3 Cell Lineages in Ontogeny and Phylogeny from 1900

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3.1 INTRODUCTION

Today, the idea that it could be useful to trace cell lineages makes perfect sense, even if the work is difficult and not many researchers are willing to invest the tremendous dedicated energy required to carry out the early kinds of cell lineage studies. Following a cell lineage in its earliest sense means, tracking a cell in an embryo through each of its cell divisions as far as possible. The *Oxford English Dictionary* (*OED*) defines cell lineage as "The manner in which the parts of a multicellular organism develop from the blastomeres of the embryo; the line of descent of a cell from a blastomere or other embryonic precursor; a population of cells sharing such a line of descent." The *OED* also gives credit to the outstanding American cytologist Edmund Beecher Wilson for introducing the term in the late nineteenth century.

The idea of cell lineage study appeared before Wilson, but only barely. Before it made sense to ask about the lineage of cells through many generations of cell division, researchers needed a reason to care about the patterns and nature of the divisions. That, as Theodosius Dobzhansky later noted for all of biology, only made sense in light of evolution (Dobzhansky 1973). We don't think in terms of lineages if we aren't

thinking in terms of descent. If cells are understood simply as dividing materially into more and more cells, but nothing about their historical background really matters, then why make the meticulous effort to trace the details of division over time?

Once scientists began thinking in terms of the evolution of species, it was a logical step to ask what kinds of observations can give evidence about past conditions and changes. How can we begin to "see" aspects of evolution when we were not there ourselves for the millions of years when evolution occurred? Perhaps cells, the patterns of their divisions, and their fates carry "ancestral reminiscences" that reflect the history and help us understand development in terms of evolution, as Wilson suggested. This paper looks at three different periods, which involve different ways researchers have explored cell lineages and their interpretations.

The first period occurred in the decades just before and after 1900. A number of researchers, especially at the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts, tracked cell lineages in the embryos of a variety of different animals. They started with questions about ancestral reminiscence and what they could learn about the evolutionary and phylogenetic relationships of animal groups. They also became interested in the cell divisions themselves, as a contributing factor in the development of individual embryos. That work was extremely time-consuming and challenging, and the researchers soon turned to other questions and other methods.

The second period of focus on cell lineage came with Sydney Brenner's idea that it should be possible to document every cell division in the nematode, *C. elegans*, and track its fate. Brenner, who later won a Nobel Prize along with John Sulston and Robert Horvitz for their work, sought an organism that was easy to observe, easy to cultivate, had a stable and predictable developmental pattern, and for which it was possible to correlate genetic mutations with structural effects.

More recently, the third wave of interest in cell lineage was discovered, which involves following the development of a lineage of particular cells from a starting point without consideration of the evolutionary past. We see this, for example, especially in efforts to interpret the causes and trajectories of cancers. Once a cancer cell is identified, questions that arise are: what does it do, where does it go, how does it develop, divide, or adapt? Are there cancer stem cells that develop into cancerous cells, and what does this even mean? These are lineage studies of identified cells as they develop in the body, but such studies typically do not look to the deeper evolutionary past to interpret the lineages. Another approach to following particular cells takes one or more cells out of the body altogether and traces the lineages of the cell lines in a culture dish. Again, the emphasis is on the particular cell and its development, division, and differentiation going forward rather than looking at the past. For this third type of cell lineage, researchers do not seek to identify or trace the ancestral factors of deep evolution that may be influencing present behavior. It's not that they would not love to be able to capture that evolutionary story, especially insofar as it would help inform understanding of the current situation. *But we don't yet have the tools to connect the individual cancer cells or cells that give rise to cell lines with evolutionary factors such as gene regulatory*

networks. Not yet. It is nonetheless worth looking at this type of cell lineage work and reflecting on what future research may bring.

3.2 CELL LINEAGE AND ANCESTRAL REMINISCENCE AROUND 1900

A Charles Otis Whitman

Though Wilson seems to have first called the study of the cleavage paths of animal embryos "cell lineage," Charles Otis Whitman had already led the way in thinking about the paths and patterns of early cell division. Whitman then took this research emphasis to the new MBL, where he became the first director in 1888. There he encouraged researchers and his own students to carry out cell lineage studies on embryos of different animals, to allow comparisons across different species and animal groups or phyla.

Whitman's work began in the 1870s, a time of eagerness to understand cells and early stages of development as far as gastrulation and germ layer formation. Ernst Haeckel had suggested that germ layers provide the start of differentiation and organization in animal embryos, which led to considerable debate about what was happening, as well as what it meant. (See Richards 2008 for perspective on Haeckel and his ideas.). In 1878, Whitman published his doctoral dissertation study of freshwater leeches (supervised by Rudolf Leuckart at the University of Leipzig) as "The Embryology of Clepsine" (Whitman 1978). Whitman described the methods used, which formed the starting point for his eventual volume on microscopical methods of the day (Whitman 1885). He also gave a rich discussion of seemingly every detail of egg preparation through early development. It is worth reviewing this first cell lineage contribution more fully.

Whitman studied several different species of the freshwater leech *Clepsine*, meticulously preparing his specimens and observing the changes over time. He embedded his discussion in the context of previous studies and interpretations. In the context of other often rather less comprehensive research of the time, Whitman's attention to detail is noteworthy. He started with each step in the formation of eggs, the role of the nucleus, the differences among his eggs, and those of other types of organisms. He was clearly working to interpret what he saw in the very earliest developmental moments. For example, he referred to early "germ spots" of 0.0037 and 0.0025 mm, and it takes 3–4 days for a full sized "primary egg cell" to develop and grow to about 0.55 mm. The mature egg takes about two weeks to develop. These several pages of detail show that much is happening to prepare an egg for fertilization and eventually being deposited outside the adult.

