The Continuing Saga of "Homeo-Madness"

Research over the past 2 years continues to point to an important role in development for the homeo box, although the proof is not yet conclusive

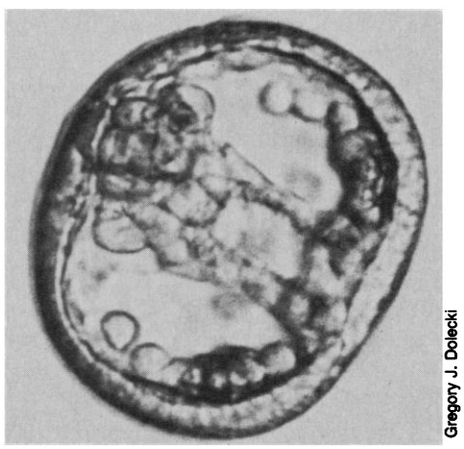
THE discovery 2 years ago of the homeo box engendered an intense interest—one researcher calls it "homeo-madness"—on the part of developmental biologists. The hope was that the homeo box, a highly conserved DNA sequence that is attached to several developmentally important genes in the fruit fly *Drosophila melanogaster*, would provide the key for unlocking the mysteries of development.

Its discovery might help explain how the fruit-fly genes work to guide the formation of the complex, segmented body of that organism. Moreover, investigators soon detected homeo boxes in several additional species, including the mouse and man. Far fewer genes for developmental control have been identified in these mammalian species than in the fruit fly, which is much more amenable to genetic study. But that situation might change if the homeo box could be used as a tag for identifying such genes in the higher species.

A great deal of research during the intervening years has been aimed at confirming the proposed function of the homeo box in *Drosophila* development and determining whether the sequence acts in a comparable fashion in the other organisms. The results continue to be encouraging. "Everything is consistent with homeo boxes having a developmental role," says Frank Ruddle of Yale University, "although that has not yet been proven." That role may be broader than originally envisioned, however.

Homeo boxes were originally discovered by Matthew Scott and Amy Weiner, who then worked in Thomas Kaufman's laboratory at Indiana University, and independently by Walter Gehring and his colleagues at the University of Basel, Switzerland, in some half dozen genes in the *Drosophila* Antennapedia and bithorax complexes. All but one of the genes in question are of the type called "homeotic" (thus the name homeo box) that specify the identities of various segments of the fruit-fly body. The remaining gene, the *fushi tarazu* (*ftz*) gene, is not a classic homeotic gene but is involved in determining segment number.

The association of the homeo boxes with genes that specify fruit-fly segmentation patterns led to speculation that the sequence might act to control segment development, even in mammals. More recent results indicate that there may not be a strict association between *Drosophila* homeo boxes and segmentation genes. The number of homeo boxes found in that organism is now approaching 20, estimates Michael Levine of Columbia University. Some of the newly identified sequences are also linked to segmentation genes. This is true, for example, of the homeo box of the *engrailed* gene, which was identified by Gehring's group and by that of Thomas Kornberg at the University of California in San Francisco, although the nucleotide sequence of the *engrailed* homeo box and those of the Antennapedia-bithorax homeo boxes are different enough to be considered members of different structural classes.



Gregory J. Dolecki

Hawaiian sea urchin embryo

Do homeo box–containing genes also guide the development of this nonsegmented organism?

However, Levine and his colleagues have identified two homeo box–containing genes that may not be involved in specifying segmentation. They are expressed near the anterior or posterior ends of fruit-fly embryos in regions that do not appear to be segmented. Moreover, Thomas Humphreys and his colleagues at the University of Hawaii have cloned a homeo box from the Hawaiian sea urchin *Tripneustes gratilla* and find that the sequence is expressed in the embyro. "There is no evidence that the sea urchin is segmented," Humphrey says. "The homeo box may be doing something more basic than guiding segmentation." Levine suggests its primary function is to specify positional identity along the anterior-posterior axis of developing embryos.

The past 2 years have also seen an expansion in the number of human and mouse homeo boxes, bringing the current total to about 15, including sequences of the Antennapedia-bithorax and *engrailed* types. The big question concerns whether the sequences contribute to pattern formation in the higher organisms as they apparently do in *Drosophila*. "That is asking a lot. There are a billion years of divergent evolution between *Drosophila* and man," Ruddle notes. Nevertheless, although the evidence so far is indirect, it is at least consistent with the proposition that the mammalian and fruit-fly homeo boxes play similar roles.

Investigators have found that the structures and organization of the mammalian homeo boxes resemble those of the *Drosophila* homeo boxes. They all contain 180 base pairs, and their structures have been highly conserved; homeo boxes of the same type, but from different species, specify polypeptides that are as much as 90% identical in their amino acid sequences.

In addition, mapping studies, which are being carried out in several laboratories, show that the mammalian homeo boxes tend to be arrayed on chromosomes in clusters just as those of *Drosophila* are. For example, mouse chromosomes 6 and 11 both have groups of four to six homeo boxes of the Antennapedia-bithorax type. Moreover, two groups, one including Ruddle, Levine, and William McGinnis, who is also at Yale, and the other consisting of Gail Martin, Robert Tjian, and their colleagues at the University of California in San Francisco, have identified a homeo box cluster on human chromosome 17, which appears to be equivalent to the one on mouse chromosome 11.

