Why Is Development So Illogical?

The more biologists learn about development the less it appears that organisms are assembled by neat, sequential processes; we should not be surprised

"People thought I was crazy," recalls Sydney Brenner, director of the Medical Research Council's Laboratory of Molecular Biology, Cambridge, England. "Jim Watson said he wouldn't give me a penny to do it. He said I was 20 years ahead of my time."

But, with the help of a group of extremely dedicated and inventive associates, Brenner is well on the way with his crazy project. He has transformed the tiny nematode *Caenorhabditis elegans* into a subject of serious science: more is known about the genetics and development of this 1-millimeter roundworm than about any other multicellular creature.

True, *C. elegans* can boast a total of only 959 cells in its entire body, 302 of which constitute its nervous system. But Brenner and his associates now know the complete developmental history of every one of these cells and can describe every connection within the nervous system. And there is now an extensive catalogue of mutants that affect virtually every aspect of the organism's albeit limited life, including mutants that disrupt patterns of development.

The object of this technically and intellectually daunting exercise, Brenner now says, was to see how far genetic analysis of cellular regulatory mechanisms could explain development and function in complex biological systems (see box). What has been achieved so far—the complete description of the anatomy and cell lineage, the genetics and an entry into the molecular biology and biochemistry—is an excellent and revealing beginning. But an understanding of how the information encoded in the genes relates to the means by which cells assemble themselves into an organism—"the mapping of genetic space onto organismic space"—still remains elusive. Brenner believes this may require a complete catalogue of the organism's molecular biology and, more important, an appreciation of what he calls the grammar of it all.

The project was conceived some two decades ago, the ambitious product of a series of conversations between Brenner and Francis Crick, who has since moved to the Salk Institute, La Jolla. Molecular biology had at that time scored outstanding successes in analyzing the genetic code and in beginning to uncover the mechanism and control of gene expression, and Brenner and Crick were impatient to explore new territories with this most powerful of sciences. "The genetics and biochemistry of control mechanisms in cellular development," was the chosen goal. It has proven to be a far less neat and tidy territory than the organized mind of the molecular biologist had contemplated.

"At the beginning it was said that the answer to the understanding of development was going to come from a knowledge of the molecular mechanisms of gene control," reflects Brenner. "I doubt whether anyone believes that anymore. The molecular mechanisms look boringly simple, and they don't tell us what we want to know. We have to try to discover the principles of organization, how lots of things are put together in the same place. I don't think these principles will be embodied in a simple chemical device, as it is for the genetic code."

There has been a persistent expectation among many molecular biologists that the guiding themes of development would somehow be encoded in a genetic program of some nature somewhat analogous to the sequential encoding of protein structure. An understanding of development would therefore entail uncovering piece by piece the details of such a program. This type of reductionist mindset has now largely been abandoned but not without a great deal of sometimes heated debate.

Brenner, who used the term "program" in his first paper on the genetics of *C. elegans* in 1973—"A thorough analysis of the effects of such mutations might throw light on the logical structure of the programme," he wrote—now eschews it. "We tend to talk loosely about genetic programs and we should be careful about the implications of this language even when used metaphorically." He prefers the term internal representation or internal description but notes that biologists face a paradox here. "The total explanation of all organisms resides within them, and you feel there has to be a grammar in it somewhere. Ultimately, the organism must be explicable in terms of its genes, simply because evolution has come about through alterations in DNA. But the representation will not be explicit. We need to understand the grammar of development to make sense of it."

Brenner offers an "amusing analogy" to make the point. "The icosahedral head of a bacteriophage is a precise geometric object. This geometry is inherited. So you say, there must be a definition of an icosahedron in the genome somewhere: where is it? You won't find a gene that says, 'make an icosahedron.' In order to understand what it means to specify an icosahedron you first have to understand the principles of molecular assembly, the way that the coat proteins interact and self-assemble.

"This is different from writing a computer program that tells the machine to draw an icosahedron. We have a different kind of hardware in the cell. The icosahedron is encoded in a distributed form throughout the entire genome. That's a pretty good picture of what we have to say when we ask, what does it take to make a hand, make a foot, make a liver. The specifications for these structures are scattered throughout the genome. It's not a neat, sequential process, like the linking together of amino

> "We should like to attack the problem of cellular development . . . choosing the simplest possible differentiated organism and subjecting it to the analytical methods of microbial genetics. Thus we want a multicellular organism which has a short life cycle, can be easily cultivated and is small enough to be handled in large numbers, like a microorganism. It should have relatively few cells so that exhaustive studies of lineage and patterns can be made, and should be amenable to genetic analysis. We think we have a good candidate in the form of a small nematode worm, *Caenorhabditis*. . . . To start with we propose to identify every cell in the worm and trace lineages. We shall also investigate the constancy of development and study its genetic control by looking for mutants."—From the Laboratory of Molecular Biology's proposal to the Medical Research Council, England, in 1963.

acids in a protein. It's everything going on at the same time, and that is something we are very bad at describing."