Clepsines are hermaphroditic, Whitman explained, and therefore raise obvious questions about how the egg undergoes "impregnation." He never observed a "sexual union" (Whitman 1878, p. 8) but became convinced that the egg must be fertilized while in the ovary, perhaps through self-fertilization. When the eggs are deposited, they are mature and ready to begin development. Now comes the excitement of

cleavage, and "so far as yet known, these changes in the egg of Clepsine are unsurpassed in variety by those of any other egg" (Whitman 1878, p. 12). He carried on with details about each sequential step of the developmental process. Polar bodies, pellucid spot, polar globules, polar activity, polar rings, and pronuclei: these are all part of the complex organization that occurs prior to the first cleavage.

At this point, Whitman acknowledged that a diversity of interpretations existed for the phenomena he was observing. While some held that a cell consists of protoplasm with a single nucleus, he could see more than one nucleus in preparation for cell division. These "free nuclei" puzzled Whitman, who was not sure about their role or whether they had an ability to cause additional cells to coalesce and/or divide. Others thought they saw additional nuclei, and Whitman noted that these might be nucleoli instead, yet questions remained. Cell theory was not so tidy for Whitman as it later became, and we see his worries later in an essay on "The Inadequacy of the Cell Theory" (Whitman 1893).

Finally, about halfway through the 1878 paper, we get cleavage. "In the fecundated egg slumbers potentially the future embryo. While we cannot say that the embryo is predelineated, we can say that it is predetermined" (Whitman 1878, p. 49). Already, in his first publication on embryology, he pointed to comparisons with other species, apparently as a way to get at patterns in the ways cells related to each other. After offering comparisons, he asked whether the similarity of cell divisions and arrangements in birds and fish can be explained in the same way as in his invertebrates. "Since the process in both cases leads to similar results, it is natural to infer that it is controlled by the same general laws" (Whitman 1878, p. 94).

For Whitman, "The egg is, in a certain sense, a quarry out of which, without waste, a complicated structure is to be built up; but more than this, in so far as it is the architect of its own destiny. The raw material is first split into two, four, or more huge masses, and some or all of these into secondary masses, and some or all of these into tertiary masses, &c., and out of these more or less unlike fragments the embryonal building-stones are cut, and transported to their destined places" (Whitman 1878, p. 50). *This is the first declaration of cell lineages, despite the underlying uncertainty about precisely what cells are and precisely what mechanisms cause them to divide.*

Whitman then went on to describe what happens during division: the change of form, the plane of division and its movement, the timing of each division, the angles as cleavage progresses. We get ectoblasts, mesoblasts, neuroblasts, entoblasts, and discussion of movements of the cleavage products as they progress on their way to becoming the germ layers that many researchers of the time considered the starting point for an individual organism. Blastula gives way to gastrula, with its infolding and reshaping of the embryo. Whitman described the stages, gave the timing of each change, and used language that reflected already accepted definitions of each stage. He was identifying known parts and processes, not discovering them for the first time. That is, he was finding, in his leeches, the developmental processes seen elsewhere by others.

Differences did arise, however; Whitman noted that *Clepsines* do not pass through a morula (solid ball of cells) stage as many other species do. Some parts come from the upper pole in some forms and the lower pole in others. Yet in comparing the *Clepsine* neurula with that of vertebrates, he found a remarkable similarity in

structure with some variations in detail and in the rate of change. *Fish, chicks, and leeches seemed to share the patterns of origin of the primitive streak, for example, and of other key developmental steps. Finding the parallels among leech, fish, chick, and other eggs reinforced the conviction that here was an "interesting remnant of the ancestral condition"* (Whitman 1878, p. 92).

Parts and organs emerge from the relevant germ layers. Ectoderm and mesoderm were clear, but he asked "Whence arises the entoderm?" that he would have expected (Whitman 1878, p. 66). He was asking about lineages and what cells gave rise to each germ layer and its subsequent parts. He needed more study to determine the origins of the entoderm, and he recognized that he might obtain clues by looking at what was known about other organisms. In his summary, Whitman explained that each cleavage had specific identifiable effects and regularities. In effect, the cells have lineages that lead from initial blastomeres to differentiated parts. "Thus it happens that, before a given ontogenetic stage is completed, the preliminary segregations and arrangements for the following stage are already more or less advanced. Thus the gastrula—and more rarely the blastula—is pre-stamped with the antimeric character of the ultimate bilateral form" (Whitman 1878, p. 79). Trace the cells backward, and it becomes clear that the later differentiated parts that makeup germ layers started in particular identifiable earlier cells predictably and reliably.

Some of the changes seem to come from physical pressures, as Wilhelm His had suggested in his emphasis on the efficacy of folding of parts in embryo formation (His 1874). Yet Whitman did not offer his own interpretative explanations but rather stuck with descriptions and comparison with the interpretations of others. As a result, his discussion comes across as entirely reliable, valuable, and significant in establishing details about the processes and progress of development. Roughly two decades later, around 1900, at the MBL, Whitman rallied a community of leading researchers to carry out more of this kind of work, or what came to be called cell lineage studies.

B Why Did Whitman Pursue This Project?

Whitman had received his Bachelor's degree from Bowdoin College, then taught natural history in high school (See biography in Morse 1912). He joined other teachers in attending Louis Agassiz's Penikese Island School in natural history and there became inspired to pursue zoology professionally. That quest took him to the leading marine research station in the world, the Stazione Zoologica in Naples, and then to the University of Leipzig to study with Leuckart, who was renowned for his excellence in microscopic techniques and his meticulous attention to morphological detail. Best known later for his work in parasitology, Leuckart studied marine invertebrates and followed the life cycles of a number of organisms. His "Leuckart charts" graced the walls of classrooms around the world, including at the MBL, and can be accessed at http://legacy.mblwhoilibrary.org/leuckart/wall_charts.html.

