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MINIREVIEW

Developmental Patterning Genes and Their Conserved Functions: From Model Organisms to Humans

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Molecular and genetic evidence accumulated during the past 20 years in the field of developmental biology indicates that different animals possess many common genetic systems for embryonic patterning. In this review we describe the conserved functions of such developmental patterning genes and their relevance for human pathological conditions. Special attention is given to the Hox genetic system, involved in establishing cell identities along the anterior-posterior axis of all higher metazoans. We also describe other conserved genetic systems, such as the involvement of Pax6 genes in eye development and the role of Nkx2.5-type proteins in heart development. Finally, we outline some fascinating problems at the forefront of the studies of developmental patterning genes and show how knowledge obtained from model genetic organisms such as *Drosophila* helps to explain normal human morphogenesis and the genetic basis of some birth defects.

Key Words: **Hox; homeotic; organ development; Pax; tinman; axial patterning; human malformations; evolution.**

All metazoans, including the writers and the readers of these lines, share a moment in their lifetime when they are nothing more than a single-cell zygote. It is remarkable to think about the astonishing variety of life forms and the intricate details of adult body plans that arise from this unicellular stage through the process of embryonic development. Exciting recent discoveries indicate that despite their variations in shape and complexity, animals are more similar to each other than meets the eye. The detection of covert similarity in diverse body plans has resulted from the great advances made in the past 20 years of developmental genetic research. For example, a series of investigations have shown that all bilateral animals, including humans, possess a common genetic mechanism for patterning the anterior/posterior (A/P) axis involving the Hox cluster genes (1–3, reviewed in 4,5).

Besides a common axial patterning system, other general architectural features in both vertebrates and invertebrates also appear to be controlled by common genetic mechanisms. Humans and insects possess organs of very diverse appearance serving similar functions, such as eyes for vision and hearts for blood circulation. Traditional views have held that these structures are analogous, i.e., convergently evolved, and are therefore likely to be specified by different genetic patterning systems (6–8). However, new evidence reviewed in this paper suggests that we now have good reason to call these organs homologous at the level of the genes that control their formation. Therefore, knowledge about the genes that control early development in human embryos can be obtained by the detailed study of "model genetic animals," such as nematode worms, fruit flies, and mice.

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This review focuses on several cases of such conservation, drawing from what we know about the function of Hox genes and other "master control genes," to shed light on the continuity of developmental gene function from *Planaria* to *Homo*. We discuss the directions of current investigations, implications for human genetics and disease, as well as some fascinating but yet unanswered questions at the forefront of the Hox research.

The Role of Hox Genes in the Determination of Segment Identity along the A/P Axis: From *Drosophila* to Humans

Homeosis was originally described by Bateson as the phenomenon in which one element of a segmentally repeated array of organismal structures is transformed toward the identity of another (9). The genetic basis for these transformations of the body plan was unknown until seminal studies were done on homeotic selector genes (now often referred to as Hox genes). Mutations in such genes often result in homeotic transformations of the body plan in one or a few segments. A large and systematic collection of homeotic mutations was assembled in *Drosophila* (10,11). A well-known homeotic gene *Ultrabithorax* (*Ubx*) was originally identified by mutations that transform halteres (small club-like balancing organs of flies) into an extra pair of wings. Another classical homeotic phenotype is produced by dominant mutations in the *Antennapedia* (*Antp*) gene, which transform the antenna on the head of a fly into an extra thoracic leg.

