Chapters 6–8 looked inside the developing organism. First, at regeneration of internal organs; then, at physiological regeneration with experiments especially on planarians; then, at issues of self-division, budding, and theories of autonomy. Through this section, Morgan expanded the range of types of regenerative phenomenon that called for explanation. And he emphasized the relations of any regenerating part to the whole—especially in cases where a relatively small part regenerated the rest of a whole organism.

Grafting, in chapter 9, introduced still further ways that regeneration occurs, through experimental manipulation and involving transplantation of parts. Different pieces from different organisms could be joined together and would develop, often in quite unusual and certainly unnatural ways. Gustav Born's grafting of pieces of tadpoles raised especially interesting questions about the relative contributions of the different parts to the resulting whole. Such work also raised fundamental questions about the extent to which the result was generation of form and function, or actual regeneration of something already existing or in the process of coming to exist in any real sense.

Morgan noted that the sort of heteroplastic grafting that Ross Harrison had done, with pieces of tadpoles from two different frog species, could be interpreted in different ways. Harrison was inclined toward the view that the resulting development was actually new growth, while Morgan felt that the result was actually a heteromorphic growth of tissue into something new as a result of the changed conditions (p. 187). He asked what would happen if two eggs could be combined at the very earliest stages before any differentiation, although that experiment had not been carried out. Such experiments might help get at the fundamental questions about how much the development is set in the egg (or at any point along the process of development) and adapts to changing conditions, or how much is actually only formed through the gradual epigenetic processes of development and differentiation.

In chapters 10 and 11, Morgan turned to look more closely at the fine structure of cells and tissues and then at eggs and embryos. Acknowledging that the only method available for determining the source of cells moving into a regenerative part was through serial sectioning, Morgan tried to piece together what he knew. Cells, tissues, germ layers: all called for more study. So did understanding of eggs and their development into embryos.

In particular, Wilhelm Roux's experiments with frog eggs, in which he had destroyed one of the cells at the 2-cell stage, led Roux to conclude that development occurs in a self-differentiating mosaic way (Roux, 1888). When it became clear that the one cell could compensate after all, Roux concluded that it "post-generated" through a process of regeneration (p. 216). Morgan carefully explained that the remaining cell had the power to proliferate cells capable of migrating to the injured side and differentiating so as to compensate for the missing material and its function. It is not the case, Morgan concluded, that cells just develop at the edge and move outward through a postgeneration, but rather the whole developing organism contributes to the development of new material (p. 227)

In this chapter, Morgan also pointed to Hans Spemann's experiments with ligatures to discover under what conditions separated

cells could develop into whole frogs and when injuries of various sorts would occur. Hans Driesch, Edmund Beecher Wilson, Oscar and Richard Hertwig, and others had carried out other experiments to get at the same set of questions about the relative contributions of different blastomeres to the emerging organism (see Gilbert, 1991 for more discussion).

In his final three chapters, Morgan considered theories of development and of regeneration, then of what conclusions followed from this discussion. These chapters provide an excellent survey of the theoretical discussions of the day, in this period before genetics, and in the context of considerable work on cytology and embryology. Morgan was clearly interested to discover the extent to which regeneration is the same as normal developmental processes and the extent to which the regenerative processes brought something different.

It is important to note that Morgan, best known for his Nobel Prize winning work on *Drosophila* genetics, rejected the prime importance of the nucleus and the idea of nuclear determinants for development. He likewise rejected mosaic ideas like Roux's and Weismann's that involved dividing up the inherited material into different cells to explain development. All the empirical evidence suggested to him the importance of the cytoplasm, which was already organized in some ways in the egg. And accumulating evidence from transplantation and other experimental studies suggested strongly that the growing and differentiating organism has from a very early stage a strong capacity to respond to changing conditions and still preserve the expected structure and function. There is, it seems, a powerful capacity to "regenerate" or to generate the normal results in the face of injury or unusual conditions through processes that could be considered regeneration.