Leuckart welcomed international scholars, including young Americans eager to gain research skills. Whitman grew from being a young student eager to learn into a professional biologist during his three years in Leipzig. He received his PhD in 1878 with the dissertation on *Clepsine*. During Whitman's time in Leipzig, Leuckart

served as rector of the university for a year, and one can only speculate whether watching that activity in his advisor inspired Whitman as he took on his own administrative and leadership roles.

Whitman went from Leipzig to a position at the Imperial University of Japan, where he established zoology as a leading field in Japan and trained a generation of Japanese microscopists in the latest techniques. He returned to the United States and spent two years at Harvard as an assistant in zoology, then moved inland to direct the Allis Lake Laboratory 1886–1889, became the first head of zoology at the research-oriented Clark University 1889–1892, and finally went on to head biology at the new University of Chicago when it opened in 1892. In addition to those administrative roles, Whitman served as founding director of the MBL, accepting the position that offered no salary because it offered the opportunity to build a kind of Naples Stazione Zoologica in America. Both the Naples station and the MBL became leading places for bringing together the study of development, physiology, and evolution. Whitman wanted to educate and encourage research in an independent collaborative environment that served as an "assembling place" for modern biology. He also edited the *Journal of Morphology* and *Biological Bulletin* to provide an outlet for scholarship.

C Cell Lineage at the MBL

As MBL director, Whitman started with a small group of seventeen instructors and students in the first year. Immediately, he began to recruit researchers to come to the MBL from the US Fish Commission, the government organization for the study of diverse fish species and their environments that was conveniently located just across the street from the MBL (Lillie 1944; Maienschein 1989). While a few other researchers in other countries carried out their own cell lineage studies, the group that Whitman assembled at the MBL carried out by far the largest and most concentrated study of cell lineages in early embryonic development. During the first decade of the MBL, Whitman recruited such outstanding biologists as Edwin Grant Conklin, Edmund Beecher Wilson, and Thomas Hunt Morgan. These three had all been graduate students at Johns Hopkins University and had visited marine stations with their advisor William Keith Brooks, including the Woods Hole Fish Commission. Each quickly found his way to the MBL, then continued to return there in summers for the rest of his life while also assuming leadership roles.

Wilson was the oldest of this Hopkins group at the MBL. He grew up in a small town of Geneva, Illinois, went to Antioch College for a year, worked while learning the basics required for Yale University, and received his Bachelor's degree from Yale (See Morgan 1940 for a biography). He then joined the U.S. Fish Commission in Gloucester, Massachusetts, in the summer of 1877, participating in dredging expeditions. From there he proceeded to the recently founded Johns Hopkins University while continuing his interest in marine studies. With a dissertation on the colonial polyp *Renilla*, for which he carried out the studies during three summers at the John Hopkins marine stations, Wilson traced the development of a single polyp into its colonial form.

Under Brooks's influence, Wilson was thinking in terms of evolution for that study; he was very much aware of the ideas of Charles Darwin, Ernst Haeckel, and others. He also learned to use serial histological sections to see inside complex organisms and cells. Wilson received his PhD in 1881 and remained at Hopkins as an assistant. In 1883, with support from a cousin, he visited England and then spent more time in Leipzig with Rudolf Leuckart, who continued to welcome American visitors as he had welcomed Whitman. From there he went to the Naples Stazione Zoologica, as Whitman had. This visit gave Wilson the chance to continue his biological research on development while also enjoying his serious interest in music. He played the cello as part of a quartet there and listened to the many concerts that the director Anton Dohrn brought to the Stazione. His visit reinforced Wilson's deep interest in the study of marine organisms, development, and evolution. Back in the US, he taught at Williams College, spent a year at MIT, co-authoring a textbook, took up a position at Bryn Mawr College in 1885 before moving to Columbia University in 1891. During the rest of his career at Columbia, Wilson traveled every summer to carry out research at the MBL.

At the MBL, Wilson learned from a colleague about the polychaete worm *Nereis*. Inspired by Whitman's earlier work on *Clepsine*, and in the hope of determining the origin of germ layers in annelid worms, Wilson reports that he had been looking for an appropriate organism. *Nereis* turned out to be useful, not just for the evolutionary question about origins, but also for following what Wilson first called cell lineage. At night, all it takes is a lantern to mimic the light of the moon and lure these colorful worms into the collecting net. From the dock on the Eel Pond, it was just a few steps to the well-equipped MBL laboratory, where Wilson could hurry into the laboratory and watch the *Nereis* eggs go through their developmental stages, including cleavages. *Nereis* eggs are transparent and relatively large, develop quickly, have visible structures to help identify which cell is which, and are easy to fix and stain (Wilson 1892, p. 363). There are good reasons that these eggs remain favorites for the MBL Embryology course today.

Wilson noted that the germ-layer theory had led to the considerable study of comparative embryology through the 1890s which, in turn, had yielded a surprising amount of disagreement in interpretations about both development and evolution. The theory held that at the point of gastrulation, an embryo develops germ layers that each lead to subsequent differentiation of different parts of the body (MacCord 2013). This raised new questions. What should count as a homology, for example: did the germ layers remain homologous with each other in different gastrulas, over time? A considerable debate centered on the significance of germ layers and their origins and Wilson concluded that the only way to resolve the various issues was "*by tracing out the cell-lineage of cytogeny of the individual blastomeres from the beginning of development*" (Wilson 1892, p. 367). Pointing to Whitman's "epoch-making" studies, Wilson saw early development as informing understanding of evolution through the impact of early variations rather than as simple mechanical proliferation of material. "*The very fact that the differentiation of the layers is effected in such a diversity of ways proves conclusively that these early stages of development are as susceptible to secondary modification as the later*" (Wilson 1892, p. 368).

