New Clues to Developmental Timing

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Within the past few years researchers have finally begun to be able to peer inside a hitherto impenetrable black box, namely, the development of complex organisms. The genes that control the commitment of embryonic cells to specific fates are now being found and characterized. A case in point is reported in this issue of *Science* (p. 409). Victor Ambros of Harvard University and H. Robert Horvitz of Massachusetts Institute of Technology have identified genes that affect the timing of developmental events in the roundworm *Caenorhabditis elegans*.

The work provides new insights into the ways that genes guide the formation of a complex organism containing many different cell types from the single fertilized egg cell. It helps to answer the question, "How is it that cells in a developing embryo do the right thing at the right time?"

But more than that, the work may also supply a genetic and cellular basis for a mechanism that has been proposed as a fundamental contributor to evolutionary change. There is a great deal of evidence indicating that new species often emerge because the timing of some developmental events is altered so that they occur later or earlier with respect to other events than they normally would. This proposed mode of evolution is called heterochrony.

The axolotl is a well-known example of an animal that appears to have evolved in this way. It is a gilled, aquatic creature that essentially resembles a salamander larva. However, the sexually mature salamander is a lung-breathing, land animal, whereas the axolotl becomes sexually mature without undergoing this metamorphosis. The development of the axolotl's somatic tissues is retarded whereas its gonads mature normally. "The key thing about heterochrony," notes Rudolf Raff of Indiana University, "is that it can achieve significant morphological change by rather small gene changes."

Genes that control developmental timing, such as those identified by Ambros and Horvitz, may be the targets of those changes. "Ambros and Horvitz have shown that, given changes in the right gene in the lineage, they can get rather important effects. For the first time they have [obtained] a set of mutants that can be used to examine how heterochronic changes actually work. They can take evolution to the lab," as Raff puts it.

Identification and analysis of the developmental timing mutations, to which Ambros and Horvitz have given the designation heterochronic, would have been very difficult, if not impossible, without an organism as well suited to the task as *C. elegans*. It is transparent and small, consisting of only about 1000 somatic cells. Moreover, the complete developmental lineage of every one of those cells has been worked out (*Science*, 6 July, p. 40). "We know the precise timing of every cell division and of many other developmental events in *C. elegans*,"

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Horvitz explains. "This knowledge has allowed us to look for mutants abnormal in specific aspects of developmental timing."

Early evidence for such *C. elegans* mutants was obtained a few years ago by Martin Chalfie, who is now at Columbia University, John Sulston of the MRC Laboratory of Molecular Biology in Cambridge, England, and Horvitz. They identified an egg-laying mutant with many abnormalities, including extra molts and the retention of a larval cuticle in the sexually mature animal. The mutation defined a new gene, which they designated as *lin-4* (*lin* for lineage abnormal). Although Chalfie, Sulston, and Horvitz did not use the term heterochronic at the time, the mutation does in fact have properties that put it in that category. It perturbs the timing of cell divisions in several lineages. In particular, some somatic cells repeated larval division patterns at a time when they should have stopped dividing and formed adult-type cells.

Because defective egg-laying appeared to be one consequence of heterochronic mutations, Horvitz and his colleag went on to examine other egg-lay mutants to see if they, too, might disp alterations in the timing of developm tal events. In the current article, Amb and Horvitz describe mutants defin three new heterochronic genes, *lin-* *lin-28*, and *lin-29*.

Of these, *lin-14* is the best charact ized. Mutations in this gene can aff development in two opposing wa Dominant mutations retard it by alter the fates of cells so that they repeat lineages of their ancestors instead generating their own more advanced eages. Recessive *lin-14* mutations, wh were originally discovered by Ed Ferguson of the Horvitz group, acc erate development in the affected eages by causing cells to express fates normally expressed by their o descendants. The dominant mutati cause an increase, and the recessive r tations a decrease, in *lin-14* gene expr sion, Ambros and Horvitz find.

Heterochronic mutations are not only ones known to alter cell fates. M tations in homeotic genes, which h been identified in *C. elegans* and in bithorax and Antennapedia complexe the fruit fly *Drosophila melanogas* also do so. However, homeotic mu tions cause the affected cells to expr fates normally exhibited by cells in ot positions in the embryo. "Heterochro mutations are the temporal analogue the previously known homeotic mu tions," Horvitz notes. "Heterochro mutations cause transformations in ti instead of space."

In any event, the current results s gest that when *lin-14* activity is at a h level, cells express early fates and wh it is at a low level, they express la fates. "One possible model is that activity of *lin-14* decreases during de opment, causing cells to express f early and then later programs," s Ambros, who had worked in the Hor laboratory until his recent move to H vard.

Many intriguing questions remain be answered about *lin-14* activity. If level of its expression conveys tempo information to cells, as Ambros and H vitz suggest, then the activity of the g itself must also be regulated in so fashion. As Ambros asks, "If *lin-14* is controlling the timing of the expression of cell lineages, then what is controlling *lin-14*? What is the clock?"

Another major unanswered question concerns the way in which heterochronic genes might be working. "We don't really have any convincing evidence about how they act," Horvitz says, "but our favorite hypothesis is that these genes function in the way that *Drosophila* homeotic genes are thought to function, namely, by specifying regulatory proteins that are expressed and act within the cells they affect." Another possibility is that the genes control the levels of hormone-like substances that act throughout the animal. Such a mechanism appears to regulate axolotl development. The body tissues of the animal remain immature because it does not produce enough thyroxine, which is needed for metamorphosis to occur.

