Perspectives

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A Century of Homeosis, A Decade of Homeoboxes

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ONE hundred years ago, while the science of genetics still existed only in the yellowing reprints of a recently deceased Moravian abbot, WILLIAM BATESON (1894) coined the term homeosis to define a class of biological variations in which one element of a segmentally repeated array of organismal structures is transformed toward the identity of another. After the rediscovery of MENDEL's genetic principles, BATESON and others (reviewed in BATESON 1909) realized that some examples of homeosis in floral organs and animal skeletons could be attributed to variation in genes. Soon thereafter, as the discipline of Drosophila genetics was born and was evolving into a formidable intellectual force enriching many biological subjects, it gradually became clear that fruit flies contained multiple "homeotic" genes (*e.g.*, *bithorax*, *aristapedia* and *proboscipedia*) (BRIDGES and MORGAN 1923; BALKASCHINA 1929; BRIDGES and DOBZHANSKY 1933), some of which appeared to be loosely clustered on the third chromosome. These genetic studies culminated in the systematic analyses of LEWIS (1978) and KAUFMAN *et al.* (1980), which provided preliminary definitions of the many homeotic genes of the Bithorax and Antennapedia complexes, and also showed that the mutant phenotypes for most of these genes could be traced back to patterning defects in the embryonic body plan.

Ten years ago, a sudden stream of papers (MCGINNIS *et al.*, 1984a,b,c; SCOTT and WEINER 1984; LAUGHON and SCOTT 1984; SHEPHERD *et al.* 1984; CARRASCO *et al.* 1984; LEVINE *et al.* 1984) introduced the homeobox to developmental genetics and sketched its basic outlines. In retrospect, each of these studies contained relatively few data for the impact they had. Putting the best face on it, one could claim they are reports of exemplary brevity. These reports defined homeoboxes as members of a highly conserved family of DNA sequences that appeared to be preferentially associated with homeotic and segmentation genes of Drosophila. Homeobox sequences were highly conserved in other animals, including mammals, and were proposed to encode DNA-binding homeodomains because of a faint resemblance to mating-type transcriptional regulatory proteins of budding yeast and an even fainter resemblance to bacterial helix-turn-helix transcriptional regulators.

The initial stream of papers was a prelude to a flood concerning homeobox genes and homeodomain proteins, a flood that has channeled into a steady river of homeo-publications, fed by many tributaries. A major reason for the continuing flow of studies is that many groups, working on disparate lines of research, have found themselves swept up in the currents when they found that their favorite protein contained one of the many subtypes of homeodomain. This was in part because the definition of what proteins belonged to the homeodomain family expanded to include proteins that had only marginal amounts of sequence similarity to the founder members in the Drosophila Antennapedia and Bithorax gene complexes. Many of the proteins that have homeodomains have nothing to do with BATESON's version of homeosis, although there is a loosely defined structural subgroup of homeodomains that is closely linked to homeotic genetic functions in animals.

The initial stream of reports immediately explained (or purported to explain) some of the burning questions concerning homeotic genes. They seemed to be a fairly closely conserved gene family, and the sequence that validated their family membership, the homeobox, provided a plausible biochemical function for their action. They were likely to be DNA-binding transcriptional regulators that would modulate the expression of many downstream genes. The conservation of very similar homeobox sequences in other animals suggested that homeotic-like genetic functions might exist in structurally homologous genes other than in Drosophila. That is, perhaps a conservation of developmental genetic circuitry could be detected at the molecular level that was invisible at the level of comparative embryological morphology. All of these things had been suggested before

in either explicit or vague terms by those with insight, prescience, and/or theoretical leanings (WOLPERT 1969; GARCIA-BELLIDO 1977; GARCIA-BELLIDO *et al.* 1979; LEWIS 1978; RAFF and KAUFMAN 1983), but a bit of molecular evidence goes a long way toward swaying opinion (especially the opinion of molecular biologists), so that much was made of the homeobox discovery.