Describing every step in detail, Wilson provided images and discussion intended to let any other researcher see the same thing he was seeing. He was looking for causes of the "organization of the egg." Those causes were at least in part hereditary, he determined, and he marveled at "the remarkable fact, and one which does not seem to be very clearly recognized" that the divisions of cells and the mechanical conditions that cause them to divide in the patterns they follow "has become hereditary" (Wilson 1892, 450; and see Guralnick 2002 for more discussion). This conclusion that mechanical conditions, rather than simply differences in form, drive the earliest cell divisions led him to further careful study of "germinal localization" in several species, and to a broader discussion of the idea of "ancestral reminiscence" in development. Those ideas received attention at the MBL when he presented a lecture on "Cell Lineage and Ancestral Reminiscence" as part of the *Biological Lectures* series in 1898, based on a paper presented earlier to the New York Academy of Sciences (Wilson 1898, 1899).

Wilson's essay resulting from that lecture captures the state of the field in the late 1890s. Each organism, Wilson noted, arises through the processes of a complicated mechanism and also of its past, including its evolutionary history. Sometimes the individual developmental ontogeny may seem to repeat the ancestral development or phylogeny, as Haeckel had argued. Wilson felt that the relationship was not one of repetition, however, but of reminiscences from past adaptations that are modified by environmental and other conditions. And, yes, cell lineage studies had shown that the reminiscences occur even at the earliest developmental stages. Cell cleavages follow an orderly and defined process, with "marvelous consistency" just as later developmental stages do. "The study of cell-lineage has thus given us what is practically a new method of embryological research" (Wilson 1899, p. 24). *In conjunction with close attention to the way the lineages give rise to the germ layers, comparative studies could show much about evolutionary relationships and about cell homologies that carry those ancestral reminiscences.*

Wilson's lecture took place in the context of work by other colleagues; studying the cell lineage of something was almost a requirement at the MBL in the 1890s. Even Thomas Hunt Morgan, known for work on regeneration, later on for research on chromosomes, and his Nobel Prize-winning work on genetics, looked closely at cell lineages and development. Morgan grew up in Kentucky, received his BS degree from the University of Kentucky, spent a year at Alpheus Hyatt's Annisquam Laboratory, then in 1886 went on to Johns Hopkins to study under Brooks. He spent 1888 in Woods Hole at the US Fish Commission, then moved across the street to the MBL in 1890. (See Sturtevant 1959 and Allen 1979 for biographies). Morgan did not carry out the detailed step-by-step cell lineage descriptions that Wilson did, but he was very much attuned to the importance of regularities and patterns as cleavage stages progressed through development.

Within the context of a look at relationships among sea spiders, Morgan focused his PhD dissertation on a particular sea spider with "A contribution to the embryology and phylogeny of the Pycnogonids" (Morgan 1891). Yet the year after he received his PhD, he also published on the larval Tornaria form of worm-like *Balanoglossus*, embryology of sea bass, and frogs. Over the next few years (1891–1904), Morgan resided as a faculty member at Bryn Mawr College, after Wilson had left the position

vacated when he moved to Columbia University. While at Bryn Mawr, Morgan pursued research on teleosts, echinoderms, sea urchins, sea stars, fish, and others, looking at whatever organism seemed likely to produce some interesting phenomenon to explore or to make itself accessible to study (Maienschein 2015). He clearly knew about and learned from the cell lineage studies his colleagues were carrying out at the MBL.

As his biographer Garland Allen has emphasized, Morgan quickly adopted an experimental approach to embryology (Allen 1979). Rather than documenting in detail every step of cell lineage development, Morgan asked about how organisms function or what conditions cause changes. In this, he adopted the experimental approach that Wilhelm Roux had announced in the 1894 introduction to the new journal that he edited, *Archiv für Entwickelungsmechanik* (which later became *Roux's Archiv* and much later *Development Genes and Evolution*).

The MBL community engaged in lively discussion of what an experimental program meant for embryology. Morgan apparently listened hard; by 1895 he was publishing descriptions of what happens to cell development after various sorts of experiments. In 1898, he wrote a short note on developmental mechanics for *Science* and suggested that "Therefore, by means of an experiment, the student of the new embryology hopes to place the study of embryology on a more scientific basis" (Morgan 1898, p. 50). Morgan's visit to the experimentally-oriented Stazione Zoologica in 1894–1895, working at a table hosted by the Smithsonian Institution, surely reinforced his experimental emphasis.

This experimental turn for Morgan took him to studies of regeneration starting in 1898 that reached a peak with his book, *Regeneration* (Morgan 1901). Chopping off pieces of planarians, earthworms, and hydra, in particular, Morgan sought to determine what happened as a result. Study of regeneration would provide a window into how development normally works, as Mary Sunderland has discussed (Sunderland 2010). Thus, while Morgan did not carry out his own detailed studies to follow the lineage of cells throughout early development, such cell lineage work informed his own studies that followed sequences of cells in order to compare normal and experimental conditions.

Edwin Grant Conklin worked most closely and in parallel with Wilson so that the two referred to details of the other's work while comparing what was similar and different. Conklin grew up in a small town in Ohio, received his Bachelor's degree from Ohio Wesleyan University, taught at the historically black Rust University, then decided that he could become a professional biologist if he received a PhD. (See Harvey 1958 for a biography). And so, in 1888 he also went to Johns Hopkins University to study under William Keith Brooks, as a number of other leaders in the US in biology did as well. Brooks had connections with the Fish Commission in Woods Hole where Conklin went in 1889. Brooks suggested that Conklin study the siphonophores that he had studied at Hopkins but soon discovered that there were none in Woods Hole.

