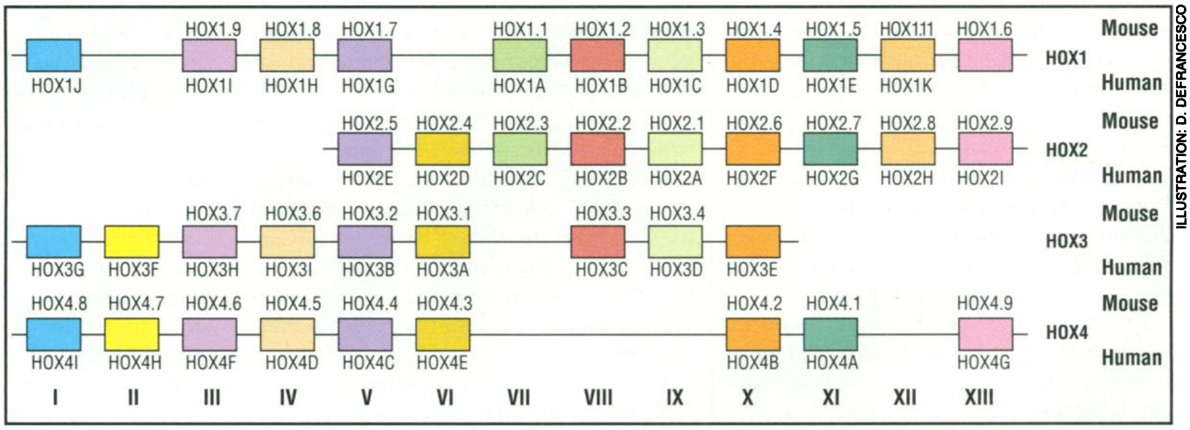
Research News

Homeobox Genes Go Evolutionary

The genes, well known as important regulators of embryonic development, may also provide a key that helps unlock the mysteries of evolution

IN THE MID-1980S, WHEN THE HOMEOBOX genes were first identified, the discovery was hailed as a major achievement in developmental biology. And rightly so. A vast amount of research has shown that these genes are extraordinary in two respects: First, they encode a sequence of 60 amino acids (the homeobox) that has been evolutionarily conserved in organisms ranging all the way from fruit flies to mice—and even to human beings. And second, they turned out to be key regulators of embryonic development. And now, as if that role weren't important enough, work in several labs is suggesting that homeobox genes may have contributed as well to evolution, perhaps even helping to generate the enormous diversity of organisms alive today.

And that's not all. The homeobox gene research is also proving to be promising in yet another sense: It is starting to bring together developmental and evolutionary biologists, a merger that is badly needed, considering the intractability of certain evolutionary puzzles, such as the long-standing quandary of how the body plan of multicelled organisms arose. Rudolph Raff of Indiana University, one of the few scientists who identify with both camps, explains that until recently collaboration wasn't happening between the two communities because they have different languages and histories. "To the developmental biologist most of what's interesting is function—how does it work? But to an evolutionary biologist what's interesting is how it got there," says Raff. But today, "the homeobox work has absolutely been the most crucial tie, unifying studies of developmental regulation and evolution." It might even shed some light on the body plan question.



		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
HOX1	Mouse			HOX1.9	HOX1.8	HOX1.7		HOX1.1	HOX1.2	HOX1.3	HOX1.4	HOX1.5	HOX1.11	HOX1.6
	Human	HOX1J		HOX1I	HOX1H	HOX1G		HOX1A	HOX1B	HOX1C	HOX1D	HOX1E	HOX1K	
HOX2	Mouse					HOX2.5	HOX2.4	HOX2.3	HOX2.2	HOX2.1	HOX2.6	HOX2.7	HOX2.8	HOX2.9
	Human					HOX2E	HOX2D	HOX2C	HOX2B	HOX2A	HOX2F	HOX2G	HOX2H	HOX2I
HOX3	Mouse			HOX3.7	HOX3.6	HOX3.2	HOX3.1		HOX3.3	HOX3.4				
	Human	HOX3G	HOX3F	HOX3H	HOX3I	HOX3B	HOX3A		HOX3C	HOX3D	HOX3E			
HOX4	Mouse	HOX4.8	HOX4.7	HOX4.6	HOX4.5	HOX4.4	HOX4.3				HOX4.2	HOX4.1		HOX4.9
	Human	HOX4I	HOX4H	HOX4F	HOX4D	HOX4C	HOX4E				HOX4B	HOX4A		HOX4G

ILLUSTRATION: D. DEFRANCESCO

How they stack up. *Closely related genes line up vertically in the mouse and human homeobox gene clusters.*

ADAPTED FROM M.T. MURTHA, T.F. LECKMAN, AND F.H. RUDDLE, *PROC. NATL. ACAD. SCI.*, 88, 10711 (1991).