Molecular analysis of the genomes of other organisms has revealed that all bilateral animals, including humans, have multiple Hox genes (Fig. 1). The proteins made from these genes all contain a similar 60-amino acid motif termed the homeodomain. Homeodomain proteins such as those of the Hox-type are transcription factors and exert their function through activation and repression of multiple target genes. Interestingly, the Hox genes are arranged so that the position and order of homologous genes (e.g., *Deformed* (*Dfd*) of *Drosophila* and *HOXD4* of humans) are preserved in the Hox clusters of different animals. The functional significance of the conserved gene order in these clusters is still poorly understood. However, a likely reason for the maintenance of the clustered arrangement for more than 500 million years is that different genes in the cluster are controlled by the same DNA regulatory regions. Therefore, it can be argued that the cluster functions as a single, complicated genetic unit (12–14). In contrast to the single Hox cluster in *Drosophila* and most other invertebrates, humans and other vertebrates have four clusters of Hox genes (*HOXA, HOXB, HOXC,* and *HOXD*), that likely evolved by two successive duplications of a primordial cluster.

In addition to conservation of primary sequence and chromosomal organization, Hox gene expression patterns are also conserved in diverse animals. Persistent expression of Hox genes in discrete zones on the A/P axis is required to remind embryonic cells of their axial position long after the initial genetic cues are gone. Hox expression zones have sharp anterior boundaries, with less well-defined posterior boundaries. The order of anterior boundaries of Hox expression along the A/P axis of the embryo and the timing of activation during development are generally colinear with the order of the genes on the chromosome (15). It is interesting to note that the same Hox gene can have a slightly offset boundary of expression in different tissues, which is especially true for vertebrate embryos (Fig. 1). Within the same tissue, however, the relative expression boundaries of different Hox cluster members are preserved.

Conservation of Hox protein sequence and expression pattern suggested that vertebrate Hox genes control axial patterning in a manner similar to that in flies (16). This was confirmed when mouse Hox mutants were obtained and homeotic transformations were found in the skeletons of mutant embryos. For example, in *Hoxc-8* homozygous mutant mice the most obvious transformations were the attachment of the 8th pair of ribs to the sternum and the appearance of a 14th pair of ribs on the 1st lumbar vertebra (17).

Studies in both *Drosophila* and mouse show that homeotic transformations in Hox loss-of-function mutants usually cause the affected body structures to resemble more anterior ones. Conversely, many gain-of-function mutant phenotypes are due to ectopic expression of more posterior Hox genes, which are capable of "canceling" the function of more anterior ones and specifying extra posterior structures. For example, when *Drosophila* Abd-A protein, which is normally confined to the posterior-most abdominal region of the fly embryo, is provided ubiquitously under the control of a heat-shock promoter, all head and thoracic segments attain a more posterior (abdominal-like) identity. The ability of a more posterior Hox gene to impose its function on more

