The Molecular Architects of Body Design

Putting a human gene into a fly may sound like the basis for a science fiction film, but it demonstrates that nearly identical molecular mechanisms define body shapes in all animals

by William McGinnis and Michael Kuziora

All animals develop from a single fertilized egg cell that goes through many rounds of division, often yielding millions of embryonic cells. In a dazzling and still mysterious feat of self-organization, these cells arrange themselves into a complete organism, in which bone, muscle, brain and skin integrate into a harmonious whole. The fundamental process is constant, but the results are not: humans, mice, flies and worms represent a wide range of body designs.

Noting that variation, biologists have often supposed that the molecular architects of body form—the genetic processes that control embryonic development in different species—would also be quite diverse. There is compelling evidence, however, that an interrelated group of genes, called *HOM* genes in invertebrates and *Hox* genes in vertebrates, governs similar aspects of body design in all animal embryos.

In at least some of the molecular systems that mold our form, we humans may be much more similar to our far distant worm and insect relatives than we might like to think. So similar, in fact, that—as our work has shown—curious experimenters can use some human and mouse *Hox* genes to guide the development of fruit-fly embryos.

The story of these universal molecular architects actually begins with the pioneering genetic studies of Edward B. Lewis of the California Institute of Technology. Lewis has spent much of the past 40 years studying the *bithorax* complex, a small cluster of homeotic genes in the fruit fly *Drosophila melanogaster*. The Greek word *homeo* means "alike," and the fly homeotic genes are so named because of their ability, when mutated, to transform one body segment of the fruit fly into the likeness of another. Mutations in *bithorax* complex genes usually cause such developmental defects in the posterior half of the fly body plan. Thomas C. Kaufman of Indiana University and his colleagues have discovered and studied a second cluster of fly homeotic genes, the *Antennapedia* complex (named for the founder gene of the complex, *Antennapedia*). Mutations in these genes usually cause homeotic defects in the anterior half of the fly body plan.

It is often the case in biology that bizarre defects in odd organisms contain the clues to solving important problems, and few biological phenomena are more bizarre than the disruptions in body design caused by homeotic mutations. For example, some mutations in the *Antennapedia* gene can cause the antennae on the head of the fruit fly to be transformed into an extra pair of thoracic legs. Surprisingly, some of the animals that develop the extra legs survive, feed and even mate with normal flies.

Antennapedia adults are rare exceptions, because most mutations in homeotic genes cause fatal birth defects in *Drosophila*. Nevertheless, even those dying embryos can be quite instructive. For instance, Ernesto Sanchez-Herrero and Gines Morata of the Independent University of Madrid found that elimination of three genes in the *bithorax* complex—*Ultrabithorax, abdominal-A* and *Abdominal-B*—is lethal. Yet such mutant embryos survive long enough to develop specialized structures that indicate all eight abdominal segments are replaced by thoracic segments. Most people would be unnerved by analogous birth defects in mammals, but these grotesque defects in flies can be observed with equanimity.

From his original genetic studies of the *bithorax* complex genes, Lewis derived two key insights. The first was that the normal function of these homeotic genes is to assign distinct spatial (or positional) identities to cells in different regions along the fly's anterior-posterior axis. That is, they "tell" cells that they are part of the fly's head or thorax or abdomen. These identities are to some extent abstract, in that the positional coordinates assigned by homeotic genes are interpreted in dissimilar ways in different developmental settings. *Antennapedia* assigns thoracic identity during both the embryonic and pupal stages of the fly's life cycle, even though the structures (sense organs, legs, wings and so on) that develop along the thorax differ in larvae and adults.

Lewis's second important insight was that the linear order of the *bithorax* complex genes on the fruit fly's chromosome exactly paralleled the order of the body regions they specified along the embryo's anterior-posterior axis.