In the original set of reports, the evidence for any of the conclusions was incomplete at best, which did not prevent the original authors from discussing them as quite likely to be true. There was even more hope expressed (and a bit of metaphorical hyperbole, at least in the titles) in a variety of review articles that suggested variously that the homeobox might be a biological equivalent of the Rosetta stone, the universal genetic key to body plan, and so on (*e.g.*, STRUHL 1984; SLACK 1984). There were even articles in newspapers and popular magazines announcing that something important had happened in developmental biology that might be relevant even to those sophisticated mammals that perform a daily perusal of *The New York Times*. All of this attention jump-started homeobox gene research in Drosophila, where it would be defined and enriched beyond anyone's wildest dreams by the rich genetics of that animal. But perhaps the most hope, and the most rapid and concerted jump out of the homeobox research starting gate, occurred in laboratories studying development in those vertebrates that had a rich history of descriptive and experimental embryology, but rudimentary genetic tool kits compared with Drosophila. Here the homeobox seemed to provide a toehold halfway up what had seemed to be a slippery and impassable barrier of developmental genetics.

Not all concurred with the blinkered enthusiasm over the meaning and utility of the homeobox homology. Some fancifully suggested that many developmental biologists were in the grip of "homeobox madness" or "homeobox fever" (RAFF and RAFF 1985; ROBERTSON 1985; WILKINS 1986), apparently a horizontally transmitted disease that caused a loss of one's critical faculties. Some geneticists and evolutionary biologists were thought to be immune to this syndrome. Many of those with cool heads who read the original homeobox papers carefully, and interpreted them critically, found some of the arguments specious. And some were, if the results within a particular paper are considered in isolation. In those days, however, the results were coming along so fast that by the time one paper was written, the results for the next, or the next two or three, were already in one's notebook. So the temptation was to "speculate" rather boldly on behalf of some of the early general conclusions described above, this being much safer than it looked since additional evidence to support them was already in hand.

Many people working on a variety of developing animals quickly realized that the homeobox, whatever its ultimate meaning, should be exploited as a useful tool to clone genes. This was especially true in Drosophila, which already had a mother lode of genetic and cytogenetic studies as a biological treasure. And this rich lode of genetics was indeed mined for all it was worth by anyone with a homeobox probe and a hypothesis (*e.g.*, FJOSE *et al.* 1985; LEVINE *et al.* 1985; REGULSKI *et al.* 1985; MACDONALD *et al.* 1986). For those of us who were doing something with homeoboxes as students or postdocs with WALTER GEHRING in Basel, Switzerland (which included MICHAEL LEVINE, ATSUSHI KUROIWA, ERNST HAFEN, ANDERS FJOSE, MAREK MLODZIK, and me), it will be difficult to forget the feeling of guilty pleasure when we realized how incredibly easy it might be to clone and identify the coding regions of the Drosophila homeotic genes and many of the segmentation genes. That this suspicion wasn't entirely a Swiss chocolate-inspired delusion was fortified by a chance conversation with GINES MORATA at a Swiss-USGEB meeting. Some of us, in collaboration with FRANCOIS KARCH and WELCOME BENDER, had found only three homeoboxes in Bithorax complex DNA (REGULSKI *et al.*, 1985). At the time, the number of protein-coding transcription units in the Bithorax complex was thought to be eight or more, but MORATA, ERNESTO SANCHEZ-HERRERO, and their co-workers had just discovered that the Bithorax complex contained only three lethal complementation groups (SANCHEZ-HERRERO *et al.* 1985), suggesting correctly that the three bithorax homeoboxes corresponded to those three lethal genes, now known as *Ubx*, *abd-A* and *Abd-B*.