Gunther Stent, of the University of California, Berkeley, a couple of years ago offered another graphic analogy to make the same point. "Consider the establishment of ecological communities upon colonization of islands or the growth of secondary forests," he wrote. "Both of these examples are regular phenomena in the sense that a more or less predictable ecological structure arises via a stereotypic pattern of intermediate steps, in which the relative abundances of various types of flora and fauna follow a well-defined sequence. The regularity of these phenomena is obviously not the consequence of an ecological program encoded in the genome of the participating taxa. Rather it arises via a historical cascade of complex stochastic interactions between various biota. . . . "

This example, like Brenner's phage icosahedron, is instructive of where in the hierarchy of things one must seek the grammar of assembly, even if the ultimate source of information is at the level of the gene: it is in the interaction of the component parts of the system.

Brenner also likes to cite the apparently highly organized and ordered set of biochemical reactions that must go on at a very high rate and with high fidelity in the growth and division of the bacterium *Escherichia coli*. Every 30 minutes each bacterium divides, following the synthesis of a large number of complex molecules from simple components, the accurate replication of DNA and the equal partitioning between two daughters. "People used to argue until relatively recently that enzymes involved in a sequential biochemical pathway must be assembled as one structure so that intermediates would pass efficiently from one processing step to the next. For electron transfer this might be true, but for most pathways it is not. People had forgotten about diffusion."

Diffusion time for the kind of molecules in question is short enough within the confines of the bacterial cell so that, even though most collisions are nonproductive, a substrate will strike the binding site of the required enzyme with sufficient frequency for even a complex biochemical pathway to run smoothly. "The heart of the matter," says Brenner, "is the binding site. Unlike a computer, where a signal is sent from one part to another by a wire connecting the two, a bacterial cell uses a broadcast system and only appropriate collisions matter; the rest are simply ignored." Counterintuitively, the complex set of reactions runs efficiently and rapidly in the absence of an overall control mechanism or superstructure. The interaction between components shapes the system. So it is, in many ways, with building organisms.

Brenner chose *C. elegans* as a tool with which to explore the grammar of development because its very small size should allow a complete knowledge of the organism: its cell number is about the square root of that for *Drosophila*, which in turn is the square root of that for humans. The architecture of the nervous system has been determined, principally by John White, by reconstruction of serial section electron micrographs. And John Sulstan has tracked the total cell lineage by watching the fate of each cell by turn through the mercifully short 3.5-day life-cycle. The publication of the complete description of this little worm will be a milestone in the study of biology.

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Those involved with the project had some preconceptions of how they thought the animal would put itself together. "Many thought that the cells were going to be powers of two, amusing mathematical symmetries, and so on," says Brenner. "What the lineage has taught us, however, is that there is hardly a shorter way of giving a rule for what goes on than just describing what there is." There is no simple logical series of cell divisions from which the groups of organ assembly might have been predicted. The lineage is, in a word, baroque.

A sequence of cell divisions might, for instance, repeatedly give rise to a set of differentiated cells, each of which is of a different type. Structures are often assembled piecemeal, with the coming together of cells from several lineages. And, most surprising of all, symmetrical structures are just as likely as not to be constructed in an entirely unpredictable, asymmetric manner. "I lost faith in the perfection of biological systems a long time ago," observes Brenner, "and so perhaps it is not surprising that they just muddle through. Anything that is produced by evolution is bound to be a bit of a mess."

Brenner does not take this to imply that all is chaos and beyond comprehension. It is, however, likely to be more difficult to penetrate than has been suspected, partly because organisms have not been designed each time from scratch, and are therefore unlikely to be put together in the most logical and most predictable manner; and partly because of the limitations of our imagination. "We tend to be more at home with hierarchical structures and sequential processes and it is common to find these in many models of development and its genetic control."

One persistent view of the organization of complex systems is the following. It has been supposed that organisms must be partitioned in some kind of molecular fashion, based on anatomical structures, physiological systems or developmental pathways. This modular organization is then thought to be the basis for modular genetic representation. Brenner acknowledges that such an arrangement is appealing both for its feasibility for control and its access to ordered evolutionary change. Large computer programs are organized in this manner in order to localize the effects of change. "Thus by analogy it is argued that genes are also arranged in closed logical packets allowing changes to take place in one subsystem without affecting the others."

Brenner offers an alternative to think about, one that involves two layers of genetic control. The first layer is a noisy, inaccurate set of processes that generates a "sort-of-worm." And the second is a set of refinement processes that tames the unruliness of the first and yields a real and recognizable worm. There is in fact the potential for many types of worm locked up in the genome, but the one that comes out is determined by the refinement genes. "Of course, in this process many changes will have 'unpredictable' consequences, but, unlike computer programming, natural selection is cheap and has plenty of time to work."

The description of the *C. elegans* lineage has been immensely instructive in simply revealing how the animal puts itself together, and, perhaps more important, how changes in the lineage can produce large morphological shifts in the adult. "What we are looking for is an understanding of how the genes participate in these processes. We came to realize that cells are the units of develop-

ment, and what we have to do is find out how the genes get hold of the cell. That is just another way of saying that the molecular biology of development is the molecular biology of the cell."