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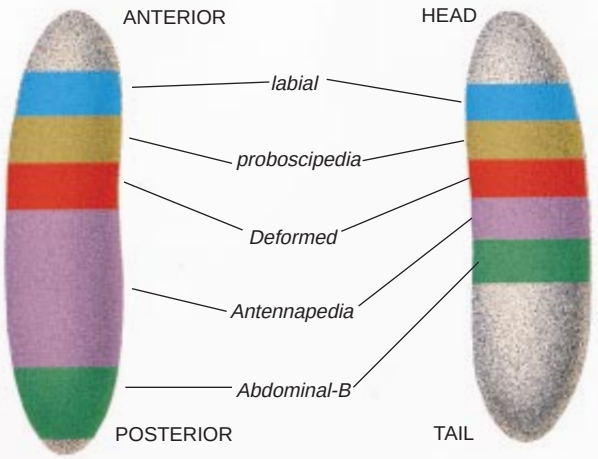
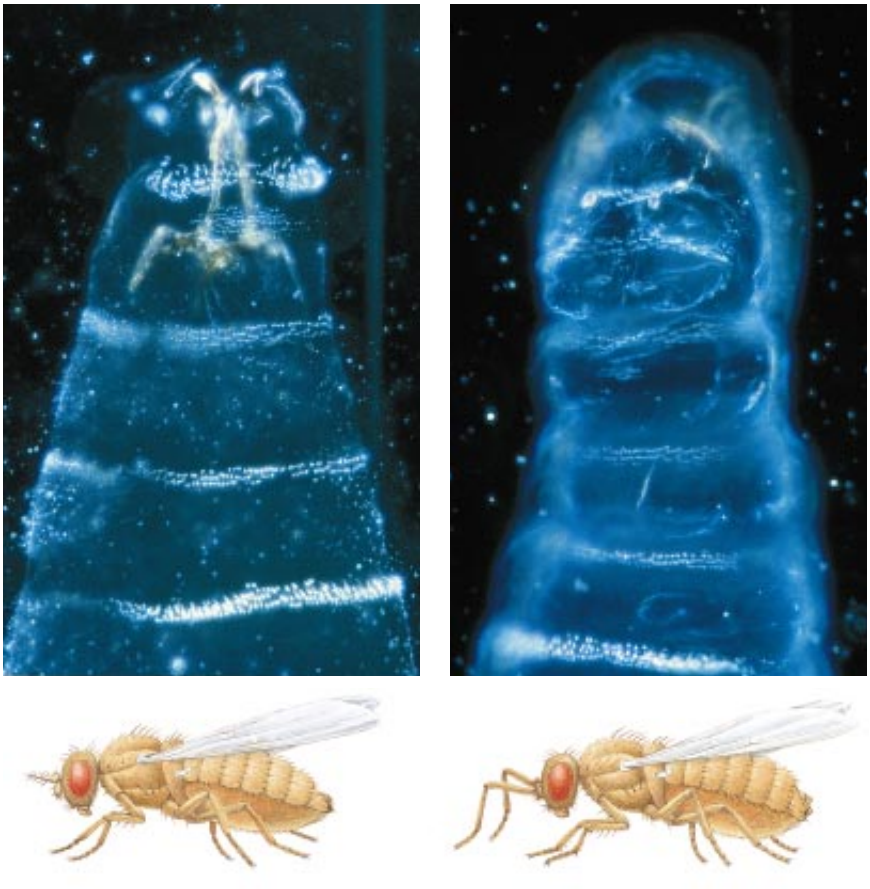


EMBRYOS of vertebrate animals as diverse as fish, salamanders, birds, rabbits and humans show great similarities early in their development. *Drosophila* fruit flies and other invertebrates develop along a very different path, yet at the earliest stages they and the vertebrates share a common pattern of expression of the so-called homeobox genes. That discovery reveals that despite the differences in the final appearance of the animals, they use closely related genes to specify parts of the body along the anterior-posterior (or head-tail) axis.

DROSOPHILA FISH SALAMANDER CHICKEN RABBIT HUMAN



NORMAL FLY

Antennapedia MUTANT FLY

HOMEOTIC TRANSFORMATIONS, in which body parts develop in the wrong positions, occur in fruit flies that have mutations in their homeobox genes. Mutations of the *Antennapedia* gene, for example, can cause belts of thoracic denticles (spikes) to appear on the heads of larvae (*top right*). Another developmental consequence of the mutation is that the mutant adults have legs growing in place of antennae (*bottom right*). A normal larva and adult are shown at the left.

The same relation also holds for the genes of the *Antennapedia* complex. United by these shared characteristics, the genes in the *bithorax* and *Antennapedia* groupings are collectively referred to as the *HOM* complex.

A partial understanding of how the *HOM* complex genes determine axial positions in the fruit-fly body plan can come from looking at where those genes are active in embryos. The *HOM* genes are present in the DNA of all of a fly's cells but are active only in some of them. When activated, the *HOM* complex genes are copied as molecules of messenger RNA, which serve as templates for the synthesis of HOM proteins. During early developmental stages, before regions of the embryo show any signs of their eventual fates, the different *HOM* complex genes are activated in successive stripes of cells along the anterior-posterior axis. Some of these stripes of activation overlap, but each *HOM* complex gene has a unique anterior boundary of activation in the body plan.