Rejecting any call on vitalistic forces or entities, such as his friend Hans Driesch's entelechy, Morgan insisted on physico-chemical processes alone. Similarly, he rejected the suggestion that the nucleus contains some sort of "Anlagen" that determine development. While acknowledging that the causes of regenerative capacities remained unknown, he was nonetheless confident that understanding them would provide a window into understanding development and differentiation (Sunderland, 2008, in press). He offered a hypothesis based on the assumption that the organism as a whole is guided by an "organization" of some sort, perhaps directed by "tensions" distributed throughout the organism from its earliest stages and the way these helped guide development (pp. 258, 272–278 on his tensions hypothesis). In retrospect, it is a decidedly unsatisfying attempt at explanation (Wolpert, 1991).

Yet Morgan's summary served as the foundation for studies of regeneration for some decades, with researchers referring back to his work. As he noted, it was not easy to get at the fundamental causes of development and differentiation. And what Morgan provided was a synthetic summary of a vast amount of research done to date, contextualized and presented in a very clear descriptive way. His work provided for embryology at the time what Wilson's *The Cell* provided for cytology. Yet as is typical in science, Morgan's work was both successful in carrying out its immediate task and also limited in anticipating later research breakthroughs because of his underlying assumptions. As discussed elsewhere, Morgan's epistemology constrained him—in ways that were extremely useful for his science at the time but also in ways that limited his vision of what was possible (Maienschein, 1991).

Like his contemporary American biologists trained at Johns Hopkins, including most notably for the history of developmental biology Wilson, Edwin Grant Conklin, and Ross Granville Harrison, Morgan remained an empiricist first rather than a theoretician. He assumed that hypothetical invisible units of heredity could not and should not be seen to play a role in causing development, as Weismann, Roux, and others argued (Roux, 1888; Weismann, 1892). Nor should the focus be on the nucleus and chromosomes, as Boveri suggested (Laubichler and Davidson, 2008). Charles Manning Child's hypothetical gradients were equally suspect (Child, 1915). In all cases, the empirical evidence was not there in Morgan's view. Like his pragmatic dissertation director William Keith Brooks, Morgan strongly disliked theoretical interpretation that seemed to run ahead of its evidence. Hypothesizing was fine, but within limits and only when carefully identified as such.

For Morgan in 1901, what he saw was the capacity of many organisms to regenerate in a variety of different ways. For him, regeneration was an epigenetic process of responding to internal and external environmental cues leading to a formed organized organism (Maienschein, 1997). Understanding regeneration would get at understanding fundamental processes of development and differentiation.

Transplantation

In the absence of clearly compelling explanations, different researchers largely emphasized other research directions and took up questions of regeneration in other contexts. Some followed chromosomes and evolutionary explanations, while others focused on cytology. One of those other lines of research, and the one most important for our look at the prospects for regenerative medicine was work on transplantation, for both basic biological and also for clinical purposes. This work was related because transplantation experiments produced conditions that called for response. Did the organism generate or regenerate normal structure and function in response to experimental manipulation through transplantation? Or what did happen, and how did it relate to both normal conditions and to other cases of regeneration?

As mentioned, experimental embryological research included Weismann's and Roux's efforts to provide evidence for his chromosome-mosaic explanation of development, in which it is the distribution from chromosomes of inherited units to different cells and their "Kampf der Theile" (or struggle of parts) that leads to differentiation. Roux sought to claim experimental embryology for his own, with his journal *Archiv für Entwickelungsmechanik der Organismen* begun in 1894. In fact, Roux recognized but tended to ignore the implications of transplantation studies. As many young scientists of the time and historians since have reported, Roux was very successful at promoting his own theoretical interpretation and subsuming other work to his point of view. This is one reason a group of leading American biologists decided to found the *Journal of Experimental Zoology*.