In principle any mutant that affects development might yield some insight into the grammar that lies between the genetic space and the organismic space. "The name of the game now is gene products: the ability to do inside-out biology." There are, for instance, mutants that cause a particular cell division cascade to reiterate. "Find out what the product of that gene is, what cells it operates in, what it does: and then you might know something about the control of these reiterative divisions." After a moment's reflection Brenner adds, "Of course, it might tell you nothing at all."

It has become clear from the *C. elegans* that there is not a simple set of obvious rules that governs development, and some critics have said that Brenner and his associates are no nearer understanding the process, that all they have done is describe what happens. "I'm not sure that there necessarily is anything more to understand than what it is," he responds. "That is a possibility for this level of complexity." Perhaps because organisms are the products of evolutionary change within certain biological and architectural constraints, simple description of how they are put together is going to yield the most profound insights. In which case, the choice of *C. elegans* has been apposite, as anything bigger would be intractable to description in the detail that is necessary.

Other critics say that the project is just too difficult, at which point Brenner's molecular biology macho comes to the fore: "I don't accept that. Peter Medawar has written that science is the art of the soluble. My reply is that molecular biology is the art of the inevitable. If you do it, it's inevitable you will find out how it works—in the end. Maybe it won't be until you are able to put the last period on the page, when you know everything, that you will be able to say, aha, now I understand! But you will get there in the end."—ROGER LEWIN

Unusual Bimetallic Catalyst Synthesized

A manganese-cobalt catalyst prepared by solvated metal atom dispersion has some unusual catalytic properties

What appears to be the first known case in which one metal activates a second metal for heterogeneous catalysis has been reported by Kenneth J. Klabunde and Yuzo Imizu of Kansas State University. These investigators have found that small pseudo-organometallic particles containing manganese and cobalt have activities for catalytic hydrogenation of alkenes that are much higher than those of similar particles containing only cobalt. The new particles also have much higher activity than commercial catalysts or similar particles containing other metals. Manganese, however, has very little catalytic activity for the reaction, indicating that the activation is somehow effected by its interaction with the cobalt.

The new Mn-Co catalyst, reported in the *Journal of the American Chemical Society* [**106**, 2721 (1984)], is but the most recent in a series of unusual catalysts produced by Klabunde and his coworkers using a technique known as solvated metal atom dispersion (SMAD). The technique, he says, was designed to take advantage of the fact that small clusters of metal atoms "are fundamentally different and more reactive than clean metal surfaces." * SMAD catalysts are typically more reactive than conventional catalysts and, in many cases, show unusual specificity.

The SMAD catalysts are prepared in a vacuum flask whose walls are cooled to −196°C by liquid nitrogen. The metal to be studied is vaporized in a small electric crucible in the center of the flask. At the same time, small quantities of a solvent are also admitted to the flask. The solvent vaporizes rapidly, then condenses on the walls of the flask along with the metal atoms. Varying heating rate and the relative proportions of metal and solvent make it possible to regulate the size of the metal clusters that are formed.

The nature of the solvent is important. It must be only weakly solvating and relatively inert to oxidative addition, abstraction, or electron transfer processes. Among the best solvents are hydrocarbons such as pentane, hexane, toluene, and tetrahydrofuran; many of the best catalysts, including the Mn-Co particles, are made with toluene as the solvent.

These "bare" metal clusters—called bare because there are no covalent metal-carbon or metal-hydrogen bonds—can be quite reactive. Aluminum atoms, for example, react with methane at 10 K; this phenomenon has not been observed for single atoms of any other metal, and Klabunde attributes the reactivity to the ^2P electronic state of aluminum, which gives it a "free-radical-like" character. Other single metal atoms react with methane at 10 K only if they have been excited by light.

Nickel clusters of unknown size react vigorously with pentane at 140 K. In fact, the nickel clusters react so extensively with pentane that pseudo-organometallic particles precipitate as a stable powder. (Klabunde calls these powders pseudo-organometallic because, even though there are extensive metal-carbon and metal-hydrogen bonds, they do not have a fixed composition and they are not soluble.) In most cases, single metal atoms are more reactive with alkanes than are clusters. Klabunde has some evidence, however, that methyl bromide reacts with clusters of magnesium but not with single atoms. If this finding is confirmed, he says, "this would be the first concrete example of an atom being less reactive than a cluster."

Catalysts can be prepared from the frozen matrix by either of two methods. In each case, the matrix is allowed to melt to form a frozen slurry. If warming is allowed to continue, the solvated metal atoms will react to form pseudo-organometallic powders, with the temperature of formation depending on the individual metal involved. The size of the particles can be controlled, at least in part, by regulating the rate of warming. Alternatively, the solvent can be allowed to permeate a support (such as zeolites, aluminas, or silicas) before the warming is completed. The same type of reaction with solvent occurs upon warming, but in this case, the pseudo-organometallic particles are deposited on the surface of the support.

*For a review of the significance of particle size in catalysis, see *Science*, 3 June 1983, p. 1032.