One of the most exciting outcomes of the early homeobox research in Drosophila was the general way it confirmed some of E. B. LEWIS's speculations about the evolution of the Bithorax complex. In an article that is oft cited but rarely read in its complex entirety, LEWIS (1978) proposed that the Bithorax complex genes were members of a gene family, having duplicated and diverged from a common ancestor and in the process having acquired divergent functions that accounted for some of the morphological differences that distinguish the Drosophila body plan from that of more primitive arthropods. Luckily for some of us, LEWIS put that speculation in the first paragraph instead of in the middle of the article among the terse and tortuous genetics. T. C. KAUFMAN (RAFF and KAUFMAN 1983) had also proposed an extension of this to embrace the homeotic genes of the Antennapedia complex controlling head and thoracic development. It is still unclear how much the variation in homeotic protein function or expression pattern can account for evolutionary changes in arthropods, but it was eventually shown that the eight homeotic genes of the Antennapedia and Bithorax complexes (now conceptually grouped as the Homeotic Complex, or *HOM-C* genes; AKAM 1989) contained eight structurally similar homeobox sequences, sometimes designated as the *Antp*-class of homeoboxes (GEHRING *et al.* 1990; MCGINNIS and KRUMLAUF 1992).

Conservation of anterior-posterior axial patterning: One important thread in homeobox gene research has been the studies on the *Antp*-class *Hox* genes in other animals, particularly in the mouse. It was found early on that *Hox* genes were expressed in discrete anterior-posterior regions of embryos (*e.g.*, AWGULEWITSCH *et al.* 1986; GAUNT *et al.* 1986), that some of the *Hox* genes mapped in clusters (LEVINE *et al.* 1985; HART *et al.* 1985), and that some Drosophila genes were much more closely related in structure to certain mammalian *Hox* genes than to other Drosophila homeobox genes (REGULSKI *et al.* 1987). But it took the comprehensive and insightful studies of BONCINELLI *et al.* (1988), GRAHAM *et al.* (1989), and DUBOULE and DOLLÉ (1989) to put it all together. All three groups provided convincing evidence that individual *Hox* genes mapped in the same relative positions in one of the four *Hox* complexes as did (some of) their homologs in Drosophila. In addition, the latter two groups showed that the embryonic expression boundaries of many of the mouse *Hox* complex genes mimicked their map order within the complexes, again strikingly similar in a general sense to the properties of the *HOM*-type homeobox genes of Drosophila.

Though many found all this to be compelling evidence that the *Hox* genes must be doing something similar to the Drosophila *HOM-C* genes, it was still correlative molecular evidence. The first strong biological evidence as to the role of the *Hox* genes came from inducing their expression anterior to their normal limits, or artificially reducing their levels of expression, both of which caused some interpretable and some uninterpretable defects in the development of more anterior regions of the frog or mouse (WRIGHT *et al.* 1989; RUIZ I ALTABA and MELTON 1989; KESSEL *et al.* 1990). By expressing Hox proteins in developing Drosophila, one could also get mouse and human Hox proteins to phenocopy specific Drosophila HOM gain-of-function mutations (MALICKI *et al.* 1990; MCGINNIS *et al.* 1990), which indicated that the Hox proteins certainly had homeotic genetic functions in the context of Drosophila cells, though still saying little or nothing about their role in mouse or human cells. However, in many recent studies performed over the past few years (*e.g.*, CHISAKA and CAPECCHI 1991; LUFKIN *et al.* 1991; LEMOUELLIC *et al.* 1992; RAMIREZ-SOLIS *et al.* 1993), mouse *Hox* genes have been mutated by gene targeting, and many of these loss-of-function mutations result in either loss of axial structures or subtle to obvious homeotic transformations of skeletal elements and/or rhombomere elements of the hindbrain. These studies have represented one of the principal success stories for the practice of "reverse genetics," a discipline that has resulted in a "reversal of fortune" for more than one long-suffering graduate student or postdoc who has not been so fortunate as to have the mouse *Hox* genes as the focus of his or her mutant screen.