In contrast to Roux's emphasis on self-differentiation in response to distribution of chromosomes, experimental transplantations suggested that organisms have a great deal of developmental plasticity and considerable capacity to respond to changing conditions and to regenerate effectively. In the 1880s and 1890s, Gustav Born used the frog eggs that were abundant near him in Breslau and began transplanting parts of one tadpole to another. What happens when parts like limb buds, that would normally become limbs, were transplanted from one organism to another—could they still develop? When it was clear that the answer was yes, Born asked whether they would develop as they normally would have, or in ways more responsive to their new conditions (Born, 1896, 1897). His successes inspired others to take up the approach, for which Hans Spemann and Ross Harrison became the leaders. Both had access to large and well-supported research labs with students and assistants to carry out the work, and both had the imagination to try new transplantations.

Spemann's studies of limb and eye vesicle transplantations have been well documented and discussed by Viktor Hamburger, who worked in Spemann's lab (Hamburger, 1988). Spemann's observations led him to conclude that there is something that guides the organism's capacity to organize development. He introduced the concept of induction, as a process whereby one part influences surrounding tissue in its differentiation. Furthermore, this led to the idea of the organizer, which served to organize the full organism and to induce the parts to differentiate and also to work together.

Developmental biologists are very familiar with textbook versions of these ideas, yet it is worth noting Hamburger's strong argument that Spemann had somewhat vitalistic leanings in this work. Spemann found it difficult to see just how the organization process could work so effectively every time, under such diverse and contrived conditions, unless there were some directive drivers. As Hamburger noted, this aspect of Spemann's work had been largely set aside in the way we remember Spemann as a proper materialist experimentalist. It might not do to have a Nobel Prize winner celebrated for his vitalistic wholistic tendencies, after all (Hamburger, 1988, pp. 64–67). Yet as Hamburger has reminded us, it is worth examining the underlying assumptions in a scientist's work, since doing so can reveal paths not taken as well as illuminating choices that were made.

Ross Harrison was the American leader in this line of research, and at first, he pursued questions of limb regeneration in frogs that followed on his study of symmetry in teleost fishes for his Ph.D. from Johns Hopkins, which had gained him a position at the Johns Hopkins Medical School Anatomy Department (Harrison, 1898). For his earliest transplantation studies, Harrison was in Bonn in 1898 and 1899 pursuing his M.D. under Rudolf Leuckart, a degree that earned him a promotion to Associate Professor on his return (Maienschein, 1991).

In Bonn, Harrison explicitly compared transplantation results to processes of regeneration and asked about the processes by which the frogs with transplantations compensate for loss or respond to added parts. In particular, he transplanted a tail from one organism to another from a different species (heteroplastic grafting), asking "whether a tail will be produced under the influence of the position of the regenerating center with regard to the whole organism, or whether the elements in the transplanted stump retain their original orientation and strive to reproduce the lost body." He needed a test and concluded that, "these experiments establish beyond a doubt the fact that the regenerative power of the tissues of the tail is very considerable in both directions" (Harrison, 1898, pp. 449, 464).

Harrison focused in particular on nerve development, asking how it could be that the nerves, which seem so subtle and complex when added up into a whole functioning nervous system, could make functional connections in transplanted tissue. He assumed that the cells play important determining roles and therefore focused on observing individual cell changes. Rather than starting with the assumption that the nerves are already formed in the tissue and therefore move with the transplanted part, Harrison concluded by observing carefully that particular cells give rise to nerves. His observations convinced him that neuroblasts differentiate as fully formed nerve cells as they stretch out their nerve fibers through protoplasmic outgrowth to establish functional neural connections. Or, he concluded, his results could be interpreted in only one way, namely that, "The nerve center (ganglion cells) is shown to be the one necessary factor in the formation of the peripheral nerve. When it is removed from the body of the embryo the latter fails to develop. When it is transplanted to abnormal positions in the body of the embryos it then gives rise to nerves which may follow paths, where normally no nerves run, and likewise when the tissues surrounding the center are changed entirely, nerves proceeding from that center may develop as normally. The nerve is therefore a product of the ganglion cell" (Harrison, 1906, 129). It is not that nerves are caused to differentiate out of and by the surrounding tissue, as some maintained, or that the neural paths are already laid down as protoplasmic bridges, as others were arguing. Nerves differentiated from ganglia, or neuroblast cells.