So far, however, the unification program is still in its early stages and can't be said to have resolved any major evolutionary issues. But one indicator of how encouraged researchers are by what they've seen is the fact that new workers are coming into the field. Invertebrate biologist Leo Buss of Yale University, for example, wants to find out if homeobox genes play any role in the unusual reproductive cycle of one of his own favorite creatures, a small marine worm.

The reason why evolutionists are so interested in development is simple enough. "To change an adult organism you have to change something in the developmental process that makes that organism," is how molecular geneticist and Nobel Prize–winner François Jacob of the Pasteur Institute in Paris put it at a meeting* held last fall on Crete. And the theory seems clear enough. But the practical problem has been to identify just what developmental changes might be important for evolution and explain how those changes came about. Unfortunately for the evolutionary biologists, they have lacked a time machine that would take them back through the aeons so that they could observe firsthand embryonic development in the ancestors of today's organisms.

Enter the homeobox genes, discovered nearly a decade ago in the fruit fly *Drosophila melanogaster* by Matthew Scott and Amy Weiner, who were working with Thomas Kaufman at Indiana University, and also by Walter Gehring's group at the University of Basel, Switzerland. In the fruit fly, the proteins encoded by the homeobox genes tell the cells in the various segments of the developing embryo what kind of structures to make, antennae for the head, for example, and legs for the three thoracic segments. "The genes are obviously most important for development and evolution. They specify the architecture of the fly and [architecture] is what evolution works on," Gehring said at the Crete meeting at which Jacob also spoke.

And while mammals such as mice don't have the same kind of segmentation that fruit flies do, the genes also specify structure formation in the higher animals. One indication of this came in work by several groups showing that the various mouse homeobox genes are active only in certain, restricted regions of the developing embryo, just as the fruit fly genes are. But the definitive proof didn't come until last year, when two groups, one including Osamu Chisaku and Mario Capecchi of the Howard Hughes Medical Institute at the University of Utah School of Medicine in Salt Lake City and the other including Thomas Lufkin, Pierre Chambon, and their colleagues in the medical school at the University of Strasbourg, France, produced mice in which they had knocked out individual homeobox genes. The resulting animals showed a variety of structural defects, chiefly in regions, such as the head and neck, where the genes are active during development.

That caught the evolutionists' attention:

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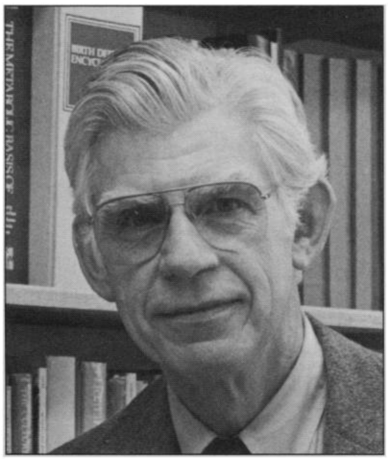
*The meeting, "Evolution and Development: Thirty Years After the Jacob-Monod Paradigm," was held in Hersonissos, Crete, from 14 to 20 October 1991.

The same group of genes participates in structure formation in species as widely diverged as insects and mammals. "What's been exciting to a number of the people in the field of molecular evolution is that the basic mode of development in animals is probably retained in all branches of the metazoans [multicelled organisms]," says Morris Goodman, a molecular evolutionist at Wayne State University in Detroit who plans to look at homeobox gene involvement in his own area of expertise, which deals with the changing patterns of hemoglobin gene control during primate evolution.


YALE

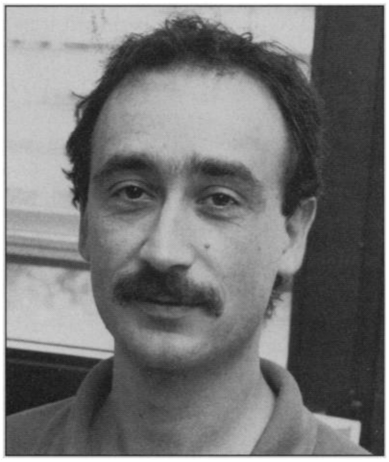
Peering into the past. *Frank Ruddle of Yale University is looking at homeobox gene evolution.*

As interesting as these functional relations of the homeobox genes are, however, it may turn out to be their specific architecture that proves most helpful in exploring their evolutionary role. The fruit fly has about 10 homeobox genes, located in two clusters on one chromosome. Mice and humans have at least 40 of the genes, grouped mainly in four clusters of about 10 genes each, which are located on different chromosomes (although additional homeobox genes are present elsewhere in the genome). Three years ago, when researchers began working out exactly how the genes are arranged in the mammalian clusters, they found the organization of all four mammalian clusters was remarkably similar to that of the fruit fly cluster. The conservation of the homeobox gene sequences and organization is "extraordinary," considering that 500 million years have elapsed since the insects and mammals diverged, says homeobox expert Frank Ruddle of Yale University.