Also adding to the same intellectual picture are the highly influential studies indicating that both beetles and nematodes encode an important part of the genetic circuitry that controls their anterior-posterior axial patterning in clusters of *HOM/Hox*-type homeobox genes (BEEMAN *et al.* 1989; WANG *et al.* 1993). With the finding that some of the most primitive animals like hydra have *Antp*-class homeobox genes that are expressed in localized body regions (SHENK *et al.* 1993), it seems possible that many or all animals use *Antp*-class genes in *HOM/Hox* clusters to assign positional identities on the anterior-posterior axis (or oral-aboral axis where head is more difficult to define). Thus, only one hundred years after BATESON finished analyzing some bizarre variations in skeletons, and insightfully grouped a class of them as homeotic variations, we now have plausible molecular explanations for the homeotic defects, and a near certainty that many of the variations that he originally noticed in a variety of invertebrates and vertebrates are due to variations in the same basic underlying genetic circuitry.

Homeodomain proteins as transcription factors: Much of the current research that concerns homeobox gene function has been substantially enriched by the work on homeodomain proteins as transcription factors. The Antp-type homeodomain proteins are a relatively small subset of the total spectrum of proteins grouped in the homeodomain family. The only criterion for admission to this family is the conservation of a few crucial amino acid residues that tend to reside in the same positions in the 60-amino-acid primary sequence of known homeodomains. Structural studies of highly divergent homeodomains suggest that most of the family members defined by these criteria will have extremely similar three-dimensional structures and similar interactions with DNA binding sites (GEHRING *et al.* 1990; KISSINGER *et al.* 1990; WOLBERGER *et al.* 1991). There are many hundreds of homeodomain proteins, in many separate subclasses (SCOTT *et al.* 1989). It seems likely that hundreds exist even within a single genome–the current count in Drosophila is >60 and climbing (KALIONIS and O'FARRELL 1993; DESSAIN and MCGINNIS 1993)–and these proteins are surely involved in a myriad of biological control circuits. Many of these are understood quite poorly at the genetic level.

Evidence was not long in coming that homeodomain proteins actually did have the ability to bind specific DNA sites and that proteins with different homeodomain sequences had different preferred binding sites (DESPLAN *et al.* 1985, 1988; HOEY and LEVINE 1988). How much these different DNA binding preferences have to do with their functional specificity is still rather mysterious. The most widely accepted model (or class of models) explaining homeodomain protein functional specificities is largely derived from the biochemical studies on yeast Matα2, mammalian Oct1 and Oct2 and other POU proteins (*e.g.*, TREACY *et al.* 1992; POMERANTZ *et al.* 1992;

VERSHON and JOHNSON 1993; CLEARY *et al.* 1993), with much support from genetic studies on chimeric HOM proteins in Drosophila embryos (*e.g.*, LIN and MCGINNIS 1992; FUROKUBO-TOKUNAGA *et al.* 1993; CHAN and MANN 1993). This model has the homeodomain presenting one face to DNA and acquiring a bit of its specificity from that interaction. The other face, a sociable but discriminating face, is free to interact with one or many other proteins either on or off DNA. Only when the right set of interactions takes place on both faces is a given homeodomain protein interpreted as part of an active or inactive transcriptional regulatory complex that is capable of flipping a developmental switch.

Some questions that might have been answered in 10 years, but might take another 10 (or 100, until BATESON's second centennial anniversary): Though the amount of research that has been done on HOM/Hox-type homeodomain proteins is enormous, it is still unknown how many genetic or cofactor inputs are *required* for a homeotic switch to be thrown that changes cells (or even a single gene for that matter) from being assigned to a head, thoracic, or abdominal fate. Another way to look at this is that the genetic and molecular interactions between the homeotic proteins and the proteins that control other equally (or more) important developmental decisions such as sex determination, muscle or nerve cell identity, the timing of developmental events, or conserved signal transduction pathways are largely unexplored and mysterious.

We still don't really understand why the *HOM/Hox* genes tend to be arranged in a colinear array that (usually) correlates with the order of their domains of expression and function in embryos. There are some appealing ideas about how these clusters might have arisen and the forces that might tend to keep them together, involving shared regulatory regions (*e.g.*, CELNIKER *et al.* 1990), but there is not enough evidence as yet to provide a convincing explanation for the persistent colinear arrangements.

