

Commentary

New vistas for developmental biology

Farewell to the First Golden Era
(The Mamas and The Papas 1967)

As the first revolution in developmental biology tapers off into frenetic normalcy, the second developmental biology revolution appears ready to take off. The first revolution has been driven by recombinant DNA technologies that have enabled us to identify and amplify minute amounts of genetic material through nucleic acid hybridization. The goals of this revolution were obvious: find the molecules that direct the transcription of specific genes, and map the signal transduction pathways that link events occurring on the cell membrane with those events they cause inside the nucleus. The results have been spectacular. We have identified paracrine factors and their receptors, kinases and their modulators, transcription factors whose activities lay dormant until activation by the transduction pathways, and various chromatin modulators that interact with the transcription factors to enhance or repress transcription. In addition, this revolution has empowered us to pinpoint the molecular causes of many human diseases, and to determine the sequence of enormous amounts of DNA. The sequence data have also led us to discover homologous genes between species, particularly between vertebrates and *Drosophila*. These technological advancements of the revolutionary “First Golden Era” of the past twenty years have given developmental biologists the identity of many of the major “players” in differential gene expression (and the means to find the others).

To explore strange new worlds . . . to boldly go . . .
(Roddenberry 1966)

The second revolution in developmental biology is fuelled by the fusion of bioinformatics, structural and functional genomics, functional imaging, DNA microarrays, and the promises of stem cell technologies and proteomics. It is not a single-minded revolution in the sense of the revolution of the past two decades. It has many disparate goals, each enabled by interactions of the new technologies. Developmental biology can be studied at any level of organization from the gene to the environment, and it will be in the interactions between these levels that much of the new revolution will occur. One of the fundamental levels will be that of the cell. The cell will become critical because it is both a part of a larger entity – the tissue – and the summation of numerous component parts – chromosomes, organelles, cytoskeleton, etc. The cell is the mediator between the genes and the body. Moreover, the importance of tissues and morphogenetic field becomes evident when we see them as integrating the cells into communities that collectively make up the phenotype of the organism. We can work both upwards from the genes to the cell, and downward from the cell (and thus from the tissues and environment) to the genes. Therefore, we can expect developmental biology to be enriched by having (at least) the following paths explored:

(i) *The physical chemistry of developmental interactions:* Now that the players are known, we will want to know what they do and how they do it. One path to follow will be to look at the interactions between ligands and their receptors and between factors that have bound to chromatin. Immunologists have paved the way to study ligand-receptor interactions, for they have shown that the difference between self-recognition and altered-self recognition (i.e. whether a T cell receptor recognizes an antigen in the cleft of a major histocompatibility protein) is in the order of Ångströms. In contrast,

developmental biology is largely devoid of quantitative measurements, and this will need to change as investigators attempt to decipher how the signals are transduced. For example, developmental biologists currently accept the fact that once a transcription factor is activated on the DNA, it can bind a histone acetyltransferase, modify the chromatin and start transcriptions. But it is not known how these events are accomplished. Similarly, the control of ligand-receptor interaction, either via stoichiometry or affinity, in the establishment of patterns and polarity remains to be clarified. Recent studies of signal transduction (for instance, the interactions of twisted gastrulation protein, BMP4, and chordin) (Chang *et al* 2001; DeRobertis *et al* 2001) clearly demonstrate that relative dissociation constants are critical in determining the fate of cells.

(ii) *The downstream apparatus of development:* One of the hot areas of developmental biology will undoubtedly be the investigation of those mechanisms by which the cell effects development. The first revolution has identified the paracrine and transcriptional bureaucrats that make the specificity decisions. It has not examined deeply the factory floor where development actually takes place (Larsen 1997). Knowledge of the “executive suite” does not imply knowledge of the “workshop”, and one of the projects of the new revolution will be to see how the decisions of the nucleus are carried out. Differentiation is *cytodifferentiation*, after all. Whereas the upstream paths from the paracrine factor receptor into the nucleus have been relatively well traversed, the downstream paths from the nucleus to the cytoskeleton and to the activities they promote on the cell surface still need to be cleared. This “black box” of intracellular development is beginning to be opened and recent research (such as that showing the dynein-dependent movement of *wingless* mRNA to the apical regions of the developing cells of the *Drosophila* blastoderm) (Wilkie and Davis 2001) clearly demonstrates the importance of “cytomorphogenesis” to subsequent development. The studies of cell polarity, cell architecture, cell–cell and cell–substrate interaction will become central to our understanding of morphogenesis. How the architecture of the tissue effects gene expression will also become a critically important area.