If deletion of a gene or some similar incident interferes with the expression of a HOM protein, then embryonic cells that normally contain high levels of that protein often undergo a homeotic transformation. That transformation occurs because of a backup *HOM* gene that is already active in the same cells and that can substitute its own positional information. For instance, if the function of the *Ultrabithorax* gene is eliminated from cells within a fly's anterior abdominal region, *Antennapedia* will take over the development of that region. As a result, structures normally associated only with the thorax (which *Antennapedia* helps to specify) also appear more posteriorly.

Homeotic transformations can also result from mutations that cause a homeotic gene to become active in an inappropriate position. The *Antennapedia* mutations in adult flies are caused by activity of *Antennapedia* in the head, where that gene is normally turned off. In summary, the genetic evidence indicates that each *HOM* complex gene is needed to specify the developmental fate of cells in a certain position on the anterior-posterior axis: the posterior head, anterior thorax and so on. More important (and more instructive about their biological function), the activity of *HOM* complex genes is apparently sufficient to determine the fate of at least some cells, even when those cells would not normally fall under a given gene's influence.

The genes of the *HOM* complex are virtually the only ones in *Drosophila* that have those properties. They also share an interesting resemblance at the structural level because all of them are members of the homeobox gene family. Homeoboxes are DNA sequences that carry the descriptions for making a related group of protein regions, all about 60–amino acid residues in size, called homeodomains. The *homeo-* prefix in the name of these domains stems from their initial discovery in *Drosophila* HOM proteins. Since then, however, homeodomains have been found in many other proteins with varying degrees of similarity. The homeodomains of the *Drosophila* HOM proteins are especially similar to one another, which suggests they are closely related. For that reason, they are often referred to as *Antennapedia*-class homeodomains.

What do these HOM proteins do at the biochemical level? Only a superficial answer can be given at present. They belong to a large group of proteins whose function is to bind to DNA in the regulatory elements of genes. The right combination of these bound proteins on a DNA regulatory element will signal the activation or repression of a gene—that is, to start or stop making that gene's encoded protein. Investigators have shown that the homeodomain region of the HOM proteins is the part that directly interacts with the DNA binding sites.

We are fascinated by the contrast between the structural similarity of the HOM proteins and their varied, specific effects. Here is a family of proteins that all bind to DNA and are presumably derived from a single ancestral Antennapedia-class protein. Yet their roles in development are remarkably diverse: one protein assigns cells to become parts of the head, another assigns cells to become thorax and so on. It seems likely that HOM proteins designate var-

ious positions along the anterior-posterior axis by regulating the expression of what may be large groups of subordinate genes. The functional specificity of the HOM proteins can therefore be defined by the differences between them that allow them selectively to regulate certain genes in embryos.

To learn more about this specificity, we decided in 1986 to construct chimeric HOM proteins that had components derived from different sources. (The chimera, a monster of Greek mythology, was part lion, part goat and part snake.) By testing the function of these chimeric proteins, we thought it would be possible to define which subregions of the HOM proteins determined their selective regulatory abilities.

For the subjects of our first experiments, we chose the HOM proteins Deformed, Ultrabithorax and Abdominal-B. These proteins have structurally similar homeodomains: that of the Deformed protein is identical to that of Ultrabithorax protein at 44 of its 66 amino acids—but they share no extensive resemblance in other regions. Each of these proteins also exerts an influence on other genes in the HOM family. Thus, the Deformed protein selectively activates the expression of its own gene; Ultrabithorax protein represses the expression of the *Antennapedia* gene; and Abdominal-B protein regulates its own gene and those of others in the *HOM* complex, including *Antennapedia, Ultrabithorax* and *abdominal-A*. We knew we could use these auto- and cross-regulatory relationships in tests of the specific functions of chimeric HOM proteins.

