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The Molecular Biography of the Cell

Essay

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The difference between radically different destinies often reflects disarmingly small variations in timing or circumstance. If the motorcade takes a different route through Dallas, if Newton sits under a different tree, or if Ilsa picks another gin joint to walk into, the story is radically altered.

In biology, perhaps the most profound example of the effects of small differences is the development of an adult organism from a single fertilized egg, a journey from inner cell mass to fully differentiated structure. The challenge of developmental biology lies in defining the influences and factors that contribute to this final destination.

As we study the life and lineage of a particular adult cell, we ask the same questions that a biographer asks of her subject: what were the critical decisions that defined the trajectory of this life, and when were they made? What was the contribution of neighbors, and what role was played by more distant influences? What was the role of chance? At what point was the final fate initially specified, and when was it ultimately sealed? In essence, we would like to understand the molecular biography of the cell.

Initial studies of development, like many early biographies, were purely observational, simply reporting what was seen. Technological advances—the discovery of the microscope and the development of cell staining techniques—led to new knowledge, including the identification of the three germ layers (from which all tissues are subsequently derived) by the German scientist Christian Pander in the early 19th century. These studies occurred only a few years after Boswell's landmark publication of *The Life of Samuel Johnson*, a work characterized by unprecedented detail and painstaking research and often regarded as the first biography of the modern era.

Over the next 100 years, the study of embryology became increasingly experimental as scientists pursued the study of "entwicklungsmeckanik," or developmental mechanics; a major goal of this research was to assess the relative contribution of intrinsic factors and environmental influences in the development of an organism. Similarly, biographers of this era, influenced particularly by Freud, began to search more intensively for their subjects' motivation and to adopt a more critical and less accepting tone.

The interrogative approach to the study of development took hold throughout the world, including the United States, where biologist Edwin Conklin's detailed examination of a snail (ascidian) found in the waters off Woods Hole, Massachusetts, led to the observation that the destinies of cells from egg to larva to final organism proceeded along what seemed to be a well-defined and reproducible path. Conklin subsequently devised the first "fate map," linking specific regions of the embryo with the structures they ultimately form in the adult. Conklin would ultimately stake out a middle ground, arguing that "increasing complexity, which is the essence of development, is caused by the combination and interaction of germinal substances under the influence of the environment."

The challenge in both biology and biography, of course, has been to identify these germinal substances and environmental factors. Fortunately, the study of cell lineage has been aided by increasingly sophisticated techniques and reagents; vital dyes, radioactivity, fluorescent labels, and viral markers have all been used to identify and follow cells in the early organism. The substitution of easily identifiable cells from related species (e.g., chick and quail) to generate informative chimeras has also contributed to important advances in this field.

These studies have led not only to new understanding but also to an appreciation for the complexity of cell fate regulation. In many invertebrates, a cell's fate seems to be largely predetermined—a program is in place, and little deviation is typically observed. In these species, factors present in the egg cytoplasm are distributed asymmetrically once cell division occurs; a classic example from Conklin's work is the localized orange "myoplasm" that is present in the ascidian egg and is inherited by blastomeres that will form the somatic muscle in the larvae. More recently, the reproducible patterns of divisions and development have been well described in the nematode, where the 959 somatic adult cells develop at the same time and the same place and following the same pattern of divisions in each animal. This regularity initially tempted researchers to conclude that cell fates were exclusively dependent on intracellular factors and not the surrounding environment. But further studies identified a role for external influences and intercellular signaling as well; for example, if the positions of cells ABa and ABp in the nematode at the four-cell stage are experimentally switched, their fates are similarly reversed (Priess and Thomson, 1987).

At the other extreme, vertebrate development—at least in its initial stages—appears to display a high degree of plasticity. Cell fates in the early vertebrate embryo are largely established by intercellular signaling (induction), as revealed by the ability of these embryos to compensate for the experimental removal of component cells. For example, it is possible to remove a blas-

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tomere from an early frog, mouse, or human embryo (in the case of preimplantation genetic diagnosis) without affecting the development of the organism; the remaining cells are able to compensate entirely. Moreover, in what may be the most striking experimental example of developmental plasticity, the nucleus of an adult somatic cell can be transferred into an enucleated fertilized egg, which can then develop into an entire organism; these studies were initially performed by John Gurdon (1962) in the frog and have subsequently been extended to many mammalian species, including sheep and mice (Latham, 2004). But here again, the story is not so clear; the success of nuclear transfer decreases dramatically as the donor cell acquires a more differentiated status, emphasizing the presence of a nuclear programming process that occurs with increased cellular specification and is ever more difficult to reverse (Hochedlinger and Jaenisch, 2003).