(iii) *Tissue engineering and regenerative technology:* Developmental biology is poised to enter medicine in a very big way. There are two important areas that are just beginning to be explored and which hold enormous potential both for understanding development and for therapeutic intervention. The first is the genetic engineering of stromal cells. Although we can do a great deal of genetic engineering with the epithelium, the same cannot be said of the stroma. This is true partly because of the relative paucity of organ-specific stromal promoters, but it is also due to the fact that because the epithelium is relatively more accessible, researchers have been less creative with the stroma. This deficiency will be remedied as biologists and physicians begin to realize the opportunities these cells provide for analysing morphogenesis and for investigating (and perhaps ameliorating) disease (Caterston *et al* 2001).

The second area of tissue engineering and regenerative technology involves pluripotent cells and paracrine factors. In addition to the embryo, the adult body still harbours progenitor cells with pluripotent, if not totipotent capabilities. Even adipose tissue from liposuction has been shown to contain stem cells capable of giving rise to other cell types of the adult body (Zuk *et al* 2001). For the practicing developmental biologist, an immediate objective would be to trace the origin and determine the phenotypic characteristics of these cells, and to investigate the molecular cues that regulate their differentiative activities. Such information is in fact crucial for the isolation and culture expansion of these cells and the formation of tissues and organs *in vitro* or *in vivo*, for regenerative medical applications. This is a rapidly emerging area of research, one that is based on developmental biology and with strong links to biotechnology, bioengineering, and surgical and internal medicine. For instance, tissue engineering of connective tissues would first require the isolation, culture, and proper differentiation of stem cells; these cells must then be efficiently loaded and optimally harboured within a biodegradable polymeric scaffold and exposed to a bioactive milieu containing a cocktail of paracrine growth factors and/or cytokines selected for their ability to induce the desired cellular differentiation. Bone marrow stroma-derived mesenchymal progenitor cells are already being actively investigated for use in skeletal tissue engineering applications, such as the promotion of fracture repair and resurfacing of degenerate articular cartilage. Identification of molecules and reagents that influence neuronal differentiation of progenitor cells has opened the door for cell-based therapies of Parkinson disease and

the possible restoration of function across formerly severed spinal nerves. Candidate stem cells have also been found that can restore heart muscle and liver function. It is reasonable to speculate that cloning technology may make it possible to generate multipotent or totipotent stem cells using one's own nuclei. Cloning a person would be difficult if not ethically unacceptable. However, taking nuclei from one's own cells, placing them into enucleated oocytes and then culturing the inner cell masses of these pre-implantation, pre-gastrulation embryos to generate stem cells may be much easier. This "therapeutic cloning" will be controversial, and different nations may enact different statutes regulating it. In some instances, the plastic cells of the adult body may still be able to respond to paracrine factors without their first having to be isolated from the body (Deutsch *et al* 2001). The discovery of those "locks" that prevent regeneration may enable the repair of bone and neural lesions (see Tessier-Lavigne and Goodman, 2000). Developmental medicine is perhaps closer than we think.

(iv) *Developmental laws and logic*: Developmental biology has remained to a large degree a "law-less science." However, mathematical and computational tools may enable a formal logic to be found within the interactions of animal development. Mathematical areas such as systems theory, network theory, bifurcation theory, complexity theory, and fractal analysis may prove useful for analysing morphogenesis, differentiation, signal transduction, and the stabilization of developmental pathways. A spate of new papers (e.g. Behera and Nanjundiah 1998; von Dassow *et al* 2000; Salazar-Cuidad *et al* 2001) demonstrate that developmental biology will no longer be the refuge of the mathematically challenged scientist.