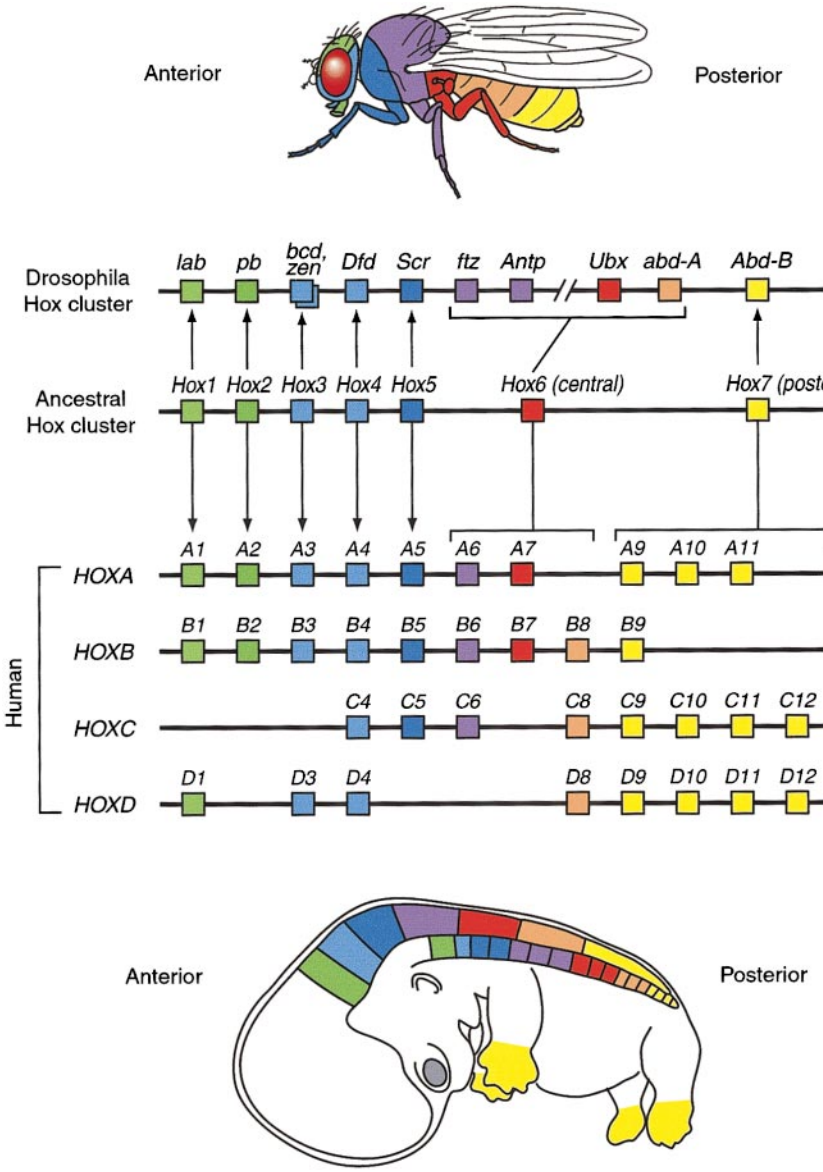

FIG. 1. Conservation of genomic organization and expression patterns of Hox genes (modified from 4,18). The lower half of the figure depicts the four clusters of Hox genes in mammals and the expression patterns (inferred from mouse expression studies) of the orthologous genes in a stage 19 human embryo. The colored fields in the expression schematic depict the anteriormost domains of expression. In actuality, the posterior boundaries of the expression domains overlap in more caudal regions. Note a shift of the anterior expression boundaries between the nervous system and the segmented mesoderm, which nevertheless preserves the relative order of Hox gene expression. Several of the posterior *HOXA* and *HOXD* genes are also expressed in the limb primordia; they are collectively indicated by the yellow color. The upper half of the figure shows *Drosophila* Hox genes, aligned with their mammalian orthologs, and corresponding expression patterns in the adult fly (the *Drosophila* Hox cluster is split into two parts, located on the same chromosome). Recent data suggest that a minimum number of Hox genes present in a common ancestor of all bilateral animals is seven (141). Such a hypothetical ancestral Hox cluster is presented in the middle, with arrows indicating the predicted evolutionary origins of insect and mammalian Hox genes. For some of the central and posterior Hox genes, it is difficult to define precise homology relationships, and groups of genes with equal homology to an ancestral gene are indicated with brackets. *Drosophila bcd* and *zen* genes are not members of the Hox A/P patterning system. They represent fast-evolving insect homeodomain genes (141).

anterior genes is called posterior prevalence, or phenotypic suppression.

Human Phenotypes Associated with Mutations in Hox Genes

Despite the scarcity of available mutations in human and mouse Hox genes, it is possible to make a few generalizations about the observed effects of such genetic lesions. In many cases, mutations involving one or several mouse Hox genes do result in homeotic transformations, but they are also associated with loss of axial structures and organs and other nonhomeotic malformations (18). Part of the reason for the highly complex mutant phenotypes is that Hox genes are involved in an elaborate system of cross-regulatory interactions and redundant functions.

Hox genes are not required solely for the proper development of the rostro-caudal main body axis. In mammals, the posterior-most members of the *HOXC*, *HOXD*, and *HOXA* clusters (*HOXC9-13*, *HOXD9-13*, and *HOXA11-13*, respectively) are expressed in the developing limb buds (reviewed in 15) (Fig. 1). Many of the same genes from the *HOXD* and *HOXA* clusters are also expressed in external genitourinary structures (19–21). The limb and genital defects observed in mice and humans that possess mutations in the posterior Hox genes indicate that Hox expression is crucial for the formation of these body parts. Table 1 summarizes the known mutations in human Hox genes and their associated phenotypes.