We have only a primitive understanding of how HOM/Hox proteins, or any other homeodomain proteins for that matter, might have the wholesale but coordinated effects that they exert on morphogenesis. In Drosophila, the HOM proteins are known to regulate the expression of other genes that encode other transcription factors, growth factors, homophilic membrane proteins, and proteins of unknown function (reviewed in BOTAS 1993), but how is this all coordinated to result in an antenna instead of a leg, or even a gut constriction?

One interesting curiosity is that despite the importance that the human *Hox* genes must have during development, there is surprisingly little direct or indirect evidence that their proper function is relevant to known human heritable developmental defects or human teratology (*e.g.*, WOLGEMUTH *et al.* 1989). In addition, to my knowledge there are as yet no naturally occurring mouse developmental defects that map to the *Hox* clusters, despite the obvious involvement of some other homeodomain protein subgroups in mouse (and human) heritable morphological abnormalities.

Disclaimer: This essay is definitely not intended to be a scholarly review of homeobox gene research over the past 10 years, just an admittedly biased look back at what happened 10 years ago, and to look at how a few of the questions that were interesting then have either been answered (or not) in the ensuing period. I've benefited enormously from talking to all of the people working on *HOM* and *Hox* genes and proteins and to many of those working on other classes of homeobox genes, and I have been influenced by nearly everyone. Thus, they all bear a highly diffuse responsibility for the opinions expressed here, though certainly no blame for the manner in which they are expressed.

LITERATURE CITED

AKAM, M., 1989 *Hox* and HOM: homologous gene clusters in insects and vertebrates. Cell **57:** 347–349.

AWGULEWITSCH, A., M. F. UTSET, C. P. HART and F. H. RUDDLE, 1986 Spatial restriction in expression of a mouse homeo box locus within the central nervous system. Nature **320:** 328–335.

BALKASCHINA, E. I., 1929 Ein Fall der Erbhomeosis (die Genovarition "aristopedia") bei *Drosophila melanogaster*. Wilhelm Roux' Arch. Entwicklungsmech. Org. **115:** 448–463.

BATESON, W., 1894 *Materials for the Study of Variation Treated with Especial Regard to Discontinuity in the Origin of Species*. Macmillan, London.

BATESON, W., 1909 *Mendel's Principles of Heredity*. University Press, Cambridge, England.

BEEMAN, R. W., J. J. STUART, M. S. HAAS and R. E. DENELL, 1989 Genetic analysis of the homeotic gene complex (HOM-C) in the beetle *Tribolium castaneum*. Dev. Biol. **133:** 196–209.

BONCINELLI, E., R. SOMMA, D. ACAMPORA, M. PANNESE, M. F. A. D'ESPOSITO *et al.*, 1988 Organization of human homeobox genes. Hum. Reprod. **3:** 880–886.

BOTAS, J., 1993 Control of morphogenesis and differentiation by HOM/Hox genes. Curr. Opin. Cell Biol. **5:** 1015–1022.

BRIDGES, C. B., and T. DOBZHANSKY, 1933 The mutant "proboscipedia" in *Drosophila melanogaster*–a case of hereditary homeosis. Wilhelm Roux' Arch. Entwicklungsmech. Org. **127:** 575–590.

BRIDGES, C. B., and T. H. MORGAN, 1923 The third-chromosome group of mutant characters of *Drosophila melanogaster*. Carnegie Inst. Washington Publ. **327:** 93.

CARRASCO, A. E., W. MCGINNIS, W. J. GEHRING and E. M. DEROBERTIS, 1984 Cloning of a *Xenopus laevis* gene expressed during early embryogenesis coding for a peptide region homologous to Drosophila homeotic genes. Cell **37:** 409–414.

CELNIKER, S. E., S. SHARMA, D. J. KEELAN and E. B. LEWIS, 1990 The molecular genetics of the bithorax complex of *Drosophila*: *cis*-regulation of the *Abdominal-B* domain. EMBO J. **9:** 4277–4286.

