

The embryonic cell lineage of *Caenorhabditis elegans*: A modern hieroglyph

The best way to acquire knowledge in Developmental Biology is to learn how this knowledge was derived

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The year was about 2,570 B.C. when Cheops, the 2nd Egyptian pharaoh of the 4th dynasty – close to his death – entrusted his architect, Hemiunu, with the building of a mausoleum as the place for his eternal rest. Hemiunu designed each and every detail of this opus to please the desire of his divine pharaoh. Now, more than 4,500 years later, his work is still standing and is the largest pyramid ever built on Earth. Two elements were important in building the pyramid: On the one hand, the precise design and thorough planning of the architect, and on the other, the massive use of workers and slaves. Things have changed over these 45 centuries and, fortunately, the work culture that supported these abuses has almost disappeared in human society. However, in parallel, valuable information regarding exactly how the pyramid was built has also been lost. As a result, construction of a replica of the Cheops pyramid using only simple machines and manual labor would be impossible today; it would necessitate the use of expensive equipment and heavy machinery. Even coordinating different teams of workers to prevent them from interfering with each other would be a challenge. Today, the most hidden details of the pyramid have

been revealed, including all of its chambers and passages, its mass, volume, orientation, etc. It is even possible to make an exact computer-generated 3D model of the pyramid. However, despite all these capabilities, the researchers are still trying to unravel a major question in archaeology and this is: How did the Egyptians build the pyramids?

An analysis of the educational curriculum and articles published in the field of Developmental Biology of the nematode, *C. elegans*, suggests that an analogous loss in transmission of valuable knowledge might be happening today. This is a very young research field, and the process of knowledge attrition seems to be taking place very quickly. This year marks the 40th anniversary of the publication of “The genetics of *Caenorhabditis elegans*” by Sydney Brenner in 1974 [1], a seminal work that stands alongside the contributions of the 19th and early 20th century embryo morphologists. Young researchers who, 40 years ago, read this work and were captivated by the enormous potential of the worm as a model for unraveling the fundamental processes governing the development of an organism, are still active in their careers. There has not even been a generation of researchers between then and now! Those researchers, in the pre-genome era, were able to articulate and define key aspects of the development of a worm.

As recently as 1983, John Sulston and co-workers published “The embryonic cell lineage of the nematode *Caenorhabditis elegans*” [2] as a frame-

work into which he envisioned future discoveries would fit. This was a descriptive article, but it demonstrated at the resolution level of a single cell – which even today is unmatched in any other model – how development of a nematode proceeds. We must not forget that the ultimate goal of the Developmental Biology of *C. elegans* is to understand how a nematode is created through an invariant pattern of cell divisions that Sulston and co-workers described. The molecular basis of this stereotyped pattern was studied from the very beginning, and that same year, Susan Strome and William Wood published an article entitled “Generation of asymmetry and segregation of germ-line granules in early *C. elegans* embryos” [3]. In this paper, Strome and Wood delved deeply into the characterization of a process that had been studied over the last few years. That process showed that the differentiation of embryonic cells was determined by internal factors that segregated asymmetrically in early cleavages. This work opened up the field of research on embryonic cell polarity in *C. elegans*. That is the point at which the idea that nematode development was controlled by internal determinants without much participation of regulatory processes gained momentum. The worm appeared as a model, different from *Drosophila* and mammalian development. It was not until 1987 that James Priess and Nichol Thomson broke this “dogma” with the publication of their work “Cellular interactions in early *C. elegans* embryos” [4], which then led to a vast amount of research dedicated to elucidating the