Several groups have reported heterozygous and homozygous synpolydactyly phenotypes that cosegregated with an expansion in a 15-residue polyalanine stretch in exon 1 of the *HOXD13* gene (22–24). A significant increase of the penetrance and severity of the phenotype correlated with increasing expansion size. Interestingly, the family with the largest expansion included affected males with hypospadias, which is not a feature of the classic synpolydactyly (SPD), but correlates with the genital expression of the gene in mammals. Correlation between the severity of the phenotype and expansion size suggests that the added alanines cause gain-of-function mutations in the HOXD13 protein. This hypothesis is further supported by the fact that the *synpolydactyly-homolog* (*spdh*), a spontaneous mouse mutation carrying a similar expansion (25), has a much more severe phenotype than the complete absence of *Hoxd-13* function (26).

Two different intragenic *HOXD13* deletions that resulted in premature stop codons have been associated with a phenotype with some features of SPD and a novel foot malformation (27). Such truncations would eliminate the function of the HOXD13 protein, which suggested that this SPD phenotypic variant was due to haploinsufficiency for the *HOXD13* gene. Finally, monodactylous limbs and abnormal genitalia were observed in two unrelated patients that were heterozygous for deletions spanning the whole *HOXD* cluster and nearby loci (28). The involvement of nearby genes in the monodactylous phenotype is suggested by the fact that less severe phenotypes are seen in mice with deletions spanning *Hoxd9–13* (26,29).

Mutations in the posterior genes of the *HOXA* cluster also result in abnormal limb and genital development. The classic hand-foot-genital (HFG) syndrome is associated with heterozygosity for a nonsense mutation in the homeodomain of HOXA13 (30). This nonsense mutation is predicted to generate a truncated protein that would be unable to bind DNA, invoking haploinsufficiency as the most likely mechanism leading to the phenotype. The importance of a diploid dose of the *HOXA* genes is further suggested by the phenotype of a patient with a large deletion spanning the *HOXA* cluster. This patient possessed features of the HFG syndrome and other anomalies, possibly caused by the deficiency of other members of the cluster (31). An apparent dominant-negative phenotype is observed in the spontaneous mouse mutant *hypodactyly* (*Hd*), with a 50 bp deletion in the coding sequence of *Hoxa-13*. *Hd* mice have more severe limb defects than the *Hoxa-13* null mutant (30,32). In another case, the expansion of a polyalanine stretch in the HOXA13 protein has been associated with a dominant HFG syndrome that includes an atypical metacarpophalangeal profile and genitourinary anomalies (33). Expansions and contractions of poly-amino acid tracts might be generated from unequal crossing over and be a common mutational mechanism for Hox genes (34).

"Master Control Genes" for Eyes and Hearts

The Hox genes are only one class of patterning genes that have similar developmental functions in simple experimental animals and humans. Another class consists of those genes that primarily control the development of one organ. The term "master control gene" has been coined to denote this class of embryonic patterning genes (35,36). Interestingly,