CHAN, S., and R. S. MANN, 1993 The segment identity functions of Ultrabithorax are contained within its homeo domain and carboxy-terminal sequences. Genes Dev. **7:** 796–811.

CHISAKA, O., and M. R. CAPECCHI, 1991 Regionally restricted developmental defects resulting from targeted disruption of the mouse homeobox gene *hox-1.5*. Nature **350:** 473–479.

CLEARY, M. A., S. STERN, M. TANAKA and W. HERR, 1993 Differential positive control by Oct-1 and Oct-2: activation of a transcriptionally silent motif through Oct-1 and VP16 co-recruitment. Genes Dev. **7:** 72–83.

DESPLAN, C., J. THEIS and P. H. O'FARRELL, 1985 The Drosophila developmental gene, engrailed, encodes a sequence-specific DNA binding activity. Nature **318:** 630–635.

DESPLAN, C., J. THEIS and P. H. O'FARRELL, 1988 The sequence specificity of homeodomain-DNA interaction. Cell **54:** 1081–1090.

DESSAIN, S., and W. MCGINNIS, 1993 Drosophila homeobox genes. Adv. Dev. Biochem. **2:** 1–55.

DUBOULE, D., and P. DOLLÉ, 1989 The structural and functional organization of the murine *HOX* gene family resembles that of Drosophila homeotic genes. EMBO J. **8:** 1497–1505.

FJOSE, A., W. MCGINNIS and W. J. GEHRING, 1985 Isolation of a homeobox-containing gene from the engrailed region of Drosophila and the spatial distribution of its transcripts. Nature **313:** 284–289.

FUROKUBO-TOKUNAGA, K., S. FLISTER and W. J. GEHRING, 1993 Functional specificity of the Antennapedia homeodomain. Proc. Natl. Acad. Sci. USA **90:** 6360–6364.

GARCIA-BELLIDO, A., 1977 Homeotic and atavic mutations in insects. Am. Zool. **17:** 613–629.

GARCIA-BELLIDO, A., P. A. LAWRENCE and G. MORATA, 1979 Compartments in animal development. Sci. Am. (July): 102–110.

GAUNT, S. J., J. R. MILLER, D. J. POWELL and D. DUBOULE, 1986 Homeo box gene expression in mouse embryos varies with position by the primitive streak stage. Nature **324:** 662–664.

GEHRING, W. J., M. MULLER, M. AFFOLTER, A. PERCIVAL-SMITH, M. BILLETER *et al.*, 1990 The structure of the homeodomain and its functional implications. Trends Genet. **6:** 323–329.

GRAHAM, A., N. PAPALOPULU and R. KRUMLAUF, 1989 The murine and Drosophila homeobox gene complexes have common features of organization and expression. Cell **57:** 367–378.

HART, C. P., A. AWGULEWITSCH, A. FAINSOD, W. MCGINNIS and F. H. RUDDLE, 1985 Homeo box gene complex on mouse chromosome 11: molecular cloning, expression in embryogenesis, and homology to a human homeo box locus. Cell **43:** 9–18.

HOEY, T., and M. LEVINE, 1988 Divergent homeo box proteins recognize similar DNA sequences in Drosophila. Nature **332:** 858–861.

KALIONIS, B., and P. H. O'FARRELL, 1993 A universal target sequence is bound *in vitro* by diverse homeodomains. Mech. Dev. **43:** 57–70.

KAUFMAN, T. C., R. LEWIS and B. WAKIMOTO, 1980 Cytogenetic analysis of chromosome *3* in *Drosophila melanogaster*: the homeotic gene complex in polytene chromosome interval 84A-B. Genetics **94:** 115–133.

KESSEL, M., R. BALLING and P. GRUSS, 1990 Variations of cervical vertebrae after expression of a *Hox-1.1* transgene in mice. Cell **61:** 301–308.