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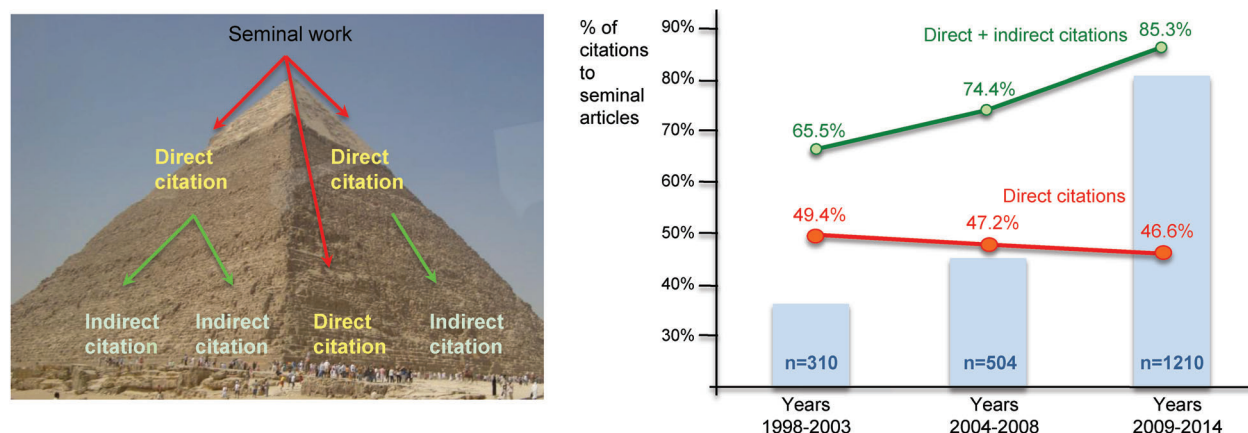


Figure 1. Representation of what is considered direct and indirect citation of a seminal work (left panel) and divergent evolution of both types of citations along the time, in the field of *C. elegans* Developmental Biology (right panel). The number of direct citations to seminal papers (red line) diminishes with time, while its deep impact, measured by the number of direct plus indirect citations (green line), scales up to virtually influence the vast majority of published papers.

nature of these inductions and signal transductions. Priess and Thomson showed that some cells in the *C. elegans* embryo were equipotent at early stages. The fate of those cells was diversified and determined depending upon interaction with other cells in the embryo. Therefore, development of *C. elegans* embryos, like those of other animals, had a regulatory component based on cell-to-cell inductions, as well as a determinative component. The only difference between this and other model organisms was the extent to which these components were present and interacted. Ten years later, in 1997, Titus Kaletta et al. established a major principle in order to explain how the embryonic cell lineage, described by Sulston and co-workers in 1983, is defined. The authors established that a series of binary decisions take place in the embryo, differentiating the fate of each daughter cell formed after every cell cleavage. In the work, “Binary specification of the embryonic lineage in *C. elegans*” [5], Kaletta et al. shifted a *lit-1* (Nemo-like kinase) thermo-sensitive mutant, after every embryonic cell cleavage, from a permissive to a restrictive temperature, and traced the embryonic cell lineage of those embryos. In all

cases, inactivation of LIT-1 led to both daughter cells having the same fate instead of two different fates, as occurs in WT embryos. The molecular details of how the Wnt pathway mediates this fate diversification were later elucidated.

In areas such as apoptosis, nervous system, ageing, etc. other articles and authors have had a deep influence on our knowledge of worm development. However, we want to stress and illustrate how cell differentiation in *C. elegans* is taught today. The five works mentioned above are pioneers in their field. Using classical and inexpensive approaches, such as genetics, cell biology or microscopy, the authors unraveled the major principles explaining how fate is specified along invariant cell lineages of the *C. elegans* embryo. Surprisingly, these articles are largely unknown to students and young researchers and (perhaps this is why) they are no longer cited in articles emanating from them in a sort of lineage of knowledge.