TABLE 1
Mutations in Human HOX Genes and Associated Phenotypes

Genes affected	Molecular nature of mutation	Observed phenotypes	References
HOXD13	Expansion of polyalanine stretch	Heterozygous synpolydactyly (SPD) Syndactyly: fingers 3–4 and toes 4–5, with polydactyly in the cutaneous web between digits Homozygous SPD Short hands and feet Complete soft tissue syndactyly of all four limbs Preaxial, mesoaxial, and postaxial polydactyly of hands Loss of tubular shape of carpal, metacarpal, and phalangeal bones Tarsal-metatarsal fusions Loss of normal phalangeal pattern Hypospadias	(22–24)
	Intragenic deletions	Some features of SPD Rudimentary polydactyly involving metatarsals 1–2 and 4–5	(27)
HOXD1-13	Deletion including HOXD cluster	Single bone in zeugopod with radial appearance Monodactyly with biphalangeal digit and absence of carpal ossification in four limbs Hypoplastic male external genitalia and cryptorchidism	(28)
HOXA13	Nonsense mutation in homeodomain	Hand-foot-genital (HFG) syndrome Small hands and feet, short great toes, abnormal thumbs Short 1st metacarpal and metatarsal, short 5th fingers, carpal and tarsal fusions, small pointed distal phalanx of 1st toe Mullerian duct fusion (bicornuate or didelphic uterus) Displaced urethral opening and displaced urethral orifices in bladder wall Hypospadias	(30)
	Expansion of polyalanine stretch	HFG syndrome with atypical metacarpophalangeal profile Urinary tract anomalies	(33)
HOXA1-13	Deletion including HOXA cluster	HFG syndrome Velopharyngeal insufficiency Persistent ductus botalli	(31)

some of these "master control proteins" also contain homeobox domains that are distantly related to the original homeobox signature found in Hox transcription factors, while others are transcription factors of other types.

One of the well-studied master control genes is required for the specification of a blood pumping organ in a wide variety of animals whose "hearts" are of incredibly diverse shapes and sizes. This work began with the study of a *Drosophila* homeobox gene that was expressed in both dorsal mesoderm and the dorsal vessel (insect equivalent of the heart) (Fig. 2A). The dorsal vessel consists of a tubular muscle that circulates hemolymph within the open body cavity (37, reviewed in 38). This gene was named *tinman*, after the character in the "Wizard of Oz" (39) who desires a heart. Mutations in *tinman* resulted in dead larvae that were missing the dorsal vessel, as well as other dorsal mesoderm derivatives (40,41).

Molecular analysis of the mouse genome revealed that mice have *tinman*-like genes, one of which is called *Nkx2.5* or *Csx*. The *Nkx2.5/Csx* gene is expressed in the fetal heart primordia (42,43)—a pattern that is strikingly similar to *tinman* gene expression in *Drosophila*. Targeted mutation of *Csx/Nkx2.5* results in embryonic lethality, and embryonic heart development is arrested at the ini-