This line of research has been discussed elsewhere (Maienschein, 1991; Witkowski, 1985) and what is most important here is Harrison's efforts to establish definitively that nerve fibers can experimentally or do normally develop by outgrowth. He reported his results in 1907. There and again in more detail in 1910, Harrison reported on his transplantation that took neuroblast cells out of the organism altogether (Harrison, 1907, 1910). This was an extension of the experimental transplantation research that depended on several underlying assumptions. First, Harrison was convinced that the cells themselves were the source of nerve fibers and therefore that research should focus on individual cells and track their movement as closely as possible. Second, he assumed that if he transplanted neuroblast cells into an artificial culture medium consisting of frog lymph, the resulting movement would be like normal development if it looked like normal processes. He fully recognized that he was making these assumptions, but felt they were valid since if they were wrong, further experimentation would show that and call for revisions (Harrison, 1912).

In 1907, Harrison reported on the successful culture of nerve fibers in the hanging drops of lymph. In retrospect, this was the first stem cell experiment, in which neural stem cells were cultured successfully to become functioning cells. Instead, what mattered at the time was that this was the first culture of cells and tissue outside the body. His results were nominated for a Nobel Prize, and the evidence is strong that he should have received a Prize (Nicholas, 1961, pp. 149–150).

Harrison did not seem to have minded too much. For above all, Harrison was an experimental embryologist driven by the desire to understand and explain development rather than by interest in medical applications. Indeed, after he moved from the Johns Hopkins Medical School to Yale University's Yale College and Medical School in 1907, he was still appointed in medicine but showed little interest in clinical innovations. Only his role as leader of the NRC revealed a medical interest (Harrison, 1944).

As a result, it was Alexis Carrel at the Rockefeller Institute in New York who took up Harrison's tissue culture methods and applied them clinically. Hannah Landecker has done an excellent job of looking at Carrel's work and placing it in the larger context of cell cultures (Landecker, 2007, chapter 2). Carrel was just as committed to developing the clinical uses of the methods as Harrison was to studying development and regeneration. He also looked at the possibilities for transplantations—in his case of organs and tissues and cells. And his emphasis on the ability to culture "immortal" cells in the appropriate culture dish helped lead to widespread assumptions about the ability of cell lines to divide forever (Carrel, 1912). The certainty about cell culture immortality provided such a strong underlying assumption that it took researchers such as Leonard Hayflick a significant effort to overcome that "truth" and move us instead to a set of assumptions about limitations on the number of cell divisions and the role of telomeres and telomerase. Whether the new orthodoxy holds, certified by the 2009 Nobel Prize remains to be seen (Brady, 2007; Nobel Prize, 2009).

Transplantation + regeneration together

Research on transplantation and on regeneration converged in various ways over time, starting most explicitly with Harrison's early work. Eugen Korschelt saw the connections and summarized relevant studies and interpretations in two impressive volumes in 1927 and 1931 of his *Regeneration and Transplantation* (Korschelt, 1927, 1931—work that deserves much closer attention than it has received and than it is possible to offer here). Other researchers looked at regeneration in terms of biochemistry (Joseph Needham), as a phenomenon of cell action and interaction (Jean Brachet), as a result of cell dedifferentiation and redifferentiation in forming regeneration blastema (Conrad H. Waddington), or as a matter of position and metabolic activity (Charles Manning Child). Each of these contributions deserves a great deal more attention. For our purposes, however, focused on the intersections of regeneration and transplantation in ways that lead to possibilities for translation, three historical episodes have brought innovations at the intersections. Cloning, chimeras, and stem cells have challenged existing assumptions and raised new questions about the resilience of developing organisms under experimental or natural attack.

