Do We Understand Development?

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Over the last 20 years, progress in developmental biology has been so dramatic that developmental biologists may be excused for having the view, possibly an illusion, that the basic principles are understood, and that the next 20 years will be devoted to filling in the details. The most significant advances have come from the application of molecular techniques and a greatly improved understanding of cell biology. So we can begin to ask questions—like whether the egg is computable.

During development, differences are generated between cells in the embryo that then lead to spatial organization (pattern formation), changes in form, and the generation of different cell types. Genes control development by controlling cell behavior. One can make a case that, given the eukaryotic cell, its elaboration to generate multicellular animals and plants was easy, compared to the evolution of the cell itself (*1*).

In a sense, the cell is more complex than the embryo, because the interactions among cells in the embryo are much less complex than interactions among the components of the cell. The response of an embryonic cell to a signal is very dependent on the internal state of the cell, which in turn depends on its developmental history. In principle, most cell-to-cell signaling could be achieved with a very small number of molecules, since the signals are always selective rather than instructive. Indeed, peptide growth factors are used as signals repeatedly during development. Such signals only select between a few possible new cell states; the complexity of development lies in the internal program of the cells.

It is surprising how few special concepts one requires to understand development. Fate maps, asymmetric division, induction, competence, positional information, determination, and lateral inhibition will adequately cover most systems. The real key to understanding development lies in cell biology, in the processes of signal transduction and control of gene expression that result in changes of cell state, movement, and growth.

Our best system for understanding how genes control pattern formation is the fruit fly *Drosophila* (*2*). This understanding has been a triumph of the combination of genetics and embryology. The two axes of the organism, the anteroposterior and dorsoventral, are initially independent of one another and are specified by maternal gene products that form gradients of positional information. After fertilization, the gradients activate a cascade of zygotic genes, mainly coding for protein transcription factors, and the embryo becomes divided into a number of regions, defined by the combination of the activities of different genes. Along the anteroposterior axis, a periodic pattern of gene activity is established—the forerunner of segments. Remarkably, each stripe of gene activity is specified independently by the local combination of proteins. Each segment also acquires a unique identity coded by the activity of a special set of genes for transcription factors known as the *Hox* genes (*3*).

Our understanding of early *Drosophila* development has had a profound influence on studies of other animal embryos with respect to approach, mechanism, and even specific genes. *Drosophila*'s importance as a model for early development would be even greater were it not that much patterning occurs before the embryo becomes cellularized, and thus interaction between nuclei is facilitated by the early and direct access of transcription factors. By contrast, in most other systems cell interactions require signal transduction across the cell membrane.

Two model systems that have provided an understanding at the molecular level of how a small group of cells are patterned are the fly eye and the nematode vulva (*4*). In the eye, a photoreceptor complex is formed from eight cells, each with a unique identity, and the proteins that determine that identity—such as *sevenless* and *bride of sevenless*—signal only from one cell to its immediate neighbor. Again, in vulval development, the interactions are local. In both systems, signal transduction involves the *ras* pathway.

Pattern formation in many animals with larger numbers of cells is based on a mechanism where the cells first acquire a positional identity, which determines their future behavior. What is particularly exciting and satisfying about pattern formation in such systems is that similar principles, and more striking, similar genes are involved in diverse organisms—hydra, flies, worms, and vertebrates. This is shown by the *Hox* genes, which provide cells with positional identity along the anteroposterior axis in many animals (*5*). Also, one cannot but be struck by, for example, the similarities between insect wing and chick wing development. In both systems it seems that there is a signaling region that gives the cells their positional identity and this region expresses *hedgehog*-related genes, first identified by their role in patterning segments in *Drosophila* (*6*).

Morphogenesis—change in form—also relies on a rather restricted number of cellular activities. There is at the cellular level a good understanding of the forces that, for example, bring about gastrulation and can cause invagination and convergent extension (*7*). However, the coordination, both biological and mechanical, of these events and their link to gene action still remains unclear. What initiates, for example, cell movement in gastrulation or neural crest migration? Although one can in principle envisage mechanisms, the details are still lacking. One obvious link between gene action and morphogenesis is through the control of expression of cell adhesion molecules.

When one considers cell differentiation, that is, the emergence of specific cell types, it is hard to discern or even expect any general principles, other than that each cell type seems to be specified by a combination of transcription factors with varying degrees of specificity. The association of the Myo-D family with muscle differentiation may be the exception rather than the rule (*8*).

