

ESSAY

The Renaissance of Developmental Biology

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Abstract

Since its heyday in the 1980s and 90s, the field of developmental biology has gone into decline; in part because it has been eclipsed by the rise of genomics and stem cell biology, and in part because it has seemed less pertinent in an era with so much focus on translational impact. In this essay, I argue that recent progress in genome-wide analyses and stem cell research, coupled with technological advances in imaging and genome editing, have created the conditions for the renaissance of a new wave of developmental biology with greater translational relevance.



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Abbreviations: BMP, bone morphogenetic protein; CRISPR, clustered regularly interspaced palindromic repeat; Dpp, Decapentaplegic; ES, embryonic stem; iPS, induced pluripotent stem; Wnt, Wingless

It is a commonly held view that the mid 1980s to 2000 represented the golden age of developmental biology. After all, it was during this period that genetic screens in worms and flies led to the discovery of the Notch [1,2], Decapentaplegic (Dpp), or bone morphogenetic protein (BMP) [3], Toll [4,5], Hedgehog [6,7], and Wingless (Wnt) [8,9] signalling pathways, and it was soon realized that these are conserved and play key roles in mammalian development and disease. Furthermore, the developmental genetics of this era led to the identification of the homeobox [10–12], the colinearity of the Hox genes in invertebrates and vertebrates [13,14], and several “master regulators” of organ or cellular identity [15,16]. Since the publication of the human genome sequence [17,18], however, the field of genomics and associated genome-wide approaches has become the hot area of research, largely eclipsing developmental biology. This change is reflected in the gradually declining impact factors of all developmental biology journals and the corresponding rise of “omics” journals [19]. Developmental biology has been further pushed to the sidelines by the growth of the field of stem cell biology following the derivation of induced pluripotent stem cells from mouse and human adult somatic cells [20,21] and the promise of rapid advances in regenerative medicine that these breakthroughs heralded. Indeed, it has almost become unfashionable to say that one is a developmental biologist, and I have been passing myself off as an “in vivo cell biologist” for a number of years. Does this mean that developmental biology is facing a continuing and inevitable decline in impact? In this essay, I contend that the opposite is the case and that, largely because of the recent

advances in genomics and stem cell biology, we should look forward to the renaissance of developmental biology.

Although “omics” papers now occupy more space in the top journals than developmental biology ones, this field has also contributed enormously to our understanding of developmental mechanisms, particularly at the transcriptional level. Firstly, complete genome sequences have made it possible to knock down every gene in the *Caenorhabditis elegans* or *Drosophila* genomes in vivo using genome-wide RNAi libraries, which is a very effective way to screen for interesting developmental phenotypes [22,23]. Secondly, advances in Chip-Seq, RNA-seq, and chromosome conformation capture techniques are providing an unprecedented view of how transcription is controlled during development [24–29]. Until recently, these approaches have been largely limited to the analysis of populations of cells, but recent advances in single cell transcriptomics are beginning to extend these techniques to specific cell types and even individual cells within developing organisms [30–32]. These approaches can therefore start to address major unanswered questions into development. For instance, whole genome sequencing of many individual cells can reveal the inheritance patterns of random somatic mutations, from which one can infer lineage relationships and construct fate maps, which are difficult to determine by other means in mammalian embryos [33,34]. Perhaps even more significant is the use of single cell RNA-seq to classify cell types on the basis of their transcriptional profiles [35]. Although the literature contains the assertion that there are about 210 distinct cell types in humans, this is almost certainly too low by at least an order of magnitude. Single cell transcriptional profiling has the potential to reveal the full repertoire of cell types that compose our bodies, which is an essential prerequisite for understanding how it is constructed.

Given the rapid advances in genomic technologies, it is conceivable that we will soon have a detailed picture of the genome-wide distribution of chromatin states, transcription factor binding site occupancy, and mRNA and noncoding RNA transcriptomes for most cells in an organism. This will provide a wealth of information about how the epigenetic landscape interacts with tissue-specific transcription factors to control cell fates. Important though this is, however, the net output will be an inventory of which genes are expressed where and when in the developing animal. Just as mapping every synapse in the human brain is unlikely to reveal the basis of consciousness, knowing the complement of expressed genes in every cell will not explain how a tissue, organ, or whole organism acquires its form and function. Interpreting this large amount of data will require understanding how this intrinsic information is integrated with external signals and cues to control cell behaviour, a topic that lies at the heart of developmental biology. In other words, the “omics” revolution can provide the raw material for generating interesting hypotheses about how animals develop, but a great deal of developmental biology research will be needed to work out how the linear information of the genome and transcriptome is transformed into a three-dimensional cellular structure. Amongst other things, this will necessitate understanding how cell shape and cell movement are controlled in different contexts, how the direction and range of cell signalling are regulated, how cells produce and are influenced by mechanical forces, and how the collective behaviours of groups of cells reproducibly generate complex structures.