Almost nothing is known about the genes with which the mammalian homeo boxes might be associated. Some mouse developmental mutations map to chromosomal locations near homeo boxes, but a direct association between any of the mutant genes and the homeo boxes has not yet been demonstrated. Nor has comparison of the DNA sequences surrounding the mammalian and fruit-fly homeo boxes of the Antennapedia-bithorax type revealed any evidence of homology, which might indicate that

similar genes had been conserved in the two species. However, according to Martin, Alexandra Joyner, also of the University of California at San Francisco, and their colleagues, a potential coding sequence to the right of a mouse *engrailed*-type homeo box does show about 80% homology with the *Drosophila engrailed* gene, a result suggesting that this gene's function may have been conserved even over that billion years of divergent evolution.

Even if information about the functions of the mammalian homeo box genes is lacking, investigators have found that the genes are active in mouse embryos, an obvious requirement for developmentally important genes. Moreover, their expression may be regulated just as the expression of the fruit-fly homeo box genes is.

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A recent case in point comes from McGinnis, Ruddle, and their colleagues who have tracked the expression of a newly discovered mouse homeo box gene that is located on chromosome 15. Messenger RNA's (mRNA's) transcribed from the gene, which has a homeo box of the Antennapedia-bithorax type, could be detected in mouse embryos from the 13th day of gestation, the earliest time checked, into adulthood. Most interesting is the investigators' finding that the transcripts are located in the spinal cords in the upper thoracic and cervical regions of the embryos. Transcripts of the fruit-fly segmentation genes, including those of the Antennapedia and bithorax complexes, also concentrate in specific regions of the embryonic nerve cord.

Mutations in the fruit-fly genes usually affect only particular segments or portions of segments. Investigators have generally shown that mRNA's made by the genes tend to accumulate during the course of development in precisely those embryonic regions that are altered by the mutations. This is what McGinnis, Levine, and their colleagues found, for example, when they mapped the locations of the transcripts of three Antennapedia and three bithorax homeo box genes. The Antennapedia gene transcripts concentrated primarily in the second thoracic and three head segments. "The pattern of expression of the mouse gene is similar to those of the *Drosophila* genes," McGinnis says. "It looks very encouraging that the genes may have analogous functions in the mouse and fruit fly." The transcript distributions of most of the other mouse homeo box genes have not yet been determined, although Brigid Hogan of the National Institute for Medical Research in London and her colleagues have found high expression of one of them in the brain and spinal cords of mouse embryos.

Studies of the activity of homeo box-containing genes in human and mouse teratocarcinoma cells provide further support for the hypothesis that the genes may be involved in development. Teratocarcinoma cells are derived from early embryonic cells that have the capacity to differentiate into many different cell types when grown under appropriate culture conditions. Investigators, including Martin, Tjian, and their colleagues, and Peter Gruss of the University of Heidelberg (Germany) and his associates, have found that some of the genes are inactive before cells have been induced to differentiate, but are then turned on.

The role postulated for the proteins encoded by the homeo box genes in fruit-fly development, and presumably also in mammalian development, is one of regulating the expression of other genes, such as those that specify the formation of the features characteristic of the individual segments of the fruit-fly body. A wealth of evidence indicates that the *Drosophila* homeo box genes also regulate the expression of one another. This may account for the tendency for the genes to be expressed predominantly in specific segments as development progresses. "The data suggest some kind of cross-regulation, but don't prove that the homeo box is involved," Levine points out.

Nevertheless, gene regulation normally involves interaction of the regulatory proteins with specific DNA sequences on the target gene and the homeo box appears capable of mediating such interactions. Scott, who is now at the University of Colorado at Boulder, noted that the polypeptide encoded by the homeo box has the potential for assuming the helix-turn-helix structure that enables a number of known gene regulators to recognize and bind to the DNA of the genes under their control.

The proposal that the homeo box has a DNA recognition function has received some additional recent support from Patrick O'Farrell and his colleagues at the University of California in San Francisco. They demonstrated that the homeo box region of the *engrailed* protein has the ability to bind to specific DNA sequences. "It is the first direct evidence of DNA binding," O'Farrell explains, "and the protein is capable of distinguishing some sequences from others." Whether the binding to DNA has functional significance is currently unclear, but the O'Farrell group found that the homeo box–containing protein interacts with sequences near the start of the *engrailed* and *ftz* genes in regions that traditionally contain regulatory sites.

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The current homeo box research largely supports an earlier suggestion by Edward Lewis of the California Institute of Technology about the workings of the genes of the bithorax complex. Those genes (and also the ones in the Antennapedia complex) are lined up on the long arm of chromosome 3 in the same order as the segments they affect. Lewis proposed that normal development features a sequential activation of the bithorax genes along the anterior to posterior axis of the fruit-fly embryo. That is essentially what McGinnis, Levine, and their colleagues found when they examined the distribution of transcripts of the three homeo box–containing bithorax genes in early embryos. However, as already mentioned, as development proceeds the transcripts tend to become restricted mainly to the particular segments disrupted by mutations in the genes.

Although the homeo box research of the past 2 years may not have revealed "a smoking gun" that conclusively proves that the sequences have an important regulatory role in development, the evidence nonetheless points in that direction. Homeo-madness seems destined to continue. ■

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ADDITIONAL READING

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Also see the November 1985 issue of *Cell* for several papers on homeo box research, including a minireview by J. L. Manley and M. S. Levine on "The homeo box and mammalian development."