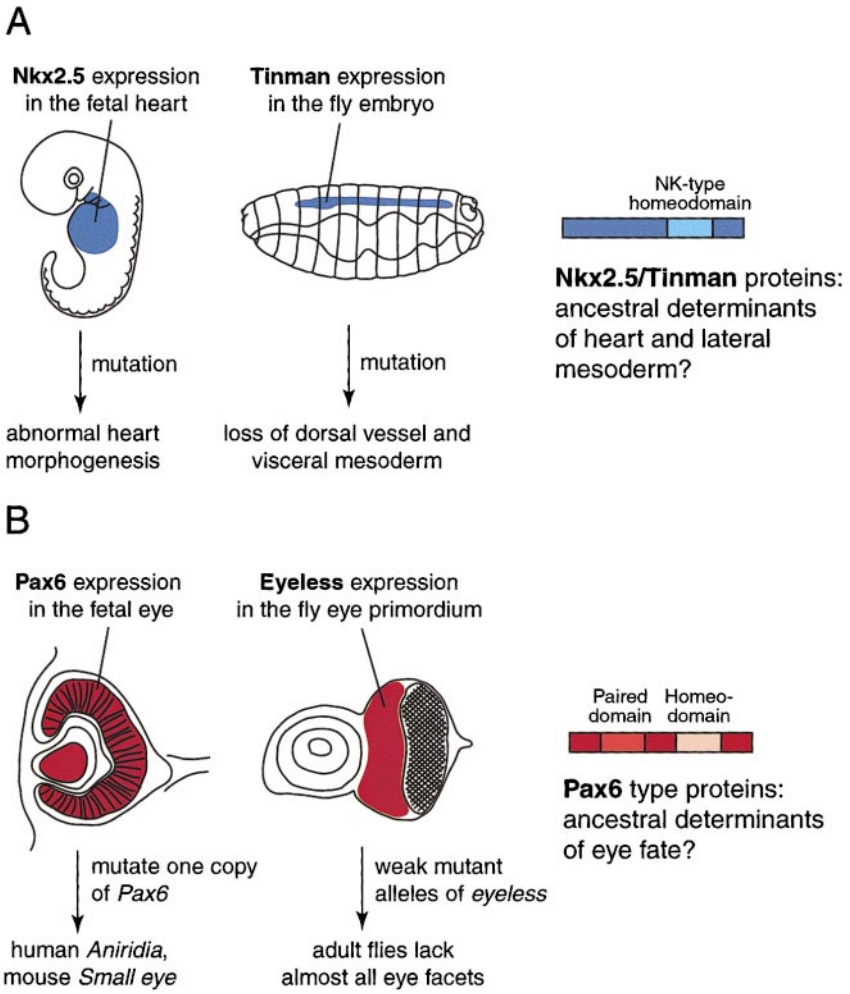

FIG. 2. Conservation of developmental patterning mechanisms involved in formation of the heart and eye primordia. (A) Schematic representation of an early mammalian embryo (left) and a *Drosophila* embryo (middle). The blue color denotes the domain of expression of the mammalian Nkx2-5 protein in the mesodermal cells that will give rise to the heart. A homologous fly protein, Tinman, is expressed in lateral mesoderm that will form the dorsal vessel, an organ performing the blood-pumping function in insects. Mutations in either of these genes result in abnormal heart morphogenesis. Nkx2-5 and Tinman share an NK-type homeodomain (right) and are thought to be ancient determinants of heart and lateral mesoderm. (B) Left panel shows the domains of expression of the mammalian Pax6 protein in the developing eye. Pax6 is concentrated in the retina and the lens. The Pax6-like protein in *Drosophila*, encoded in the gene called *eyeless*, is also expressed in the eye primordia (middle). Loss-of-function mutations in *Pax6* are associated with syndromes affecting eye development, and weak mutant alleles of *eyeless* result in loss of eyes in adult flies. Pax6-like proteins contain paired domain and homeodomain signatures and are found in all higher metazoans (right). Pax6-type transcriptional regulators have been involved in eye formation since the early origins of all bilateral animals.

tial stage of heart looping (44). There is also evidence from human genetics indicating that the human *NKX2-5* gene (localized to chromosome 5q35) is required for normal heart morphogenesis. Several cases of familial congenital heart disease with defects in the morphology of the atrial septum and in atrioventricular conduction were associated with both haploinsufficiency and gain-of-function mutations in the *NKX2-5* gene (45). These observations led to a conclusion that the Csx/NKX2-5/Tinman-like proteins are ancestral determinants of heart and surrounding visceral mesoderm (Fig. 2A). Recent data indicate that a pathway controlling early heart development, involving several signaling molecules and transcription factors, is similar between *Drosophila* and vertebrates (38,46,47). Even though