KISSINGER, C. R., B. LIU, E. MARTIN-BLANCO, T. B. KORNBERG and C. O. PABO, 1990 Crystal structure of an engrailed homeodomain-DNA complex at 2.8 Å resolution: a framework for understanding homeodomain-DNA interactions. Cell **63:** 579–590.

LAUGHON, A., and M. P. SCOTT, 1984 Sequence of a Drosophila segmentation gene: protein structure homology with DNA-binding proteins. Nature **310:** 25–31.

LE MOUELLIC, H., Y. LALLEMAND and P. BRULET, 1992 Homeosis in the mouse induced by a null mutation in the *Hox-3.1* gene. Cell **69:** 251–264.

LEVINE, M., G. RUBIN and R. TIJAN, 1984 Human DNA sequences homologous to a protein coding region conserved between homeotic genes of Drosophila. Cell **38:** 667–673.

LEVINE, M., K. HARDING, C. WEDEEN, H. DOYLE, T. HOEY *et al.*, 1985 Expression of the homeo box gene family in Drosophila. Cold Spring Harbor Symp. Quant. Biol. **50:** 209–222.

LEWIS, E. B., 1978 A gene complex controlling segmentation in Drosophila. Nature **276:** 565–570.

LIN, L., and W. MCGINNIS, 1992 Mapping functional specificity in the Dfd and Ubx homeodomains. Genes Dev. **6:** 1071–1081.

LUFKIN, T., A. DIERICH, M. LEMEUR, M. MARK and P. CHAMBON, 1991 Disruption of the *Hox-1.6* homeobox gene results in defects in a region corresponding to its rostral domain of expression. Cell **66:** 1105–1119.

MACDONALD, P. M., P. INGHAM and G. STRUHL, 1986 Isolation, structure and expression of *even-skipped*: a second pair-rule gene of Drosophila containing a homeo box. Cell **47:** 721–734.

MALICKI, J., K. SCHUGHART and W. MCGINNIS, 1990 Mouse *Hox 2.2* specifies thoracic segmental identity in Drosophila embryos and larvae. Cell **63:** 961–967.

MCGINNIS, N., M. A. KUZIORA, M. REGULSKI and W. MCGINNIS, 1990 Human *Hox-4.2* and Drosophila *Deformed* encode similar regulatory specificities in Drosophila embryos and larvae. Cell **63:** 969–976.

MCGINNIS, W., and R. KRUMLAUF, 1992 Homeobox genes and axial patterning. Cell **68:** 283–302.

MCGINNIS, W., M. LEVINE, E. HAFEN, A. KUROIWA and W. J. GEHRING, 1984a A conserved DNA sequence found in homeotic genes of the Drosophila Antennapedia and Bithorax complexes. Nature **308:** 428–433.

MCGINNIS, W., R. L. GARBER, J. WIRZ, A. KUROIWA and W. J. GEHRING, 1984b A homologous protein-coding sequence in Drosophila homeotic genes and its conservation in other metazoans. Cell **37:** 403–408.

MCGINNIS, W., C. P. HART, W. J. GEHRING and F. H. RUDDLE, 1984c Molecular cloning and chromosome mapping of a mouse DNA sequence homologous to homeotic genes of Drosophila. Cell **38:** 675–680.

POMERANTZ, J. L., T. M. KRISTIE and P. A. SHARP, 1992 Recognition of the surface of a homeo domain protein. Genes Dev. **6:** 2047–2057.

RAFF, E. C., and R. A. RAFF, 1985 Possible functions of the homeobox. Nature **313:** 185.

RAFF, R. A., and T. C. KAUFMAN, 1983 *Embryos, Genes, and Evolution.* Macmillan, New York.

RAMIREZ-SOLIS, R., H. ZHENG, J. WHITING, R. KRUMLAUF and A. BRADLEY, 1993 *Hoxb-4* (*Hox-2.6*) mutant mice show homeotic transformation of a cervical vertebra and defects in closure of the sternal rudiments. Cell **73:** 279–294.