Digital graphics technologies are already beginning to make it easier to plot developmental changes in transcription patterns. Theoretical calculations of morphogen concentration and cellular signaling molecules can now be graphed on the computer (Jernvall *et al* 2000; Meinhardt *et al* 2001). Even more exciting is the application of "Hollywood-esque" digital animation technology to the theatre of development. Developmental biologist should be able to "dial-in" incremental changes to the characteristics of the "players", in this case cells, morphogens, transcription factors, cellular and molecular activities and then dynamically visualize the three-dimensional, spatial and temporal consequences of such changes on morphogenesis. For example, this type of approach should help developmental biologists determine for a given morphogen, in a virtual context, how parameters such as its concentration, diffusion properties, structure-and-activity relationship (e.g. point mutations), act together to effect the final phenotype. Application of these technologies should lead to an explosion of models relevant to developmental biology. This approach will also revolutionize teaching. In addition to tweaking the parameters of developmental systems, we can expect students of the next generation to use virtual reality tools to explore development. Studying amphibian gastrulation may involve "sitting" on a dorsal blastopore lip cell and riding it into the embryo.

(v) *Evolutionary developmental biology*: Evolutionary developmental biology (evo-devo) is the science that seeks to discern how changes in development effect evolutionary changes and how evolution is constrained by the mechanisms of development. Just as development connects genotype with phenotype, so heritable changes in development can cause new phenotypes that can be retained through natural selection. Recent advances in this field have been made possible by DNA sequence databases and the cloning technologies that enable one to look for homologous genes in a variety of organisms. The computer-assisted modelling of morphogen concentrations (mentioned above) will also aid in evolutionary developmental biology.

This area of developmental biology has just become organized and a number of new journals have emerged to publish its findings. However, relatively few organisms have been studied. In the future, evolutionary developmental biology will spread across all the living kingdoms to enable us to read (at least in its grand outlines) the entire history of life on this planet. Evo-devo is genealogy writ enormous. It will not only show the history of life on earth, but also the mechanisms generating the wealth of organic diversity. One of its major projects will be to determine the developmental and evolutionary origins of cell types such as the mesoderm, lymphocytes, neural crest cells and cnidoblasts. It will also throw light on how these cell types became different from their precursors (Holland and Chen 2001; Rodaway and Patient 2001). Other projects will include determining the mechanisms by which new gene expression patterns can produce new morphological structures and how gene

expression can form robust patterns that can both facilitate and resist change. We can readily envisage a new era of comparative developmental biology, wherein regulatory regions of developmentally important genes will be compared to one another and to the different morphological structures they help produce.

(vi) *Ecological developmental biology*: The microevolutionary component of evo-devo, ecological developmental biology, seeks the interactions between the developing organisms and their respective habitats (Gilbert 2001). There is not only a circuitry of interactions within the developing organism, but also between the developing organism and its biotic and abiotic environment. Temperature, nutrition, population density, and even infectious microorganisms can determine the sex of some animals, and morphological changes can be wrought by predators, competitors, and even physical stress. Ecological developmental biology will look at the plasticity that each organism inherits and how the environment elicits particular phenotypes. Ecological developmental biology can change our worldview. Rather than seeing us as individual organisms developing in isolation, ecological developmental biology sees our development as a coming-into-being of a consortium (McFall-Ngai, personal communication). Organisms develop an intimate symbiosis with other organisms that can alter one's gene expression patterns and sometimes even alter one's anatomical phenotype. Ecological developmental biology also concerns teratology, since environmental effects on development need not always be adaptive. As industries release new compounds into the world's air and drinking water, the monitoring of developmental changes becomes an increasingly important area of study. How the environment regulates cell growth, cell division, and cell death becomes a critically important issue for developmental biologists, conservation biologists, and public health biologists.

The revolution in plant developmental biology started later than that of animal developmental biology, and the second revolution in plant developmental biology is more continuous than its zoological counterpart. Even so, since knowledge of plant developmental biology is more critical to our welfare than knowledge of animal development, and since widescale genetic modification of plants is relatively easy (both procedurally and economically), plant developmental biology will experience both growth and inflorescence in the next decade. The mechanisms by which animals and plants coordinate their development (and by which plants protect themselves by altering the development of predatory animals) will provide an interesting intersection point for plant and animal traditions of developmental biology.