Cloning

As early as 1938, Spemann saw nuclear transplantation as the ultimate transplantation or an "experiment which appears, at first sight, be to somewhat fantastical," though it was not yet called cloning (Spemann, 1938, p. 211). Since it was possible to transplant various body parts, why not transplant a nucleus, he asked? Such an experiment could begin to test the relative contributions of nucleus and cytoplasm to development. As we know, Spemann did not fully carry out the experiment himself, but Robert Briggs and Thomas King did (1952) with frog embryos in the earliest stages of cell division. Then John Gurdon showed that it was possible to do successful transplantations with somewhat later embryos, but the question remained whether later developmental stages were already too determined to be transplanted in this way (Maienschein, 2003).

By the 1960s, embryologists and developmental geneticists had become persuaded much more than earlier generations that development works through a process of progressive differentiation. Cells become differentiated and presumably determined into specialized roles, and the assumption became part of textbook thinking that the arrow of development goes one direction only. Presumably, the emphasis on the genetic dogma with its arrows from inherited DNA to RNA to protein reinforced the idea that time's arrow goes in one direction. Only in recent decades have researchers been challenged sufficiently to overcome this strong underlying assumption.

Chimeras

Another assumption was that an organism is an individual, and although it might be possible to move around parts of lower organisms like hydra, and even sponges (as H.V. Wilson had shown, 1907), surely this would not be possible for mammals. Charles E. Ford et al. reported in 1956 on experiments with transplanting of hematopoietic cells from a normal mouse to one lethally injured by radiation. The result led to recovery by the recipient, and the question concerned why. Was it the transplanted donor cells that were being taken up by the host and incorporated into a new chimera? As Ford's group put it, "Although the very term chimera points to the antiquity of the idea, it is believed that the experiment reported here provides the first decisive evidence in animals that normal cells of one species may, in special circumstances, not merely survive and multiply in another, but even replace the corresponding cells of the host and take over their functions" (Ford, et al, 1956).

Beatrice Mintz from the Institute for Cancer Research in Philadelphia went further. In the 1960s, she developed improved techniques for harvesting cells from different mice and "shoving them together" as the technique was sometimes described. An abstract from the 1962 summer meeting of the American Society of Zoologists explained that, "A method has been developed whereby some or all of the blastomeres from two mouse embryos in early cleavage stages may be readily combined: these cells, or whole eggs, quickly reassort to form a single embryo which continues normal development. The best survival is obtained, and the simplest procedures required, when entire eggs are united at approximately the 8-cell stage." The procedure only worked after the zona pellucida was removed, and Mintz's contribution included a method for removal that did not harm the cells. She found that the cells combine in about an hour, and the mosaic, as she called it, could be made from normal embryos or even with lethal mutants (Mintz, 1962).

In retrospect, it is rather surprising that this ability to construct embryos out of cells from different eggs/early preimplantation embryos did not raise more public interest at the time, with its implicit possibilities for creation of human chimeras. The familiar striped mice that showed their genetic mosaicism so clearly seem very photogenic. This line of research has largely escaped the attention of bioethicists, presumably because it does not immediately affect humans, and until recently, bioethicists remained focused on human medical issues. More recently, bioethics debates have focused largely on human/non-human chimeras, but surely for those who believe that life begins with fertilization, the ability to separate and combine cells at later stages should at least raise ethical if not practical questions. Yet this is another topic, for further exploration (Robert and Baylis, 2003).