The need for a topic led him to look at many different species, while also working out a research question. Conklin settled on cell lineage of the slipper snail *Crepidula*. Like Wilson, he carried out extremely meticulous work and described in detail the changes with each cell cleavage. The snails behaved in some ways different, and

in some ways the same as Wilson's worms and the comparisons enriched both of their studies. Conklin reported that Brooks was not at all convinced that this was a reasonable topic, nor a useful methodology. Staring at cells and the mechanics and structures of their changes during cleavage did not seem likely to have morphological significant, Brooks complained.

Yet the next summer working again at the Fish Commission, Conklin met Wilson working at the MBL and began close communication with him that led to a long friendship. Conklin completed his dissertation, and Brooks is said to have commented: "Well, Conklin, this university has sometimes given the doctor's degree for counting words; I think maybe it might give one degree for counting cells" (Harvey 1958, p. 63). Whitman approved and agreed to publish Conklin's dissertation in the *Journal of Morphology* that Whitman edited, even though the 226 pages, 9 plates, and 105 color figures almost bankrupted the journal. "What Is Money for?" Whitman asked in making it clear that of course, he would support publication of Conklin's work, as reported in an interview in the last days of Conklin's life. (Bonner and Bell 1984; the interview is deposited with the American Philosophical Society archives.)

In 1905 Conklin published one of the last major cell lineage studies with his "The Organization and Cell-Lineage of the Ascidian Egg" (Conklin 1905). There he noted that the system of nomenclature and descriptions of variations used for annelids and mollusks would not work for ascidians, and required adjustment, although it was not yet clear just how. The complexities help show why researchers largely set cell lineage studies aside in favor of other methods and questions.

While Wilson emphasized the dual influences of physical and mechanical factors and ancestral reminiscences, Conklin was a Darwinian first. Indeed, he had entered biology because of a fascination with Darwinian evolution, and his conviction that his Methodist beliefs were perfectly compatible with a proper understanding of evolution. The mechanics of development played a secondary, though necessary, role. Biology was at root evolutionary for Conklin.

Whitman's own graduate students carried out lineage studies as well. Frank Rattray Lillie, who became the second director of the MBL and the second chair of zoology at the University of Chicago after Whitman, reported having been recruited right away both to study with Whitman and specifically to carry out cell lineage work. Whitman assigned Lillie to work on the freshwater mussel *Unio*, which required him to lug his assemblage of buckets and waders to a pond nearby Falmouth. Aaron Treadwell studied the polychaete worm *Podarke Obscura* Verrill, and A. D. Mead looked at annelid worms, which provided valuable material for comparison.

D Why Did Cell Lineage Work End—For a While?

Robert Guralnick has noted that although Conklin continued the longest with cell lineage work most others set the approach aside earlier. They had learned that there was much to learn, he suggests, and only Conklin was such a committed Darwinian that he saw cell lineage as supporting evolutionary biology and as worth pursuing to illuminate phylogenetic relationships (Guralnick 2002). Other factors have undoubtedly played a role in the move from cell lineage as well. Cell lineage work has always

been hard, requiring many hours of tedious and careful observation, watching, describing, drawing, and sometimes preparing, preserving, fixing, staining, and so on. The researcher invests a tremendous amount of work before knowing what the results will be. Today's techniques for tracking cells and labeling have helped with part of the work, but a cell lineage researcher still has to invest time, energy, and attention to the work.

One student in the MBL Embryology course some years ago commented on how much harder she thought embryology is today, where "you have to know so many molecular techniques and work in the lab a lot." Yet when asked about what she thought Wilson had to do in order to carry out his cell lineage studies of *Nereis*, she imagined that he had to "watch an egg or maybe a few" that he had probably ordered from the supply department, and then "write down and draw the stages." The Wilson work, she imagined didn't sound so hard to her. More recently, the Embryology course director Alejandro Sánchez-Alvarado has had the students go down to the Eel Pond dock at night with collecting nets and lights, just as Wilson did. They take the worms back into the lab, identify the eggs that have been fertilized, and watch cell divisions. They watch all night, carefully observing to see the changes with each cell division. They try drawing and also learn that, in fact, much of the work is not just watching a few embryos develop and observing everything directly. Cells have to be collected, fixed, stained, sectioned, and so on. Each step requires more work and different skills. The students today acknowledge that cell lineage work is still demanding.

Wilson, Conklin, and the others did not have the molecular tools we have available today, but they drew on many other techniques. The MBL Archives still has boxes of slides that Conklin made for the 1939 Embryology course, and the many slides, each have many sections taken from hundreds of embryos. It becomes obvious that the work was highly skilled and difficult, and also that interpreting from all those many different individual images to discover what is "normal" involves careful interpretive work. Diversity across individual organisms and across types of organisms complicated the interpretations. Furthermore, Wilson's and Conklin's enthusiasm about their choice of organisms shows that actually following the lineages is much more successful in some species than in others, which were set aside as not so useful. The eggs have to be large and visible enough, develop fast enough, and have stable enough patterns: all factors that Wilson had pointed to in his first *Nereis* studies. The reasons just described are negative factors that may have pushed researchers away from further cell lineage work.

More positive factors also pulled researchers in new directions; these involved new methods that lured biologists both to ask different questions and to study them in different ways. Some have pointed to genetics as an alternative research program at the time, but cell lineage had already given way before genetics attracted many followers. Another consideration was that Wilson and Conklin, and others at the time, found other ways to study other details about cells, cell division, and the relative roles of nucleus and cytoplasm, for example. Wilson, in particular, published his *Atlas of the Fertilization and Karyokinesis* in 1895, which drew on photography to show what chromosomes were doing, step by step during early cell divisions (Wilson 1895).

A year later, the first edition of his magnificent textbook *The Cell in Development and Inheritance* appeared (Wilson 1896). With two follow up editions of *The Cell* and additional rich studies of cells and chromosomes, Wilson was recognized as the leading cell biologist for decades. This work drew on some of his cell lineage studies but even more importantly on other methods for fixing, staining, and observing the details inside cells. As a result of this body of work, the American Society of Cell Biology awards the E. B. Wilson Medal, and the MBL offers an E. B. Wilson History and Philosophy of Science Lecture. Conklin had a similar impact and adopted additional methods, and the Society for Developmental Biology recognizes Conklin for his contributions to embryology.