Mutations in *lin-14* affect many cell lineages and produce widespread alterations in *C. elegans*. It will also be interesting to determine whether the gene acts by controlling the activity of other genes. According to the current view, development is controlled by hierarchies of genes, with those in the higher ranks turning on or off, as the case may be, the more specifically acting genes in the lower ranks. *lin-14* might be one of the higher ranking genes in the *C. elegans* developmental hierarchy.

In contrast to the situation with *lin-14*, the alterations caused by mutations in *lin-29* are much more restricted in scope. They are limited to certain cuticle-forming cells of the fourth larval stage. Genes with such specific effects might then be lower in the hierarchy and under the control of genes, such as *lin-14*, with more diffuse effects.

To determine whether heterochronic mutations might have played a role in nematode evolution, Ambros and Horvitz have begun examining roundworm species that are related to *C. elegans* to see whether their cell lineages show variations in timing analogous to those occurring in the heterochronic mutants just identified. If this turns out to be the case, then the work on heterochronic genes may help provide genetic and molecular explanations not just of the temporal regulation of development but also of a common mechanism of evolution.

—**Jean L. Marx**

Additional Reading

1. S. J. Gould, *Ontogeny and Phylogeny* (Belknap, Cambridge, Mass., 1977).
2. R. A. Raff and T. C. Kaufman, *Embryos, Genes, and Evolution* (Macmillan, New York, 1983).
3. M. Chalfie, H. R. Horvitz, J. E. Sulston, *Cell* **24**, 591 (1981).

How Fast Is Oil Running Out?

An expert at the Library of Congress's Congressional Research Service (CRS) has projected a 17 percent decline in U.S. oil production by the year 2000.* That is his optimistic prediction. If discoveries do not pick up soon in the more promising oil provinces, production will drop 29 percent, he says. To those who hold out hope that production might be maintained if only industry is given a fair profit and a free hand, he says they must be wrong.

To test the optimistic hopes for constant production, Joseph Riva, a CRS resource analyst who has searched for oil here and abroad, estimated how much oil would have to be found to maintain the 1982 production level of 2.95 billion barrels per year. That production requires a certain amount of known reserves that can be drawn on. A relatively new oil field can be tapped for about 10 percent of its remaining oil each year (reserves-to-production ratio of 10:1). Pump harder and the field can be ruined, the way sucking too hard on a straw can spoil a milk shake. As a field is depleted, its ratio of reserves to production will drop. Riva assumed that the present domestic ratio of 9:1, which reflects America's already dwindling oil resource base, would drop farther to 8:1 by 1995.

In order to maintain 1982 production under these conditions, the oil industry would have to discover almost 45 billion barrels of oil during the 18 years between 1982 and 2000, according to Riva's calculation. That, he points out, is 70 percent of all the oil left undiscovered in 1982. In comparison, drillers found only 44 percent of the undiscovered oil of the preceding 18 years. And it is harder to find oil now than it was then. Except in Alaska and offshore California, most of the big, easily found fields have been discovered already. To find that much oil in the remaining fields, drillers would have to discover fields six times faster than in the past.

To calculate the seemingly inevitable decline, Riva combined, on a region-by-region basis, present estimates of oil left to be discovered and past performance in finding oil. The estimates of undiscovered oil (and oil to be found by expansion of known fields) are derived from those of the U.S. Geological Survey. In well-explored, mature regions such as West Texas, Riva chose to use the mode of USGS estimates rather than the mean in order to moderate the influence of rare, large finds, unlikely events in such thoroughly drilled areas. Riva's total estimate of undiscovered oil was about 64 billion barrels. He then assumed that drillers would find the same or a larger percentage of undiscovered oil (and field expansion oil) in the next 18 years as they did in the past 18 years. Regions like the Rocky Mountains, where hopes are high that the best finds are yet to come, were assumed to have higher discovery rates than past experience indicated. In this projection enhanced oil recovery—the thinning of oil before pumping—would not increase, contrary to some forecasts of a doubling.

By this calculation, Riva projects a 17 percent drop in production by 2000. All major producing regions would decline except the Rocky Mountains–Northern Great Plains region. Alaska's production would drop 16 percent, West Texas's 40 percent, and the Gulf Coast's 44 percent. Riva considers this 17 percent decline " a very optimistic projection." He and many others consider the USGS estimates of undiscovered oil in the Rocky Mountains, off the Atlantic coast, and perhaps even in Alaska to be overly optimistic, especially in light of recent drilling disappointments (*Science*, 27 January, p. 382). It is certainly optimistic to expect future finds to be as easy as past discoveries, most of the big fields having already been found.

A more likely production decline, Riva says, is 29 percent. That assumes that no oil is found off the Atlantic coast or in the Oregon-Washington region and that things do not improve in the Rocky Mountains–Northern Great Plains region. That decline still assumes an Alaskan discovery rate equal to the one that included the discovery of the supergiant Prudhoe Bay field. A distinct downward trend in U.S. oil production should show up in a few years, says Riva, as field depletion overwhelms the unprecedented drilling of production wells of the past 5 years.—**Richard A. Kerr**

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