Stem cells

Another line of research, which, at the time, also seemed to be addressing rather different questions, concerned stem cells. Concepts of stem cells derived from "Stammzellen" had been introduced as early as the late 19th century, originally in plants and by the mid-20th century also in animals (Maienschein, 2003). They were taken to be undifferentiated cells, which had the capacity to differentiate in various ways under different conditions. Stem cells acquired major significance in the 1960s, with the recognition that bone marrow transplants could restore lost function in patients, presumably because the hematopoietic cells in the bone marrow that were not fully differentiated could give rise to several different types of cells including blood cells (Ford, et al., 1956; Becker et al., 1963). These hematopoietic cells are a special type of stem cell but raised questions about whether other cells might also be sufficiently undifferentiated and have enough plasticity to respond to new needs under changing conditions.

That question led many researchers to explore the plasticity of different cells. Given the background of research in cell and tissue culturing, it is not surprising that researchers would soon successfully culture a diversity of cell lines ranging from the cancerous human HeLa cells to embryonic stem cells in mice (Landecker, 2007). Leroy Stevens's work on mouse stem cells starting in the 1950s at the Jackson Laboratories introduced pluripotency. The Stevens story most often told (Lewis, 2000) explains that he began doing research funded by the tobacco companies, exploring in mice whether it was the tobacco itself or perhaps some other factor such as the paper in cigarettes that caused medical problems. Fortunately, Stevens discovered mouse strain 129 that developed teratomas in the testes. The mix of tissues included teeth, hair, and other cells that definitely should not have been there under normal conditions. Stevens recognized the value of this strain and bred them to determine what cells were leading to the strange results.

In 1970, Stevens reported on the results of long years of careful examination of many different cell types to discover the origin of the teratomas. The cells from the blastocyst's inner cell mass, taken from 3- and 6-day-old mouse embryos, were pluripotent, that is, they were able to proliferate indefinitely and also capable of being differentiated as any kind of cells including the cells that made up the teratomas. When Stevens grafted the embryos to the testes of adult mice, the result was teratomas that "resemble in every respect the spontaneous testicular teratomas characteristic of strain 129/Sv" (Stevens, 1970, p. 381).

Mintz and Karl Illmensee reportedly visited Stevens to borrow some of his mice and learn more about his methods. They applied the techniques and showed "an unequivocal example in animals of a non-mutational basis for transformation to malignancy and of reversal to normalcy. The origin of this tumor from a disorganized embryo suggests that malignancies of some other, more specialized stem cells might arise comparably through tissue disorganization, leading to developmental aberrations of gene expression rather than changes in gene structure" (Mintz and Illmensee, 1975, 3585). They showed that the embryonic stem cells could, under the right conditions, give rise to whole organisms and not just to teratomas, and Ricki Lewis suggests that "Their surprise announcement of this feat at a meeting floored Stevens, a story unto itself" (Lewis, 2000, p. 5).

The more familiar stem cell research, including establishment of mouse pluripotent stem cells lines in culture, starts in 1981 and builds on Stevens's foundations. Martin J. Evans and Martin H. Kaufman in Cambridge and Gail Martin at the University of California San

Francisco, both reported on their successes (Evans and Kaufman, 1981; Martin, 1981). Evans and Kaufman concluded definitively that "We have demonstrated here that it is possible to isolate pluripotent cells directly from early embryos and that they behave in a manner equivalent to EC cells isolated from teratocarcinomas. The network of inter-relationships between the mouse embryo and pluripotent cells derived from it has previously lacked only the direct link between the embryo and cells in culture for completion. We have now demonstrated this." Further, as Martin concluded, "Given these results, it seems likely that there will soon be available pluripotent, embryo-derived cell lines with specific genetic alterations that should make possible a variety of new approached to the study of early mammalian development" (Martin, 1981, p. 7638).

The story of stem cell research reaching from mice to men is well known and we need not repeat it here, except to remind readers that the work of James Thomas and John Gearhart demonstrated in 1998 that the same ability to culture pluripotent stem cell lines was available in humans as well as mice (Maienschein, 2003). The major difference between 1981 and 1998 was not extension of techniques to human cells, however, but the advances in understanding of the genetic basis. Molecular genetics had progressed to the point that in less than a decade after the first isolated human embryonic pluripotent stem cell lines were established, researchers had discovered ways to isolate the essential genetic factors and induce pluripotency in cells that were not from embryos.