Most of those who wanted to study development took up experimental methods along with Morgan. Experimental methods allowed new phenomena to be discovered, and allowed comparisons by altering one or another factor and observing the different results. Much has been written about the history of experimental embryology, and we need not repeat it here. *The point to emphasize is that cell lineage studies were not so much seen as a dead end or worthless. Rather, the approach did not seem to address questions about developing embryos as well as experimental approaches at the time. Nor did it seem to illuminate understanding of evolution. In effect, the study of development and evolution had diverged by 1910, but not forever.* Cell lineage brought them back again in the 1960s with the nematode worm *C. elegans.*

3.3 CELL LINEAGE AND CAENORHABDITIS ELEGANS

The tiny nematode worm *C. elegans* is sometimes described in terms of negatives: it is not infectious or parasitic or hazardous or pathogenic. It also lives in soil and feeds on microbes. It is just the sort of organism that Wilson had sought with his cell lineage studies. It is very small (and 1 mm), has a short life cycle, it is prolific in producing eggs, it is easy and relatively inexpensive to cultivate in the laboratory. It is also transparent, so it is possible to watch the process of development as its cells divide. The worms typically reproduce through self-fertilization, but they can be crossed as well, which offers breeding advantages. We also know now that the cell lineage is essentially invariant under normal circumstances. An individual worm has a predictable 959 somatic cells in one sex and 1031 in the other, with 6 chromosomes, and over 100 million base pairs.

As Bruce Alberts wrote in his introduction to the 1222-page volume *C. elegans II*, studying this worm makes sense because "This simple creature is one of several 'model' organisms that together have provided tremendous insights into how all organisms are put together. It has become increasingly clear over the past two decades that knowledge from one organism, even one so simple as a worm, can provide tremendous power when connected with knowledge from other organisms. And because of the experimental accessibility of the nematodes, knowledge about worms can come more quickly and cheaply than knowledge about higher organisms" (Alberts in Riddle et al. 1997; also Brenner and Wood 1988). The worm network has developed a valuable resource for researchers to share results and methods through WormBase (Stein et al. 2001).

In 2002, the Nobel Prize Committee awarded the Prize in Medicine or Physiology to Sydney Brenner, H. Robert Horvitz, and John E. Sulston "for their discoveries concerning genetic regulation of organ development and programmed cell death" (Nobel Prize 2016). Their work brought a return to cell lineage studies. Why? Why was it thought to be productive to take up such studies?

Brenner had already established his reputation with his contributions to the discovery of messenger RNA, which led to a Lasker Award. Historian/philosopher of biology Rachel Ankeny explains very nicely how Brenner went on to establish *C. elegans* as a model organism for research in general, and the role of cell lineage studies in that work. Ankeny explains that the extremely creative Brenner wrote a letter saying that he felt that "nearly all the 'classical problems' of molecular biology have either been solved or will be solved in the next decade... the future of molecular biology lies in the extension of research to other areas of biology, notably development and the nervous system" (Ankeny 2001). Brenner saw *C. elegans* as promising for both development and neurobiology. He needed something tractable, with a small number of cells and the ability to track the cells. He sought a way to carry out cell lineage studies that would track each cell throughout its development (Brenner 1973).

Others argued that the nematode was not a good choice. Perhaps it is too simple. Perhaps the worm is just a tube of material without enough morphological structure to observe the differences and track cells. Perhaps its nervous system is not complex enough to track interesting features. Yet, Brenner documented in 1974 about 300 mutants and over 100 genes. Brenner started with the deceptively simple statement: "How genes might specify the complex structures found in higher organisms is a major unsolved problem in biology." With a universal genetic code, how does the sameness turn into difference? As yet, "we know very little about the molecular mechanisms used to switch genes on and off in eukaryotes. We know nothing about the logic with which sets of genes might be connected to control the development of assemblages of different cells that we find in multicellular organisms" (Brenner 1974, p. 71).

To study nervous system development requires both tracking structural effects of genetic differences to understand how genes specify the nervous system and also tracking how the nervous system produces behavior. Such research requires an organism with a simple enough system, much simpler than *Drosophila* or other favorites. Brenner concluded with understatement that "*C. elegans* is a favorable organism for genetic analysis" (Brenner 1974, p. 91). This was just the beginning, leading to the massive report in 1986 on "The structure of the nervous system of the nematode *Caenorhabditis elegans*," by Brenner and others (White et al. 1986). There we learned that 302 neurons work in a structure that does not vary across organisms, and they are coordinated with 5000 chemical synapses, 2000 neuromuscular junctions, and 600 gap junctions. The research required the kind of meticulous, time-consuming, and at times almost obsessive dedication to tracking every detail, just as the earlier cell lineage researchers had done.

Sometimes the researchers described the work in terms of mapping the architecture of the worm, mapping the genome, developing wiring diagrams. More recent studies have tied *C. elegans* systems to gene regulatory networks of the kind that Eric Davidson and Britten first introduced in 1969 (Britten and Davidson 1969).

Brenner's first emphasis was on the nervous system. Meanwhile, John Sulston worked on development more generally in a paper published in 1977 showed that the post-embryonic cell divisions carry out a very precise and predictable sequence, with very strictly specified cell fates (Sulston and Horvitz 1977). The early embryonic stages had already been laid out, and Sulston and Horvitz sought to complete the picture. They observed division after division and tracked the details, just as they acknowledged that Wilson and colleagues had done.