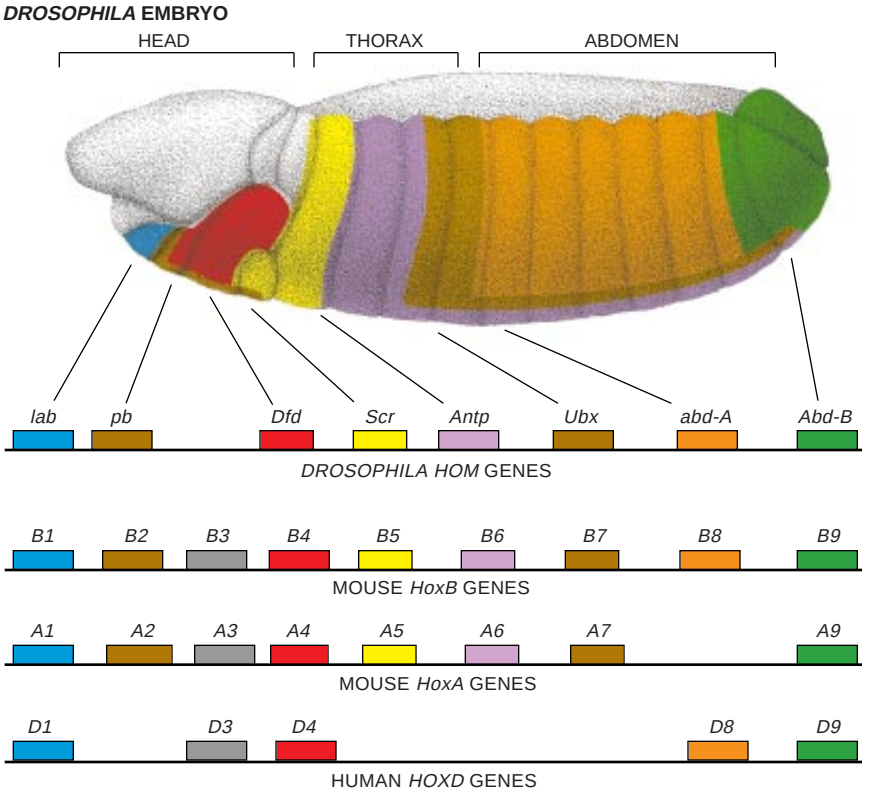
The first challenge was to create genes that would make the chimeric homeotic proteins we desired. Recombinant DNA techniques make that feat possible through the splicing of bits and pieces of genes at the DNA level. If the gene engineering is done with care, protein domains can be very precisely moved from one protein to another while retaining their functional characteristics. We then had to make sure that the chimeric genes would be active in all embryonic tissues. We therefore used a method worked out a few years previously by Gary Struhl, now at Columbia University, that involves attaching the gene to regulatory DNA sequences that can be activated by a mild heat shock. Finally, we inserted our heat-inducible *HOM* gene chimeras into *Drosophila* chromosomes by a technique called P-element transformation.

The *Drosophila* flies that we transformed in this way thereafter carried the chimeric genes in every cell of their body, and those genes would produce chimeric proteins at any stage of development if we simply raised the temperature of the flies' growth chamber to 37 degrees Celsius for a brief period. (*Drosophila* prefer to live at 25 degrees C but can tolerate 37 degrees C for an hour or two with no ill effects.) Using these animals, we could assay the ability of the chimeric proteins to act on the regulatory elements of target genes in their normal chromosomal positions and in their natural embryonic environment—a demanding test that closely mimics the usual conditions under which these proteins operate.

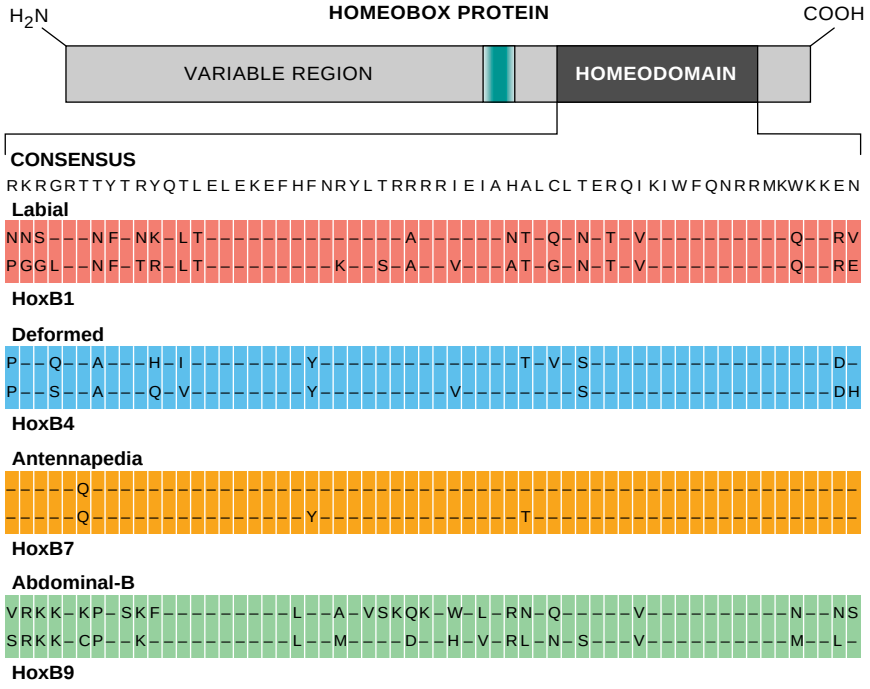
Because HOM proteins have highly similar homeodomains, they bind to nearly identical DNA sites when tested in the laboratory. It therefore initially seemed likely that the features giving each protein its functional specificity would be found outside the homeodomain—in the parts of the proteins that were most individual. Yet as often happens when simple deductive reasoning is applied to biological problems, that expectation was wrong.