The field of stem cell biology is probably even hotter than that of genomics, with many countries targeting significant proportions of their research budgets towards this strategic area. Indeed, a third of this year’s applications for European Research Council Starting Grants in Cell and Developmental Biology were on stem cell-related topics. Stem cells are central to developmental biology, since they are the founder cells of most, if not all, developmental lineages. At present, however, most stem cell research is more applied and uses tissue culture assays to investigate questions such as the nature of “stemness” or which factors can induce pluripotent stem cells to differentiate into specific cell types. This has caused heated discussion in the

developmental biology community about what its relationship should be to the stem cell field. For example, the British Society of Developmental Biology recently held its longest general meeting for years to consider whether we should change our name to the British Society of Developmental Biology and Stem Cells, a motion that was narrowly defeated after much debate [36].

This existentialist angst is partly driven by a desire to be associated with a well-funded and exciting field but also reflects the profound links between developmental biology and stem cell research. Takahashi and Yamanaka [21] did not pick transcription factors at random when trying to induce pluripotent stem cells, as two of the four factors, Oct4 and Sox2, were chosen because previous work in the early mouse embryo had shown that they are required for the pluripotency of inner cell mass cells [37,38]. One of the most remarkable recent advances in the stem cell field has been the development of multistep protocols that use scores of factors and inhibitors to induce embryonic or induced pluripotent stem cells to differentiate into specific cell types, most notably insulin-secreting pancreatic β -cells [39,40]. Many steps in these extremely complicated protocols are based on understanding and recapitulating the normal development of the pancreas, including in vivo studies that identified the signalling factors that induce endoderm, then foregut, then pancreatic endoderm, and finally endocrine precursor cells [41–46]. Finally, in vivo studies are beginning to reveal that the special properties of stem cells are not so unique, and that differentiated cells can de-differentiate and regain pluripotency. For instance, multicellular germline cysts in *Drosophila* and mice can fragment, de-differentiate, and become germline stem cells [47–49], while ductal cells of the liver and even newt muscle syncytia can revert to a stem cell state in response to damage [50,51].

For me, one of the most exciting aspects of the recent explosion of stem cell research is the amazing amount of self-organisation that can take place in vitro under appropriate culture conditions. No one would ever have predicted that a uniform starting population of embryonic stem (ES) cells could spontaneously undergo morphogenesis in culture to give rise to an optic cup with a stratified neural retina [52], that ES or induced pluripotent stem (iPS) cells could be cultured to form cerebral organoids, or “mini brains,” with defined cortical regions [53], or that a single *Lgr5*⁺ intestinal cell could form an intestinal epithelium with crypts and villi [54,55] (Fig 1). The number of organ-like structures that can now be grown in tissue culture is increasing exponentially and now includes most regions of the gut [55–58], liver [50], pancreas [59,60], salivary gland [61], skin [62], prostate gland [63], Rathke’s pouch [64], neural tube [65], lung [66], and even embryoid bodies that break symmetry and recapitulate some of the cell movements of gastrulation [67]. Although it will be important to confirm that these systems recapitulate normal development, the complexity of the structures produced and their similarities to the real in vivo organs suggest this is likely to be the case. They therefore provide fantastic models for studying mammalian and, particularly, human development.

The study of human development has been hampered by the inaccessibility of the embryo in utero inside the mother and by the obvious ethical rules against performing experiments on foetuses. Both of these problems are overcome by organoid systems, several of which can be grown from iPS cells [66, 68–70]. We are therefore now in a position to study how normal and diseased human tissues develop in vitro. This means that they can be observed by time-lapse imaging and analysed with the whole array of sophisticated tools that have been so successful in model organisms. A better understanding of the mechanisms that allow organoids to self-organise and undergo morphogenesis should also inform more translational research into regenerative medicine and disease modelling. After all, one wouldn’t want to use a technology on oneself without understanding how and why the components work.