To analyze the impact of these articles on the field of *C. elegans* Developmental Biology over the last years, we quantified citations of the seminal works in full text and open access articles published in Pubmed Central (<http://www.ncbi.nlm.nih.gov/pmc/>) with the query: (caenorhabditis elegans[ti] OR caenorhabditis elegans-[ab] OR caenorhabditis elegans-[majr]) AND (embryology OR development) AND (differentiation OR “fate specification”). This is approximately one third of the total published articles on this topic and, therefore, a significant and theoretically unbiased sample of total published articles. The articles were grouped into four periods of time: years

1974–1997 (the period during which the pioneer articles were published) and three consecutive sets: 1998–2003 ($n = 310$), 2004–2008 ($n = 504$), 2009–2014 ($n = 1210$). When considering direct citations to the five seminal articles, there is a slight decrease in the number of direct citations over the time. 49.4% of articles published on *C. elegans* development from 1998–2003 cited at least one of the five seminal papers, 47.2% in 2004–2008, and 46.6% in 2009–2014. However, if for each period we consider not only the direct citations, but also the citations to other articles, which in turn cited any of the seminal papers (indirect citations), the tendency is quite different: 65.5% of the articles published on *C. elegans* development from 1998–2003 were directly or indirectly based on one or more of these five seminal papers, 74.4% in 2004–2008 and 85.3% in 2009–2014. As a control for this analysis, we generated 1,000,000 random combinations of five articles taken from the seminal papers group and performed the same analysis. The number of either actual direct citations or indirect citations was significantly lower than for the five selected seminal papers: for the period from 2009–2014, we obtained a mean of 19.27 ± 12.1 direct citations (1.59%) and 390.88 ± 225.8 direct + indirect citations (32.30%) (Fig. 1).

In summary, the vast majority of actual works published on *C. elegans* development is based on five seminal papers; however, less than half of current articles in the field acknowledge them directly. We are not saying that these authors are intentionally neglecting them, but rather that these seminal

works had such a deep impact on the field that they have become part of the accepted paradigm and a direct acknowledgement is not considered necessary. The problem arises when talented and eager students base their education only on recent articles. Their formation then becomes skewed and devoid of a foundation that contextualizes the recent discoveries and techniques. This gap must be corrected during the education of the students.

In teaching Developmental Biology, we can follow a mechanistic approach: we can explain the processes at a molecular level in a sequential manner. Thus, cell signalling would be the result of a cell secreting a ligand, activation of a receptor, signal transduction through adapter proteins which are phosphorylated, and, finally, modification of the activity of a transcription factor for expressing tissue-specific genes in the target cell. According to this approach (which is dominant in the academic field today), knowledge of how development occurs is achieved by the sum of the knowledge of the molecular details involved in it. The more details we know, the clearer our knowledge should be. It should be noted, however, that these molecular functions are in fact common to many other biological processes: when referring to cell signalling, neither protein secretion nor phosphorylation nor gene transcription are developmentally specific mecha-

nisms. Therefore, it seems important to understand a “higher level” of organization in the development of an organism into which to fit the molecular details that ultimately are responsible for it all happening. This cellular level of organization is the subject of the works we have mentioned earlier. Applying a classic principle in Developmental Biology (ontogeny recapitulates phylogeny) to its own teaching, we would say that the best way to acquire knowledge in Developmental Biology is to learn how this knowledge was derived. This approach, which today is old fashioned, provides a general framework in which the molecular features that we mentioned above acquire a biological sense in building an organism.

An understanding of the seminal works is key to educating future developmental biologists, and should be part of their curriculum. This is true not only for *C. elegans* research. Directly or indirectly, all articles that are handled daily in writing papers, dissertations or publications are based on these seminal articles, which can provide the student with a clean outline of Developmental Genetics, and which exemplify a spirit that allows them to face enormous challenges with few resources. We believe that this discussion can be applied to other disciplines. Nowadays, information is easily accessible through Internet databases. But keep in mind

that we are speaking about practical knowledge that leads us to design experiments or propose hypotheses. This knowledge is acquired through education and leads to a mindset that is exercised in the daily approach to problems that arise in the lab. It is not stored in a database. Let us not forget that the ancient Egyptians also documented their achievements, but they did not leave us with details of how they built their pyramids.

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