We found that if we removed the native homeodomain from a Deformed protein and put an Ultrabithorax homeodomain in its place, the chimeric protein lost the ability to regulate *Deformed* gene expression in embryos. Instead the new protein acted on the expression of the *Antennapedia* gene—much as a normal Ultrabithorax protein would. By transferring the Ultrabithorax homeodomain to Deformed, we had apparently also transferred its selective regulatory abilities. Another homeodomain swap experiment gave us similar results. A Deformed protein carrying an Abdominal-B homeodomain instead of its own mimicked the regulatory specificity of an Abdominal-B protein.

The chimeric proteins did not behave exactly like the protein from which their homeodomain was derived. Both the Deformed/Ultrabithorax chimera and the Deformed/Abdominal-B chimera activated expression of their target genes, whereas the normal Ultrabithorax and Abdominal-B proteins repressed expression of the same genes. Presumably, regions of the Deformed protein outside the homeodomain region supply a strong activation function that can work with any of these HOM homeodomains. Consistent with



HOMEOBOX GENE COMPLEXES have been identified in both invertebrates and vertebrates. *Drosophila* have *HOM* genes, which occupy the same order on the fly chromosome as the anterior-to-posterior order of body regions whose development they control. Mice and humans have *Hox* genes, which are closely related to members of the *HOM* complex and show the same spatial and functional arrangement.



HOMEODOMAINS are the highly similar 60–amino acid regions of the proteins made by all homeobox genes. Each letter in the consensus string represents an amino acid; deviations from that consensus are shown for several closely related HOM and Hox proteins.

this notion, Deformed does have a few regions of protein sequence that are rich in the types of amino acids characteristic of "activation domains" in other gene regulatory proteins.

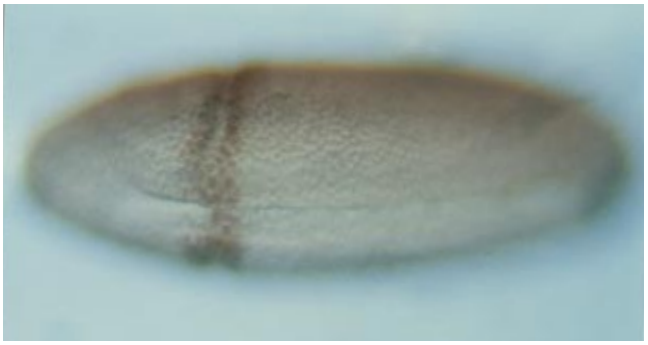
Similar experiments on the functional specificity of HOM proteins have also been carried out by Richard Mann and David S. Hogness of Stanford University and by Greg Gibson and Walter J. Gehring and their colleagues at the University of Basel. Their experiments were based on evaluations of the homeotic transformations that mutant and chimeric HOM proteins induced in developing flies. Because they were looking at the developmental effects of the HOM proteins rather than just at their effects on gene expression, those investigators were using a more demanding measure of HOM protein function than the one we applied. Yet their results, too, support the idea that much (though not all) of the functional specificity of the HOM proteins resides in the small differences within or immediately adjacent to the homeodomain regions.

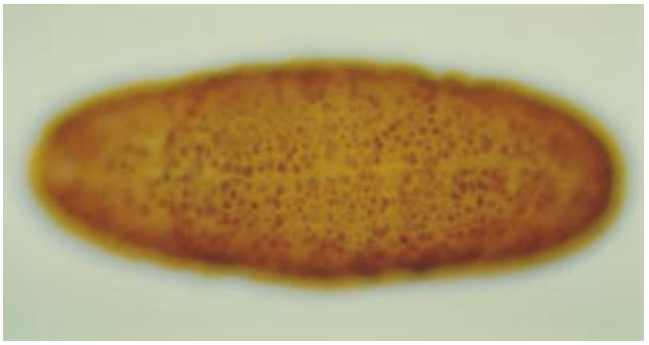
To us, all those findings also suggested that certain long-shot experiments already ongoing in our laboratory, for which we had only faint hopes of success, actually had a chance of yielding interpretable results. Those experiments involved functional assays of mouse and human homeodomain proteins in *Drosophila* embryos. To convey the significance of those tests, we need to review what is known about the mammalian *Hox* genes.