The different homeobox genes in the fruit fly and mammalian clusters can be distinguished because the homeobox sequences vary somewhat from gene to gene. And when researchers, including Denis Duboule of the European Molecular Biology Laboratory in Heidelberg, Germany, Robb Krumlauf of the National Institute for Medical Research in London, England, and Ruddle, compared the order in which the different genes line up, they found something remarkable: It was precisely the same in the mammalian and fruit fly clusters.

And that isn't the only parallel between homeobox organization in flies and mice that was discovered in the past few years. Several studies showed that there is a relation between the positions of the genes in the fruit fly homeobox cluster and the regions of the embryo where the genes are active. Those on the 3′, or right-hand, end of the cluster are expressed in the most anterior regions and the zones of expression move progressively farther back in the embryo the more leftward the position of the genes in the cluster. When researchers began studying the expression patterns of the mammalian homeobox genes, they found that the same was true for them, too. As Ruddle says, the result "very much suggests that the genome is not just a bag of genes that you can shake up and rattle around."

What's more, the parallelism in homeobox sequences and organization means it's easy to compare the homeobox gene complements of different species, from the simplest to the most complex, to see when they arose in evolution and how they changed as organisms evolved. That's one of the main directions that the Ruddle group is now taking, and they've found that these genes arose very early indeed.



Homeobox gene tracer. *EMBL's Denis Duboule follows their expression pattern in embryos.*

How early? Well, other researchers had already identified homeobox genes in such simple organisms as sea urchins and the worm *Caenorhabditis elegans*, but in the December *Proceedings of the National Academy of Sciences* Ruddle, with Michael Murtha and James Leckman, also of Yale, reports that they've identified homeobox genes in the most primitive multicelled organisms yet—the cnidarians, which include jellyfish, and the freshwater hydra, the small tentacled creature most people meet in high school biology lab. Buss' group also reports detection of cnidarian homeobox genes in the December *Journal of Experimental Zoology*. "We think the beginning of the homeobox systems predated the divergence of vertebrates and arthropods [which include the insects]," Ruddle says.

That notion is in accord with a model previously proposed by Ruddle and postdocs Claudia Kappen and Klaus Schughart on the basis of a computer comparison of the sequences of the known homeobox genes. The trio concluded that the primordial metazoan ancestor probably had four or five homeobox genes. Because the cnidarian homeobox genes most closely resemble those on the 3′, or anterior, ends of the fruit fly and mammalian clusters, Ruddle says, the original genes were probably involved in the formation of the most anterior structures of the metazoan body plan. The genes needed for formation of more posterior structures apparently came later, presumably by duplication of the preexisting genes to yield a cluster with about 10 genes in the primordial ancestor of the fruit fly and vertebrates.

The continued evolution of the complexity of the vertebrates may also have involved the addition of more posterior genes. Duboule's group, for example, has recently identified new mouse homeobox genes that are located at the 5′ end of the fourth homeobox cluster and are expressed in the posterior regions of the mouse embryo. The genes have no close relatives in the fruitfly, indicating that they arose after the vertebrates diverged.

The Yale group's model also predicts that during the course of vertebrate evolution, the whole cluster duplicated at least twice, possibly as a result of chromosome duplication, as species became more complicated, ultimately producing the four clusters seen in mammals. The sequence comparisons lead the researchers to estimate that these duplications took place about 350 million to 400 million years ago, a timeframe consistent with the idea that they contributed to the increasing complexity of the vertebrates. Frank Schubert, a postdoc in the lab of developmental biologist Peter Gruss at the Max Planck Institute of Biophysical Chemistry in Göttingen, Germany, has produced a similar model.

Tantalizing as they are, these models remain quite speculative. Confirmation of them can only come from the laborious work of analyzing the homeobox gene complements of vertebrates to see if they do increase in number as their body plans become more complex. While homeobox genes have been found in a wide range of organisms, most of the work has concentrated on the fruit fly and the higher mammals, which leaves enormous gaps in the record. The Ruddle group is now looking at the homeobox genes in the wormlike amphioxus, the most primitive chordate, and the lamprey, which is more complex than amphioxus, but less so than mammals.

Meanwhile, preliminary evidence in support of the idea that more complex species have a greater number of homeobox genes comes from Peter Holland of Oxford University in England. The homeobox gene Holland is analyzing does not belong to one of the four main complexes, but he has found that advanced vertebrates, including the mouse and the zebrafish, have three forms of the gene, whereas the fruit fly and an ascidian have only one. By comparing the sequences of the genes, Holland says, “you can tentatively conclude that there’s been a duplication of that gene about the time of origin of the vertebrates.”