As Sulston and Horvitz put it, based on their observation of living nematodes: "As in embryogenesis, the pattern of these divisions is rigidly determined; essentially invariant postembryonic cell lineages generate fixed numbers of neurons, glial cell, muscles, and hypodermal cells of rigidly specified fates. These lineages reveal the ancestral relationships among specific cells of known structure and function; they thus complement the classical embryology, which defined the ancestral relationships among different organs" (Sulston and Horvitz 1977, p. 110).

Their work was very much in the tradition of cell lineage work around 1900. But where that earlier work had been largely set aside for reasons discussed, now these researchers saw the way forward in answering a wide range of developmental questions. As the authors noted, because they could observe every cell all the way through development, they could track cell migration in details. They could observe synapse formation and other functional steps with cell differentiation. Programmed cell death would be observable, and they could track the effects of particular mutations to get at genetic effects. All the exciting possibilities for cell lineage from around 1900 seemed accessible with this tiny worm in the 1970s. The full sequencing of the *C. elegans* genome in 1998 opened even more opportunity for study and secured this nematode as an NIH-approved model organism (*C. elegans* Sequencing Consortium 1998).

Now we come back to intersections of development with evolution. David Fitch and W. Kelley Thomas wrote the chapter "Evolution" for the massive 1997 volume *C. elegans II*. They reminded us why this is an excellent organism for studying evolution as well as development. They noted that "many evolutionary changes are similar to mutant phenotypes, suggesting that much of evolution may proceed by changes at the kinds of regulatory loci defined by genetic studies" (Fitch and Thomas in Riddle et al. 1997, p. 815). Following a review of phylogenetic relationships more generally, they then provide summaries of the evolution of various characters, looking at homologies, the different developmental stages, and other factors.

Fitch and Thomas note in their conclusion that study of worms is not likely to "divulge the precise developmental genetic changes that transformed our hominoid ancestors into humans only a few millions of years ago." Yet "it will provide models for how evolution works with development to make living forms. From models arise predictions. Only then can we evaluate and incorporate notions about general mechanisms into the body of explanatory principles being built by integrative approaches in biology" (Fitch and Thomas in Riddle 1997, p. 850). We have, as they note, just begun the search to understand that evolutionary past. Or as Wilson might have put it, we still see ancestral reminiscences but have not yet worked out causal and explanatory connections even though we have so many

more tools and have made so much progress since Wilson's day; thanks to advancing tools available to address old questions.

3.4 CELL LINES

The third type of cell lineage work looks at lineages of particular cells, not at all cells from the earliest embryonic stages. Lineages of particular cells in the body give rise to one kind of research. Cancer cells are one of the favorite examples. Most of the time, obviously, we do not pay much attention to the particular individual cells in our body. We let them do their work and assume they are doing the job right. When cells become cancerous, however, we are concerned both about where they go next for clinical purposes, and where they came from for research purposes. Tracing the lineages forward can occur by watching metastatic growth throughout the body. For example, a cancer cell or cells can produce a tumor, then migrate to other places to produce other tumors, and so on. This is a kind of lineage, even though it is usually not possible to trace every cell division over time. Cancer stem cells also initiate new lines of cells—cancer cells—which has significant implications for what therapeutic approaches are likely to prove effective, as Lucie Laplane discusses in her 2016 book *Cancer Stem Cells. Philosophy and Therapies* (Laplane 2016).

In his 2007 book on the *Dynamics of Cancer: Incidence, Inheritance, and Evolution,* Stephen Frank offered insight into this first type of research approach to cancer cell lineages with his discussion of cell lineage history in Chapter 14. Frank sought not just to study cancer cells and their future cell divisions, but also to understand the accumulation of past mutations and heritable changes that have led to the cancer cells being studied. He acknowledged that the "present studies remain crude, but hint at what will come" (Frank 2007). Genetic sequences would provide just a start, Frank suggested and also were starting to be correlated with methylation patterns, microsatellite sequences, and other factors to understand what has shaped the cells to date and drives them forward in particular ways. This approach to somatic mapping and evolution of cell lineages has made some advances and continues to hold considerable promise. Yet to make still more progress, we will need more researchers to embrace the study of evolution for medicine, and to develop new techniques for identifying and connecting the various factors involved in the complex adaptive systems of cancers.

Much more widely adopted are techniques for culturing cells outside the body. Instead of trying to trace those cancer cells or their effects inside the body, put the cells in a culture dish where they are relatively easy to watch, track changes, and follow lineages. This approach had already begun in 1907, when Ross Granville Harrison took neuroblast cells out of a frog and placed them in a medium of frog lymph, then watched. He wanted to determine whether the cells would be able to grow and differentiate into nerve cells, and he assumed that if they did that they would be following the patterns of normal nerve cells. The story of Harrison's first ever tissue culture research, which was also the first ever stem cell research, has often been told, so we need not repeat it (Maienschein 1983; Witkowski 1985). Here the important message is that, over a century ago, researchers began culturing cells. Harrison himself did not pursue tissue culture research, but others immediately

recognized its tremendous promise and pursued different ways to culture tissue, clusters of cells, and also individual cells.

Hannah Landecker has asked how we should think about those cultured cells. In *Culturing Life. How Cells Became Technologies* and more recent articles, Landecker argues convincingly that we have come to think of the biotechnological creation in the laboratory as life. Indeed the cell cultures are alive, they do consist of cells, and yet they are not and have never themselves been parts of organisms. The cells have, indeed, become technologies (Landecker 2007). Different kinds of such technologies include cell lines like HeLa cells that were taken initially from an individual's cancer cells, or cell lines starting from undifferentiated cells like human embryonic stem cells.