During the past nine years, genes that contain *Antennapedia*-class homeoboxes have been found in the chromosomes of many animal species besides *Drosophila*. Such genes have been carefully studied in frogs, mice and humans, where they are called *Hox* (short for "homeobox") genes. In both mice and humans, *Hox* genes cluster into four large complexes that reside on different chromosomes. In their organization and patterns of embryonic expression, the genes of the *Hox* complexes share intriguing likenesses to the genes of the fly *HOM* complex. For example, one can identify *Hox* genes that structurally resemble the *HOM* genes *labial, proboscipedia, Deformed, Antennapedia* and *Abdominal-B.* The equivalent *Hox* and *HOM* genes are arranged in the same linear order within their respective complexes.

A further parallel has been observed both by Denis Duboule of the University of Geneva and Pascal Dollé at the CNRS Laboratory of Eukaryotic Molecular Genetics in Strasbourg and by Robb Krumlauf and his colleagues at the National Institute for Medical Research in London. They have assembled convincing evidence that the patterns of expression for the two types of genes are alike. That is, the *Hox* genes are activated along the head-tail axis of the early mouse embryo in the same relative order that the *HOM* genes are activated on the anterior-posterior axis of *Drosophila*.

Structural similarities between the mouse and fly proteins are mainly limited to the homeodomain regions. Fly Antennapedia and mouse HoxB6 are nearly identical in the amino acid sequence of their respective homeodo-





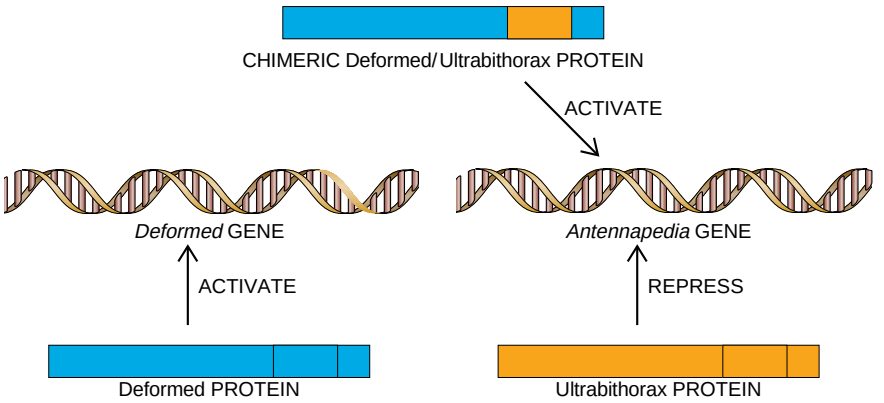
EXPRESSION of the *Deformed* gene in fly embryos, as revealed by a brown dye, is normally confined to a band of cells that become posterior head structures (*left*). Genetically engineered embryos that carry heat-inducible *Deformed* genes, however, will produce the Deformed protein in every cell of their body after a brief exposure to heat (*right*). The developmental abnormalities found in such embryos can be used to infer *HOM* gene function.

mains (they differ at only four of 61 positions), which means that these two proteins resemble each other more than Antennapedia does any other fly HOM protein. In an evolutionary sense, this information argues that *HoxB6* and *Antennapedia* are structural homologues—that is, they descended from a common ancestral gene different from the one that gave rise to, say, *Abdominal-B* or *Deformed.*

By the same reasoning, the similarity between the entire *HOM* complex and the *Hox* complexes argues that the most recent common ancestor of *Drosophila*, mice and humans—a wormlike creature that lived about 700 million years ago, give or take a few hundred million years—had a protocomplex of *Antennapedia*-class homeobox genes. The exact type and arrangement of genes in that complex remain a mystery. Nevertheless, we can be confident, using the modern *HOM* and *Hox* complexes as guides, that the ancient protocomplex contained structural homologues of *labial, proboscipedia, Deformed, Antennapedia* and *Abdominal-B.* This overall view of *HOM* and *Hox* gene evolution is strongly supported by research on beetle homeotic genes by Richard W. Beeman of the U.S. Department of Agriculture and Rob E. Denell of Kansas State University and by recent reports from many laboratories that the primitive roundworm *Caenorhabditis elegans* also has a *HOM* complex distantly but recognizably related to the *Drosophila HOM* and vertebrate *Hox* complexes.

All this structural evidence, though suggestive, still does not directly tell us whether HOM and Hox proteins do serve the same developmental function in embryos. After all, the mouse and fly gene complexes have been in different evolutionary lineages for hundreds of millions of years, with plenty of time to evolve new or divergent abilities. So the similarities in structure and expression might be historical quirks and not trustworthy indicators of functional resemblance between present-day HOM and Hox proteins.