Nevertheless, researchers clearly have a long way to go in documenting the role of the homeobox genes in evolution. One question they want to answer concerns how the structural organization of the gene clusters has been maintained so rigorously over millions of years. The leading view at present is that the regulation of all the genes in a cluster has to be closely coordinated. Krumlauf’s group has evidence, for example, that the regulatory elements of one gene may overlap with those of another. If that’s the case, then if the cluster were disrupted, the whole system might break down. Hence only organisms in which the genes maintained the correct order could survive.

And to uncover the actual mechanisms by which the homeobox genes might have contributed to evolution, researchers will have to try to correlate variations in the genes and their patterns of expression with the developmental changes that give rise to different body structures. Achieving that goal will require a great deal of work, but it’s at least getting under way. In one of the early examples, Michael Akam’s group at the Wellcome/CRC Institute of Cancer and Developmental Biology in Cambridge, England, is comparing the expression patterns of various homeobox genes in the fruit fly and in another insect, the locust *Schistocerca gregaria*. “We wanted to see if we can relate evolutionary diversity to the genes or whether they are irrelevant,” Akam explains. In preliminary work, the researchers have seen subtle differences in the expression patterns of some of the genes in the two species, which might account for a structural difference in their last abdominal segments.

Such findings are only the earliest indications that biologists will be able to find homeobox differences that can explain why two species’ embryos develop in different ways—and hence, ultimately, why those species diverged in evolution. While the final marriage of developmental biology and evolutionary theory is clearly some way off, perhaps one day it will produce an offspring that can explain, in satisfying molecular detail, how new species evolved. ■ JEAN MARX

ADDITIONAL READING

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Jawing With Our Georgian Ancestors

Not only are old cold war secrets being unearthed in former Soviet republics these days, but the citizens of these new nations also are digging up other surprising relics from their (very ancient) past. Word is just now spreading through the scientific world of a startling new discovery in the republic of Georgia, where archeologists began excavating the cellar of a ruined medieval house last summer and came upon a lower jawbone. What’s surprising is its age—it may be the earliest hominid remnant ever found in Europe and possibly the earliest anywhere outside Africa. If the oldest preliminary date for the jawbone—1.6 million years—holds up, the find could throw new light on how and when early hominids migrated outward from Africa.

Although rumors of a major new find had been circulating in the archeological community for months, the discovery was made public only last month at a meeting on the human ancestor *Homo erectus* in Frankfurt, held to commemorate the 100th anniversary of the discovery of the million-year-old Asian find called Java Man. At a workshop, Leo Gabunia of the Georgian Academy of Sciences produced the mandible, which he kept in a tobacco tin.

Those who got a good look say it is a remarkably complete mandible that is clearly a hominid in origin and archaic. “It’s not just a recent Georgian who fell into a pit,” says Philip Rightmire, an anthropologist at the State University of New York at Binghamton, who is an expert on *Homo erectus*. Adds Christopher Stringer, head of the human origins section at the Natural History Museum in London, “It is a very significant find.”

The large and heavy mandible, with all 16 teeth still in place, was embedded in the foundation of a house in the long-deserted city of Dmanisi, along with archaic stone tools, the skulls of two saber-toothed tigers, and the rib of an elephant. A team of Georgian and German scientists, led by Vachtang Dzarparidce of the Georgian Academy of Sciences and Gerhard Bosinki of the University of Cologne, has dated the surrounding sediments and animal remains, and they claim the mandible is from a *Homo erectus* who lived 900,000 or 1.6 million years ago—but not in between.

The reason the German-Georgian team argues that the jawbone is unlikely to be of an intermediate age is that their dating method relied on measuring the orientation of the earth’s magnetic field in a lava flow underneath the jawbone. As the lava cooled, metallic particles in it “locked in” the orientation of the earth’s magnetic field, which reverses over long periods. The particles’ orientation fits a date of either 900,000 or 1.6 million years, but not the intervening period—when the magnetic field was reversed.

Either date could make the new find of great significance, because of the scarcity of early hominid remains outside Africa. Although Java Man is a million years old, the oldest accepted hominid in Europe—the Mauer mandible—is only a half-million years old. But million-year-old stone tools have been found in France, and paleoanthropologists have long wondered who made them and what relation they had to humanity’s African origins.

If the 900,000-year-old date for the Georgian jaw holds up, it would help answer the question of who made the ancient European tools. If the earlier date of 1.6 million years turns out to be correct, the jaw would throw light on even deeper questions—providing support for those who believe *Homo erectus* began migrating northward out of Africa not long after it evolved as a rival for an earlier human predecessor, *Homo habilis*, 2 million years ago. The older date, however, remains controversial—in part because the data from the German-Georgian team have not yet been published. Until they are, everything about the new find will no doubt be the subject of heated debate in the world of paleoanthropology. ■ ANN GIBBONS