REGULSKI, M., K. HARDING, R. KOSTRIKEN, F. KARCH, M. LEVINE *et al.*, 1985 Homeo box genes of the antennapedia and bithorax complexes of Drosophila. Cell **43:** 71–80.

REGULSKI, M., N. MCGINNIS, R. CHADWICK and W. MCGINNIS, 1987 Developmental and molecular analysis of *Deformed*: a homeotic gene controlling Drosophila head development. EMBO J. **6:** 767–777.

ROBERTSON, M., 1985 Mice, mating types and molecular mechanisms of morphogenesis. Nature **318:** 12–13.

RUIZ I ALTABA, A., and D. A. MELTON, 1989 Involvement of the Xenopus homeobox gene Xhox3 in pattern formation along the anterior-posterior axis. Cell **57:** 317–326.

SANCHEZ-HERRERO, E., I. VERNOS, R. MARCO and G. MORATA, 1985 Genetic organization of Drosophila bithorax complex. Nature **313:** 108–113.

SCOTT, M. P., and A. WEINER, 1984 Structural relationships among genes that control development: sequence homology between the Antennapedia, Ultrabithorax, and fushi tarazu loci of *Drosophila*. Proc. Natl. Acad. Sci. USA **81:** 4115–4119.

SCOTT, M. P., J. W. TAMKUN and I. G. W. HARTZELL, 1989 The structure and function of the homeodomain. Biochim. Biophys. Acta **989:** 25–48.

SHENK, M. A., H. R. BODE and R. E. STEELE, 1993 Expression of *Cnox-2*: a HOM/HOX homeobox gene in hydra, is correlated with axial pattern formation. Development **117:** 657–667.

SHEPHERD, J. C. W., W. MCGINNIS, A. E. CARRASCO, E. M. DEROBERTIS and W. J. GEHRING, 1984 Fly and frog homeo domains show homologies with yeast mating type regulatory proteins. Nature **310:** 70–71.

SLACK, J., 1984 A Rosetta stone for pattern formation in animals? Nature **310:** 364–365.

STRUHL, G., 1984 A universal genetic key to body plan? Nature **310:** 10–11.

TREACY, M. N., L. I. NEILSON, E. E. TURNER, X. HE and M. G. ROSENFELD, 1992 Twin of I-POU: a two amino acid difference in the I-POU homeodomain distinguishes an activator from an inhibitor of transcription. Cell **68:** 491–505.

VERSHON, D., and A. D. JOHNSON, 1993 A short, disordered protein region mediates interactions between the homeodomain of the yeast α2 protein and the MCM1 protein. Cell **72:** 105–112.

WANG, B. B., M. M. MULLER-IMMERGLUCK, J. AUSTIN, N. T. ROBINSON, A. CHISHOLM *et al.*, 1993 A homeotic gene cluster patterns the anteroposterior body axis of C. elegans. Cell **74:** 29–42.

WILKINS, A. S., 1986 Homeobox fever, extrapolation and developmental biology. Bioessays **4:** 147–148

WOLBERGER, C., A. K. VERSHON, B. LIU, A. D. JOHNSON and C. O. PABO, 1991 Crystal structure of a MATα2 homeodomain-operator complex suggests a general model for homeodomain-DNA interactions. Cell **67:** 517–528.

WOLGEMUTH, D. J., R. R. BEHRINGER, M. P. MOSTOLLER, R. L. BRINSTER and R. D. PALMITER, 1989 Transgenic mice overexpressing the mouse homoeobox-containing gene *Hox-1.4* exhibit abnormal gut development. Nature **337:** 464–467.

WOLPERT, L., 1969 Positional information and the spatial pattern of cellular differentiation. J. Theor. Biol. **25:** 1–47.

WRIGHT, C. V. E., K. W. Y. CHO, J. HARDWICKE, R. H. COLLINS and E. M. DE ROBERTIS, 1989 Interference with function of a homeobox gene in Xenopus embryos produces malformations of the anterior spinal cord. Cell **59:** 81–93.