Destiny often seems precariously contingent upon the presence of a key influence at the right place, at the right time, and in the right amount. For example, the differentiation of spinal cord neurons in the mouse depends upon the exposure of cells within the neural tube to precise concentrations of TGFβ-family factors diffusing down from the roof plate above and Sonic Hedgehog diffusing up from the notocord below (Lee and Jessell, 1999). The timing of these signals is equally critical; the same neuronal progenitors that differentiate into neural crest cells in the presence of either BMP-4 or Activin A will instead differentiate into D1 and D2 neurons if exposure to the same factors is delayed by 24 hr (Liem et al., 1997). Similarly, specification of the mesendoderm in zebrafish is dependent upon a delicate titration of nodal signaling, which is modulated by secreted factors that can activate (squint, cyclops) or inhibit (lefty1, lefty2) the inductive signal (Schier, 2003).

Just as the availability of new resources helps biographers refine their original understanding of their subject, biologists have also benefited from recently developed approaches, particularly the opportunity to examine the fate of cells expressing specific genes by using the regulated expression of the bacteriophage enzyme CRE recombinase to alter and indelibly mark the DNA of these cells (and their progeny), or by deleting selected cells through the expression of a potent toxin. In the pancreas—an area of particular interest to our research group—these approaches have led to a number of unexpected findings. For example, cells expressing both insulin and glucagon were widely believed to represent the penultimate stage of α and β cell differentiation before elegant experiments by Herrera (2000) revealed that these double-positive cells were in fact developmental dead ends. Similarly, the proximity of islets to pancreatic ducts led many to conclude that β cells were derived from ducts; recent studies from Dor et al. (2004) demonstrated that, despite this proximity, new β cells in the adult are derived not from ducts but rather from the proliferation of existing β cells.

Yet, for all the remarkable opportunities afforded by these new technologies, as a discipline we have a long way to go before we can claim to understand how a cell's fate is determined, let alone control the process by experimental intervention. For example, we know that future β cells, like all cells in the adult body, are initially part of the inner cell mass, from which the epiblast is derived; we also know that one of the first major decisions made by future β cells in the epiblast is the assumption of endoderm identity (specifically, definitive endoderm). But determining precisely what makes a cell in the epiblast become endoderm remains a formidable scientific challenge; to resolve this puzzle, we try as best we can to understand the experience of a cell in the epiblast, to place ourselves in the position of our subject. We seek to define both the external influences present in the microenvironment—the morphogens, physical forces, and attachment substrates—and the internal constitution of the cell at the time, including an appreciation of which signaling pathways are activated or primed for activation and which pathways are not yet functional. We also recognize that cells typically are not passive recipients of information but rather interact dynamically with their environment, a process that can directly shape the ultimate fate of all the cells involved.

The monumental challenge of sorting through such a complex array of contributing factors to determine the most important influences has humbled biographers and biologists alike. But there is a crucial difference: biologists have the advantage of being able to watch the same story unfold multiple times, providing the opportunity to observe the event from different perspectives, to capture and analyze intermediate stages, and, perhaps most importantly, to interact with the process itself in an effort to determine with greater precision the factors responsible for the final fate.

Moreover, developmental biologists arguably have at least one clear standard by which to measure their success—the directed differentiation of embryonic stem (ES) cells into a specific adult cell type. For some purists, directed differentiation sounds more like a tangential engineering project than a rigorous scientific standard. But what better way to evaluate our understanding of the biology while also generating therapeutically important knowledge—and ultimately clinically useful reagents—in the process? Although we remain a long way from this goal, progress is clearly being made; the generation of motor neurons from mouse ES cells stands out as an especially promising proof of principle (Wichterle et al., 2002).

Biographers are often surprised, and occasionally alarmed, by the implications of their research. Perhaps because the study of origins is so fundamental, perhaps because development itself is so fragile, or perhaps because the language we employ is so encumbered, the study of cell lineage has also raised difficult questions of a broader nature. In a discipline that seeks to define the contribution of genetic factors and environmental influences in the determination of character and the shaping of destiny, extrapolation beyond the embryo may be unavoidable. The French biologist Laurent Chabry, for example, was dismayed by his discovery that the development of a sea squirt appeared relatively predetermined, concerned that he might lend credence to political theories supporting the inheritance of social inequality (Gilbert, 1994). Similarly, Conklin, an ordained lay preacher, struggled to reconcile his scientific observations with his deeply felt religious beliefs, ultimately concluding that "The real dig-

nity of man consists not in his origin but in what he is and in what he may become."

Andre Maurois, the French critic, remarked that "a great biography should, like the close of a great drama, leave behind a feeling of serenity." When we are able to write a definitive biography of the differentiated cell, when we are knowledgeable enough to generate such a cell from an embryonic stem cell in a culture dish, and when we are wise enough to appreciate the responsibility associated with such an accomplishment, then we, too, will be entitled to experience such a feeling of serenity. But until then, let us continue to agitate.

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