(vii) *Developmental neuropsychobiology*: The development and evolution of neural and glial cells is already an area of special concern for developmental biology. Gene Wilder said it loudly in *Young Frankenstein*: "Hearts and kidneys are tinker toys. I'm talking about the central nervous system!" (Wilder and Brooks 1974). A liver cell may have connections with a dozen other cells. A neuron in the brain may be in specific contact with tens of thousands of other neurons. The continuation of neural growth rate into childhood, the creation of new synapses by experience, and the creation of memories all contribute to the biological basis for consciousness and cognition. The development of human cortical functions will likely be an area where developmental biology, psychology, anthropology, and philosophy will meet and form a new science of human nature. The ability of computers to model neural networks will most likely be expanded to acquire and facilitate the study of emergent properties unexpected from the component parts of the system.

Come together . . . right now (Lennon and McCartney 1969) – *Cell-based developmental medicine*

As mentioned above, progenitor or stem cell based therapies have opened exciting possibilities and represent the basis for regenerative medicine. The central player here is again the cell, this time as a functional conduit to restore biological function to the host tissue/organ by the process of neomorphogenesis. The regenerate tissue is, in effect, a microcosm of developmental biology, capable of recapitulating the programme of highly coordinated activities responsible for the formation of the tissue/organ in the first place, and adapting to the functional demands of the mature host tissue/organ. Thus, tissue engineering requires an expedited and "coming-together" developmental program of

exquisitely high fidelity. How to translate the basic information gathered from studies of embryonic development into practical parameters that can be manipulated to achieve the expedited and high fidelity process of tissue neo-morphogenesis is an exciting challenge for the modern developmental biologist. Again, the cell (and groups of cells) is at the interface between being the passive recipient of growth factors and the active agent of morphogenesis. If growth factors are to become “the magic potions of the twenty-first century” (Slack 1999), then it will be because the adult host cells (or their stem cells) can be induced to respond to them.

With great power comes great responsibility
(Lee 1963)

The new revolution in developmental biology is going to provide us with remarkable power. We may be able to alter the course of human development, human evolution, and even the evolution of the biosphere. Julian Huxley predicted that humans would fill the position of “business manager for the cosmic process of evolution” (Huxley 1953). However, on-the-job-training usually means learning from mistakes, and these would be tremendously costly. Moreover, as investigations into regeneration, stem cells, and tissue engineering produce areas of “applied developmental biology” and “developmental medicine”, we can expect the market to play a larger role in deciding which areas of science need funding and who gets to fund them. This has already occurred in the *in vitro* fertilization research for animal breeding and human infertility. What is private knowledge and what is public knowledge may also become a new “bill-of-rights” issue in developmental biology (already the case in genomics). Public policy will affect research directions, and perhaps we will see “off-shore” developmental biology laboratories pursuing research not sanctioned in some scientifically advanced countries. Developmental biology will demand not only intellectual creativity and technical expertise, but also considerable wisdom in establishing its own regulations.

Whoever predicts the future of science is foolish; but one can be emboldened by some successful prognostications of the past. In 1966, at the lag phase of the first revolution in developmental biology, Joshua Lederberg was asked to write the introduction to the new series, *Current Topics in Developmental Biology*. One of the points he made was that developmental biology should focus on new model systems, and he suggested that the mouse and the nematode would be good choices. He also made the observation that “embryology is the branch of biology closest to human affairs”, and that experimental techniques for the manipulation of development may be possible within a generation and the length of the human life span may be a matter of technology. A similar multifaceted revolution occurred in the 1890s, as new microscopic, photographic, and chemical procedures were combined with genetics and cytology. E B Wilson, whose landmark volume *The Cell in Development and Inheritance* (1896), helped establish the intimate relationship between genetics, embryology and cytology, concluded his book with a statement which is relevant even today:

“The splendid achievements of cell-research in the past twenty years stand as a promise to its possibilities in the future, and we need set no limit to its advance. To Schleiden and Schwann the present standpoint of the cell-theory might have seemed unattainable. We cannot foretell its future triumphs, nor can we doubt that the way has already been opened to better understanding of inheritance and development.”