The discovery of induced pluripotent stem cells in mice and then humans challenged what had earlier been fundamental underlying assumptions about development. First, the central genetic dogma that DNA gives rise to RNA gives rise to protein. Second, the arrow of differentiation that goes only in one direction. It did not take long to recognize that the paths of gene expression were much more complicated than the initial models had suggested, and that many factors direct gene expression and interactions with other factors in the organism's internal and external environments. But it took longer to overcome the idea that cells are undifferentiated and become differentiated, gradually over time. The old epigenetic assumptions hold true most of the time under normal conditions, but it has become increasingly clear that dedifferentiation occurs not infrequently, and redifferentiation as well. A pluripotent stem cell may give rise to a differentiated somatic cell, which in turn may be reprogrammed as a pluripotent cell that seems for all practical purposes to act like a stem cell (see Lanza, et al, 2009 for a compendium of discussions of stem cell biology).

Translation

This is not the place to repeat the story of translational medicine, nor to discuss the practical and ethical ramifications of a perhaps over emphasis on translation. Yet the research traditions are importantly connected, with regeneration and transplantation leading to emphases on translational medicine (see Maienschein, et al, 2008 for discussion of the implications). It will be a wonderful thing when researchers can take cells, cause them to become the right kind of tissue, transplant that, and bring about regeneration of lost function and perhaps even structure. This is the goal, of course, and the push for clinical applications is not surprising. It is important that the research community not let the political demands for results get ahead of the science, of course, but there is considerable excitement in beginning to make progress with that science and to being to see answers to some of Morgan's questions about how regeneration works.

What difference does the history make?

Of course, the most common use of history is in gaining perspective, through stories about past people and practices. The stories can inspire young researchers and serve to help "humanize" science, since research rarely is as neat and tidy and linear as science textbooks might like to suggest. Beyond such stories, however, the more important value of revisiting the history is to illuminate underlying assumptions and examine the choices made (Laubichler, et al, 2008). Instead of the oft-encited purpose of studying history so as not to repeat it, looking at historical work can actually allow to us to repeat it. Or rather, we can repeat the parts and approaches that it makes no sense to pursue.

In the case at hand, we can return to Morgan's questions and assumptions. He rejected the mosaic hereditarian ideas of Roux and Weismann, and in retrospect, we might be surprised by the fact that he then became the most prominent and first Nobel Prize winning geneticist. Yet if we look more closely, we see Morgan aware of the complex interactions of organisms with their environmental conditions. We see Morgan as asking instead about the ways that cells and tissues accommodate to change: do they grow new material and new parts, he asked, or transform existing material and parts? This remains a central question for developmental biology, and all the work since 1901 has not given us definitive answers. It is worth returning to some of the transplantation experiments of Ross Harrison to look at the lines of research set aside at the time.

Similarly, it is fascinating to revisit Stevens's experimental production of teratomas and see how far the research took him and why he did not go farther. His studies are ripe for exploration of genetic causes, but he lacked the molecular techniques needed to knock out and otherwise regulate gene expression. If researchers go back to where he was, what might we be able to learn from picking up the lines of thinking and research? Stevens understood a lot about differentiation of cells in ways that could have kept a generation of researchers from making what turn out to have been bad assumptions about the relative plasticity of cells, for example. We could go on, looking back at what Mintz was trying to accomplish with her studies of chimeras and some of the lines of research set aside as others seemed more promising. It would be great fun to ask not "how would science be different if history were different than it was," but rather "how can science be different now if we go back in time and reflect on the reasoning, the underlying assumptions, and the ways of doing research at various exciting lines of research into transplantation and regeneration."

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