One approach to the problem is to explore the biological effects of *Hox* genes in vertebrate embryos and to compare them with what is known about the effects of *HOM* genes in invertebrates. For instance, does the inappropriate activation or specific inhibition of *Hox* gene function during mouse development cause homeotic transformations? In one effort to answer this question, Peter Gruss and his colleagues at the Max Planck Institute for Biophysical Chemistry in Göttingen created strains of mice whose embryos produce HoxA7 protein in the head and anterior cervical region. Normally, HoxA7 protein (which is similar to the Antennapedia and Ultrabithorax proteins of the *HOM* complex) is most abundant in the posterior cervical and anterior thoracic regions and is excluded from more anterior parts. Some mice in which *HoxA7* is expressed inappropriately develop deformities of the ear and palate and occasionally have homeotic transformations of the cervical vertebrae.



GENETIC TARGETS of homeodomain proteins are largely determined by the homeodomain regions of those proteins. For example, a chimeric Deformed protein carrying an Ultrabithorax homeodomain acts on the same genes as does Ultrabithorax. Yet the regulatory effect of the chimera—activation—is more like that of Deformed because of protein regions outside the homeodomain.

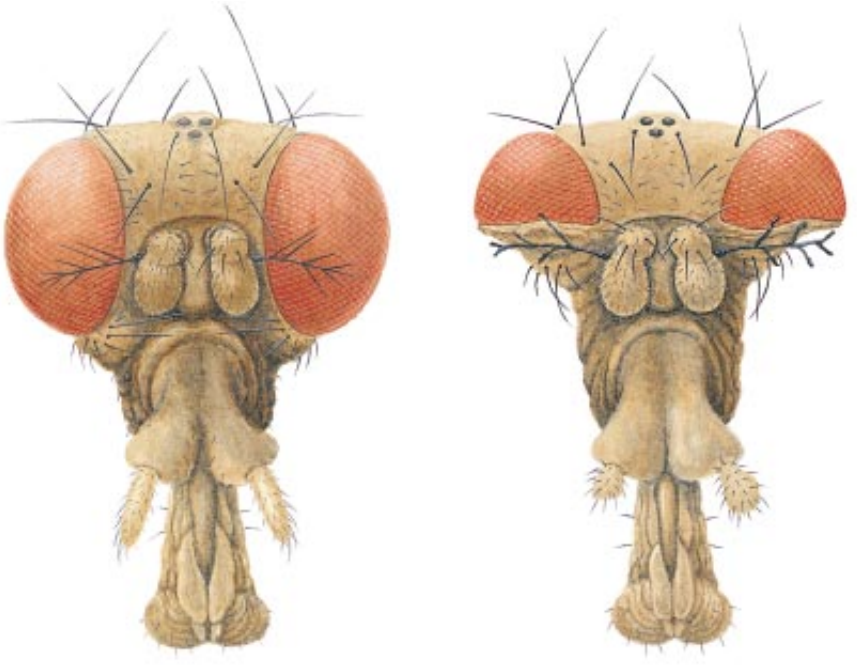
The difficult converse experiment—knocking out *Hox* gene function—has been accomplished for *HoxA3* by Osamu Chisaka and Mario R. Capecchi of the University of Utah and for *HoxA1* by Thomas Lufkin and Pierre Chambon and their collaborators at CNRS in Strasbourg. Their work has shown that some structures in the anterior regions of mouse embryos do depend on those genes. Mutation of the *HoxA3* gene results in mice that die just after birth with a complicated set of head and neck deformities, including abnormally shaped bones in the inner ear and face and the absence of a thymus.

Such deformities are reminiscent of a human congenital disorder called DiGeorge's syndrome, raising hopes that the study of *HOM* and *Hox* genes will be of practical benefit in explaining some human birth defects. Much more research needs to be done before biologists have a good understanding of how *Hox* genes participate in the developmental design of mice and humans, but these and other initial experiments certainly suggest that the *Hox* and *HOM* genes serve comparable purposes.

In our own work, we have tried to make a direct comparison by testing whether Hox proteins can take the place of HOM proteins in developing *Drosophila* embryos. Ideally, one would accomplish this swap by completely replacing the *HOM* gene of a fly with its *Hox* homologue; the *Hox* gene might then be expressed only when and where the *HOM* gene would be ordinarily. Unfortunately, such an experiment is not yet feasible, because the genes in their entirety are too big to be manipulated by current technology. Still, we could do the next-best possible thing: by using *Hox* DNA sequences linked to heat-inducible regulatory elements, we could make all the cells of a developing fly express a Hox protein.