References

- Behera N and Nanjundiah V 1997 Trans gene regulation in adaptive evolution: a genetic algorithm model; *J. Theor. Biol.* **188** 153–162
- Caterson E J, Nesti L J, Albert T, Danielson K G and Tuan R S 2001 Application of mesenchymal stem cells in the regeneration of musculoskeletal tissues; *Medscape MedGenMed* (<http://www.medscape.com/medscape/GeneralMedicine/journal/2001/v03.n01/mgm0205.cate/mgm020>)
- Chang C, Holtzman D A, Chau S, Chickering T, Woolf E A, Holmgren L M, Bodorova J, Gearing D P, Holmes W E and Brivanlou A H 2001 Twisted gastrulation can function as a BMP antagonist; *Nature (London)* **410** 483–487

- De Robertis E M, Wessely O, Oelgeschlager M, Brizuela B, Pera E, Larrain J, Abreu J and Bachiller D 2001 Molecular mechanisms of cell–cell signaling by the Spemann–Mangold organizer; *Int. J. Dev. Biol.* **45** 189–197
- Deutsch G, Jung J, Zheng M, Lora J and Zaret K S 2001 A bipotential precursor population for pancreas and liver within the embryonic endoderm; *Development* **128** 871–881
- Gilbert S F 2001 Ecological developmental biology developmental biology meets the real world; *Dev. Biol.* **233** 1–12
- Holland N D and Chen J 2001 Origin and early evolution of the vertebrates new insights from advances in molecular biology, anatomy and palaeontology; *Bioessays* **23** 142–151
- Huxley J 1953 *Evolution in action*, Mentor series (New York: New American Library) p. 116
- Jernvall J, Keränen S V E and Thesleff I 2000 Evolutionary modification of development in mammalian teeth: quantifying gene expression patterns and topography; *Proc. Natl. Acad. Sci. USA* **97** 14444–14448
- Selänne L 1999 Laser confocal microscopy and geographic information systems in the study of dental morphology *Paleontologia Electronica* 2; http://www.wodp.tamu.edu/paleo/1999_1/confocal/issue1_99.htm
- Larsen E 1997 Evolution of development The shuffling of ancient modules by ubiquitous bureaucracies; in *Physical theory in biology* (eds) C Lumsden, L Trainor and W Brandt (Singapore: World Scientific) pp 431–441
- Lederberg J 1966 Remarks; in *Current topics in developmental biology 1* (eds) A Monroy and A A Moscona (New York: Academic Press) pp ix–xiii
- Lee S 1963 *The amazing spiderman 1* (New York: Marvel Comics)
- Lennon J and McCartney P 1969 “Come together” in *Abbey Road*; Apple Records, SO-383
- Meinhardt H and Gierer A 2001 (accessed) <http://www.eb.tuebingen.mpg.de/abt.4/meinhardt/theory.html>
- Rodaway A and Patient R 2001 Mesendoderm: an ancient germ layer?; *Cell* **105** 169–172
- Rodenberry G 1966 *StarTrek* (Paramount Television)
- Salazar-Cuidad I, Newman S A and Solé R V 2001 Phenotypic and dynamical transitions in model genetic networks I. Emergence of patterns and genotype–phenotype relationships; *Evol. Dev.* **3** 84–94
- Slack J M W 1999 *Egg and ego: An almost true story of life in the biology lab* (New York: Springer) p. 37
- Tessier-Lavigne M and Goodman C S 2000 Regeneration in the Nogo zone; *Science* **287** 813–814
- The Mamas and the Papas 1967 *Farewell to the First Golden Era* (MCA Records) MCA-709
- von Dassow G, Meir E, Munro E M and Odell G M 2000 The segment polarity network is a robust developmental module; *Nature (London)* **13** 188–192
- Wilder G and Brooks M 1974 *Young Frankenstein* (California: 20th Century Fox)
- Wilkie G S and Davis I 2001 *Drosophila wingless* and pair–rule transcripts localize apically by dynein-mediated transport of RNA particles; *Cell* **105** 209–219
- Wilson E B 1896 *The cell in development and inheritance* (New York: Macmillan) p. 330
- Zuk P A, Zhu M, Mizuno H, Huang J, Futrell J W, Katz A J, Benhaim P, Lorenz H P and Hedrick M H 2001 Multilineage cells from human adipose tissue: implications for cell-based therapies; *Tissue Eng.* **7** 211–228

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