Thanks to the excellent bestselling book by Rebecca Skloot, a wide audience has heard about Henrietta Lacks and the HeLa cell line derived from her cervical cancer cells (Skloot 2010; Landecker 2007, Chapter 4). Back in the early twentieth century when Alexis Carrel was inspired by Harrison to culture cells from chicken hearts and had reached the conclusion that he could create what he came to believe were immortal cell lines, his results seemed both plausible and yet worrisome. Years later, when researchers realized that they could culture cancer cell lines "immortally," as from the cells of Henrietta Lacks, the possibilities seemed exciting and important. Laboratories in vastly different places could work with what were presumed to be the same cell lines and could then compare results. The HeLa cells became a "body of knowledge," as Landecker puts it. Standardized methods for culturing, freezing, sharing, and recording were set up to develop a network around the cells.

Yet already by the 1960s, some researchers had begun to worry about contamination in some of the cell lines being used extensively in biomedical research, and they raised questions. Are the HeLa cells around the world today really part of the same "immortal" line that started with Henrietta Lacks? Surely not. Mutations have occurred, environmental conditions have made a difference, people have made mistakes, things have happened. John Masters asked in a summary article in 2002 why researchers continue to accept ignorance about the quality of their cells, and perhaps even to engage in known fraud in knowingly putting forth cell lines such as HeLa as something they are not (Masters 2002). A more extensive historical reflection essay in 2009 carried the critique further and asked whether HeLa cells might not have evolved so far as to have become something else and need a new name (Lucey et al. 2009). Here we are looking at evolution that has occurred after the cell lines have been long established, not as ancestral reminiscences from the past in Wilson's or Conklin's terms. To some degree, the laboratory practices themselves are causing evolution to occur.

Stem cell lines raise similar questions, especially embryonic stem cell lines because they begin with undifferentiated or pluripotent stem cells (those defined as cells that have the capacity to become any kind of cells). Discovered in 1998 in humans, embryonic stem cell lines have achieved considerable notoriety for the ethical questions they have raised and the political posturing surrounding them. (For more discussion, see Maienschein 2014). Actually, culturing the stem cells, or using them for any research or medical purposes necessarily involves manipulating or even killing the embryo from which they came, which worries those who regard

the embryo as something we should not be manipulating. For our purposes here, there are questions about the cell lineages that result when the stem cells are harvested from a blastocyst that plays the central role. What do these cultured cells tell us about life? What do they tell us about development or heredity or evolution?

Landecker argues rather forcefully that, "Biotechnology changes what it is to be biological" (Landecker 2007, p. 232). She made that comment initially with respect to the change in attitudes following cloning of Dolly the sheep and cryopreservation techniques involved. Cloning and freezing change the timescale. Not only can we culture cells for long periods of time; we can also freeze them and use them much later, which makes them asynchronous with respect to the organisms from which they came in the first place. In this light, cells began to seem highly plastic and changeable. Is life equally plastic under normal conditions, and what does it even mean to be normal? Landecker concludes her book by asking, "Once we have a more specific grasp on how altering biology changes what it is to be biological, we may be more prepared to answer the social questions that biotechnology is raising: What is the social and cultural task of being biological entities—being simultaneously biological things and human persons—when 'the biological' is fundamentally plastic?" (Landecker 2007, p. 235).

In another line of reasoning that is not immediately related to our story about cell lineages here but is nonetheless worth noting, both Landecker and also philosophers John Dupré and Maureen O'Malley point to cell-lineages as clusters of cells that indicate organisms. They focus on questions about what turns cells and cell lineages into organisms, and they all see metabolism as the force that makes those cells into an organism and a living individual. Dupré and O'Malley explain that "Our central argument is that life arises when lineage-forming collaborate in metabolism" (Dupré and O'Malley 2009). Landecker's study of metabolism follows similar lines in suggesting that we need a much richer understanding of metabolism and of what it means to be biological and alive. These reflections suggest one way in which the study of cell lineages informs and helps shape our understanding of life more generally, and are worth exploring further in other contexts.

3.5 CONCLUSION

We have come a long way from the cell lineage work of Whitman, Wilson, and Conklin, and yet perhaps not so far. In the 1890s, it looked like cell lineages might be quite predictable and follow reliable patterns so that each cell had a knowable fate. Experimental work by Morgan and others quickly showed that individual organisms, and the cells in them, respond to experimental conditions in ways that can change the normal patterns while still leading to functional living organisms. The patterns may be predictable under normal circumstances, but also subject to adaptation and revision. Despite their ancestral reminiscences, individual cells, and the organisms that result from the sequence of cell divisions are highly responsive to changing conditions. Indeed, perhaps the adaptability and plasticity also result from ancestral reminiscences of changing conditions and the advantages of the ability to adapt. The more-in-depth evolutionary background directs the normal patterns as well as the diversity of adaptive responses to changing conditions in ways that call for further investigation into how and why.

C. elegans studies show a very high degree of predictability in both normal and experimental adaptations. The expected patterns, as well as the plasticity, all reflect what it is to be biological, to start as an inherited egg cell that has some organization already in place, is then fertilized, and responds to particular conditions at hand through a lineage of cell divisions that are more or less predictable and fixed.

We have looked at three different approaches to cell lineage here: documentation of cell lineages in the early sense at the MBL, documentation of the details and patterns of every cell through every division revealed with work on *C. elegans*, and understanding of the forces and factors that allow cell lines to continue through many generations. These are three different approaches to understanding life. The researchers involved have brought different underlying assumptions to their work, and they have made different choices—or organisms for study as well as of questions to ask or methods to use. Whitman and his cell lineage crew had an evolutionary past in mind, providing what Wilson called those ancestral reminiscences. Brenner and the worm crew were focused on the phenomena in front of them, tracing how cells make up a functioning nervous system. Clinical studies of cancer cell lines emphasize the cells and how they behave after they become cancerous. As we see, there are different reasons to follow cell lineages. Taken together, they help give us a rich understanding of the complexities of life, as well as of the way in which research can make those complexities accessible through the hard work of paying close attention to one detail after another, as one cell divides into another.

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