An additional reason for the relative decline in the popularity of developmental biology in recent years has been the mounting pressure in many countries to focus on translational research, as this increasingly means research on humans, whereas most research on animal

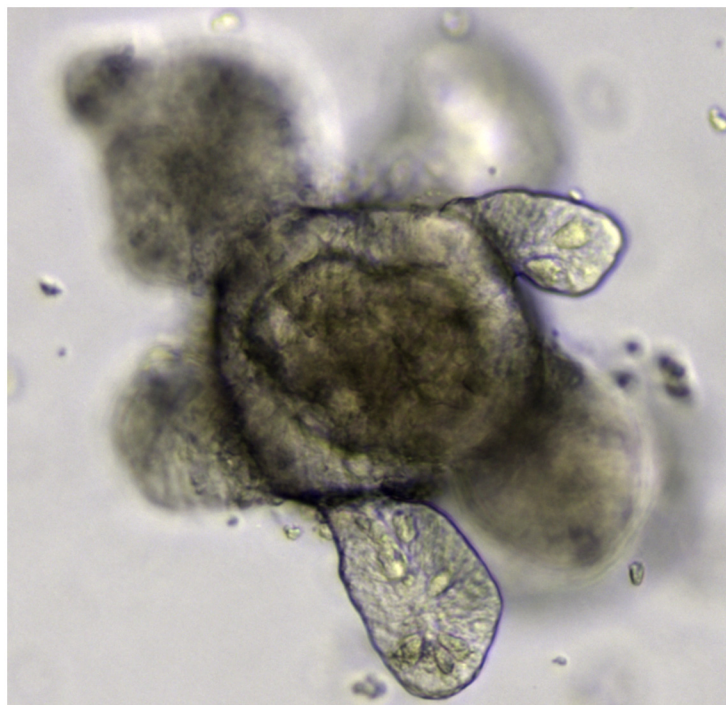


Fig 1. An intestinal organoid grown from Lgr5⁺ stem cells (courtesy of Meritxell Huch).

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development until now has been carried out using worms, flies, fish, frogs, and mice. Studying the development of human organoids therefore provides a way for the field to meet this translational agenda. This does not mean we should stop research on the classic model organisms: they still provide the most amenable systems for understanding many key developmental processes and are the only systems where one can examine the development of the whole organism or the relationship between development and physiology. Nevertheless, many insights in development have come from comparisons between organisms and using each system for the experiments to which it is most suited. The addition of organoids to the developmental biologist's repertoire will not only provide "human relevance" but will inform and enhance our understanding of developmental processes.

Both the "omics" and stem cell revolutions have been based on technological advances: for the former, the development of high throughput sequencing [71,72], and for the latter, the discovery of the Yamanaka factors [20,21,73] that proved that cellular reprogramming is possible. Developmental biology has not undergone such a dramatic revolution in recent years, perhaps because a wider diversity of techniques and approaches are needed to investigate how cells and groups of cells change fate, shape, and position during the formation of complex structures. Nevertheless, two recent advances are likely to enormously enhance our ability to analyse the complexity of development. Firstly, new microscopy techniques, such as light sheet, imaging make it possible to perform live imaging of thick specimens, such as embryos or organoids, at video rates in three dimensions [74–76], while advances in super-resolution microscopy will soon allow the observation of developmental processes with molecular precision in real time [77–80]. Secondly, the recent adaptation of clustered regularly interspaced palindromic repeat (CRISPR)/CAS9 technology to eukaryotes promises to transform many aspects of the field [81,82]. CRISPR can generate gene knock-outs in almost any organism or cultured cell line and can even be used to perform genome-wide screens in tissue culture using lentiviral libraries

[83–85]. Furthermore, because the double-strand DNA breaks induced by the Cas9 nuclease/guide RNA complex stimulate homologous recombination, one can easily introduce specific mutations or fluorescent tags into a gene of interest by providing appropriate repair templates [86,87]. This will allow sophisticated imaging and genetic analysis in nonstandard organisms and organoid systems. We therefore have an increasingly effective collection of tools to answer the exciting questions that recent advances in stem cell and “omics” research have raised.

The field of solid state physics was revitalized a few years ago by changing its name to condensed matter physics, and it may be that the developmental biology would benefit from rebranding itself in much the same way, for example, by becoming development and regenerative medicine, or four dimensional biology, or something even more catchy. Nevertheless, whatever name the field settles on, I predict that the power of these new approaches and the significance of the problems still to be solved will make the next decade a golden age for developmental biology.

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