In general then, it can be argued that the principles of development are understood, although many crucial details at the molecular level are missing. For example, there is not a single case in all of vertebrate development where an intercellular signal has been unequivocally identified, even though there are excellent candidates like activin and fibroblast growth factor in early amphibian development (*9*), and fibroblast growth factor and retinoic acid in vertebrate limb development (*10*). We also still do not know how signals are propagated and whether there are, for example, graded distributions of diffusible morphogens, even though the evidence for graded properties in positional identity is substantial (*2, 11*). The downstream targets of the *Hox* genes still remain elusive: How, for example, does the change in just one gene change the an-

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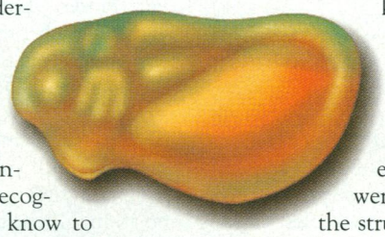
tenna of the fly into a leg? It may well be that no general principles are involved in the control of morphogenesis and cell differentiation. Even so, we do not yet have an example where we understand in detail the development of a single adult organ. We remain largely ignorant of timing mechanisms and how the size of different structures is controlled. It also has to be recognized that we do not yet know to what extent the principles of animal development apply to plants, although recent progress has been dramatic, and genes have been identified that control the identity of floral structures (*12*).



How many genes control development—as distinct from providing the housekeeping functions of the cell? The answer is not known, but one can guess. Analysis of early insect development suggests that only about 100 genes are involved in controlling patterning during early development. And in the nematode at least 50 genes are known that control vulva development (*13*). If one thinks of, say, 100 genes for each multicellular structure in the adult, then 50 different structures in *Drosophila* would require 5000 genes. For mammals, for which there are some 350 distinct cell types, tens of thousands of genes might be needed. Understanding the function of so many genes is made even more difficult by cases of apparent redundancy. That is, it is possible to knock out certain genes in mice without there being any obvious effect on the phenotype. It is likely that true redundancy is illusory and merely reflects the failure to provide the correct test for an altered phenotype. It may thus be very difficult to work out the true function of such genes.

Will the egg be computable? That is, given a total description of the fertilized egg—the total DNA sequence and the location of all proteins and RNA—could one predict how the embryo will develop? This is a formidable task, for it implies that in computing the embryo, it may be necessary to compute the behavior of all the constituent cells. It may, however, be feasible if a level of complexity of description of cell behavior can be chosen that is adequate to account for development but that does not require each cell's detailed behavior to be taken into account.

An analogy to some of these problems is found in the analysis of protein folding, which seems a much simpler problem, but where it may not be possible to work out the final structure from the sequence information by using first principles. Rather, the solution will come from homology (*14*). As with protein folding, homologies drawn from an extensive database could provide the best basis for making predictions about development. It is not unreasonable to think that enough will eventually be known to program a computer and simulate some aspects of development. We will, however, understand much more than we can predict. For example, if a mutation were introduced that altered the structure of a single protein, it is unlikely that it will be possible to predict its consequences.

So what will the next 20 years bring? Undoubtedly powerful new techniques will be invented that will enable us to understand the details of gene action and the biochemistry and biophysics of cell behavior. Working out the detailed action of all those genes, proteins, interactions, and kinases will be a hard slog and often tedious. It is unlikely that any new general principles will be discovered. However, the current excitement will continue as we come to understand the detailed mechanisms, and as more and more similarities between apparently different developmental systems emerge. Almost certainly there will be new ways of integrating particular aspects of development, and so we will learn, for example, the logic underlying the apparently varied mechanisms for generating periodic structures and the reasons for the variety of mechanisms for setting up the axes in early development. We can also look forward to great progress in the area of evolution and development. We may then see the solution to grand problems like how basic body plans emerged, how they are conserved, and the origin of developmental novelty. We will thus come to understand how development constrains and directs the form of all multicellular organisms.

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Of Flies and Fishes

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In vertebrates, the single most successful approach for identifying genes of importance in development is based on the surprising finding that important control genes, or at least stretches of sequences of control genes, are conserved through evolution. Thus, for many genes discovered in the invertebrate model systems *Drosophila melanogaster* and *Caenorhabditis elegans*, "homologs" in frog, mouse, and chicken have been identified, and their functions in vertebrate organisms tested with loss-of-function mutations made by embryonic stem (ES) cell–mediated homologous recombination (*1*). Mouse genes with similarity to selected *Drosophila* genes frequently show severe loss-of-function phenotypes. This result is in contrast to what one generally finds for biochemically characterized vertebrate proteins, where in many instances the loss-of-function phenotype shows little or no visible abnormality in the development or patterning of the animal.

Why do many *Drosophila* genes make a fortune in vertebrate embryology? To answer this question, a brief review of the way in which they were identified in *Drosophila* is necessary. In flies, mutants were systematically sought; single genes essential for embryonic pattern formation were identified by virtue of their loss-of-function phenotype. Such saturation screens were possible principally because *Drosophila* is so ideal for genetic research. In particular, the small number of chromosomes, and the existence of giant chromosomes of the salivary glands provided a unique physical measure for the numbers of genes and the analysis of chromosomal aberrations. *Drosophila* has about 6000 "essential" genes, of which 5000 mutate to lethality (roughly one-third each are embryonic, larval, or

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