The first protein that we and our laboratory colleague Nadine McGinnis tested in this way was the human HOXD4 protein, the equivalent of a mouse HoxD4. (When referring specifically to human genes, the *HOX* label is capitalized to conform with standard genetic nomenclature.) The gene for this human protein, which has a homeodomain like that of the fly Deformed protein, was isolated and characterized in 1986 by Fulvio Mavilio and Edoardo Boncinelli and their colleagues at the Institute for Genetics and Biophysics in Naples.

In *Drosophila,* when the *Deformed* gene is expressed outside its normal anterior-posterior limits, the adult flies suffer a variety of head abnormalities, such as the absence of a ventral eye. We were amazed to find that the human HOXD4 protein, when expressed in developing fly cells, caused the same deformities. We could not attribute these changes entirely to the human protein, however: our experiments indicated that the human protein was



NORMAL FLY *Deformed* MUTANT FLY

***DEFORMED* MUTANT FLIES have a variety of head abnormalities, including the absence of the lower half of the compound eyes. The same abnormalities can be induced by making the immature flies express the human HOXD4 protein. This human protein resembles the Deformed protein and seems to have a similar function.**

promoting the expression of the fly's *Deformed* gene as well. (Remember that one normal effect of the Deformed protein is that it activates its own gene, in a cycle of positive feedback.) The human HOXD4 protein was therefore mimicking the effects of inappropriate *Deformed* expression because—at least in part—it *was* causing inappropriate *Deformed* expression. Nevertheless, we could see that *HOXD4* did act like a weak but specific replica of its *Drosophila* homologue.

Encouraged by this result, Jarema Malicki, a graduate student in our laboratory, tested the function of the mouse HoxB6 protein in developing flies. HoxB6, which Klaus Schughart and Frank H. Ruddle identified and characterized a few floors away from us at Yale University, has a homeodomain that is highly similar to Antennapedia protein. The effects of HoxB6 protein expression in developing fly cells was spectacular and unmistakably homeotic. In *Drosophila* larvae the HoxB6 protein caused much of the head region to develop as if it were thoracic: instead of a larval head skeleton, the transformed flies produced denticle belts, rows of spikes that are usually arranged on the bellies of *Drosophila*. In *Drosophila* adults, HoxB6 caused a homeotic transformation of the antennae into thoracic legs. Both the larval and adult homeotic transformations were much like those caused by the inappropriate expression of Antennapedia protein throughout the body.

What can one make of these evolutionary swap experiments? First of all, they reinforced our conclusions that the homeodomains themselves determined much of the regulatory specificity of the proteins: the homologous fly and vertebrate proteins have little in common outside the homeodomain region. In addition, the experiments suggested that from a functional standpoint, the homologous proteins are at least somewhat interchangeable and have similar "meanings" for early embryos. The system for determining anterior-posterior axial positions has evidently changed little in the past 700 million years.

If one were to imagine the complicated network of interactions between gene regulatory proteins inside an organism as a jigsaw puzzle, then the homologous fly and mammal proteins are pieces that can fit in the same places. Looking at the *HOM/Hox* system in this way also highlights how much we still have to learn: the other puzzle pieces that enable the HOM and Hox proteins to regulate genes and to have a specific function have yet to be identified.

In a way, these experiments also hark back to the classical observations of Karl Ernst von Baer, who in the 1820s concluded that if one examined early embryonic morphologies, all vertebrate forms seemed to converge toward a common design. The story, which sounds too good to be true, is that von Baer came to this epiphany after the labels fell off some of his bottled specimens of early embryos, and he realized with some chagrin that he could not be sure whether the embryos were lizards, birds or mammals. The structure and function of the *HOM* and *Hox* gene systems suggest that this developmental convergence embraces the early development of a great many animal species. But only at the level of molecular pattern can the developmental convergence of such different embryos be "seen."

Sometime between 600 million and a billion years ago, the *HOM/Hox* system evolved; it has proved so useful that many animals have since relied on its fundamental abilities to determine axial position during development. Is it the only developmental genetic system that has been so conserved? That seems unlikely. Researchers have found hints that some other regulatory genes in flies and mice are highly similar in structure and are activated in the same or homologous tissues. Exploring the functions of those novel genes, and how they interact with the *HOM/Hox* system, promises to reveal many more fascinating insights into the evolution and mechanism of the ancient genetic systems that serve as the molecular architects of animal body plans.

FURTHER READING

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