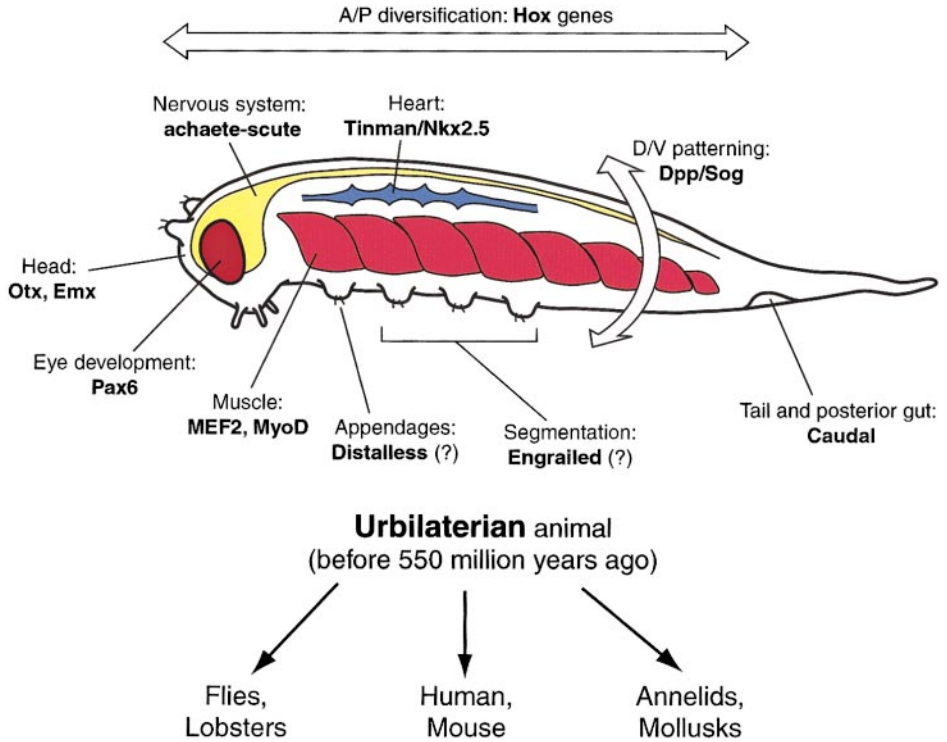

FIG. 3. A common ancestor of all bilateral animals possessed a complete set of genetic functions involved in formation of major organ systems. This schematic reconstruction of an Urbilaterian (a hypothetical common ancestor of bilateral animals) (65) was inspired by, but is only loosely based on, an upside-down drawing of a lobster made by Geoffroy Saint-Hilaire (142). Ancient genetic patterning systems, common to all extant bilaterally symmetrical metazoans, were already present in this creature (74). Major organ systems are indicated, accompanied by conserved regulatory proteins involved in their formation (shown in bold). The bottom part of the figure shows that this Urbilaterian animal gave rise to all major metazoan adult body plans, including chordates. See text for references concerning the individual genes.

the morphologies of insect and mammalian hearts are dramatically different, the underlying genetic machinery for the specification of a mesodermal zone that develops into a blood-pumping organ appears to be well conserved.

In addition to heart primordia, the mesodermal layer of the embryo gives rise to muscle, bone, and connective tissues. While the earliest events in specification of the mesoderm vary in different animal groups, one common denominator has been found in the development of skeletal muscle cells: a MADS box gene, *MEF2* (*D-MEF2* in the fly), is an early marker of skeletal muscle lineage in both insects and vertebrates (48). In vertebrates, MEF2 enhances and stabilizes the expression of such well-known muscle-specific genes as the basic-helix-loop-helix homologs *Myf5*, *MyoD*, *MRF4*, and *Myogenin* (49). In *Drosophila*, mesoderm fates are initially controlled by Twist and Snail proteins, and Twist directly activates *D-MEF2* (48,50). D-MEF2 and its vertebrate homologs are required for the completion of myogenesis in all muscles (49,51). Key features of this system have been preserved through millions of years of evolution. Such features include the conservation of the MEF2 MADS domain, which mediates sequence-specific DNA binding, and conservation of DNA target sites in regulatory regions of the muscle-specific genes (48).

Another example of conservation of developmental patterning pathways was shown in a series of experiments that revealed a striking similarity in the mechanisms underlying the formation of eyes and photoreceptor cells in many different taxa. For most animals, the visual system is crucial for survival, and indeed it has been argued that primate brains receive most of their information through the eyes (52). As is often the case in genetics, relevant mutations proved crucial for unraveling the molecular pathways underlying eye development. Two such mutations have been known for quite some

time: the *Aniridia* defect in humans (53–55, reviewed in 56), and the *Small eye* (*Sey*) mutation in mice and rats (57–59). The human *Aniridia* syndrome is characterized by a reduction in eye size and the absence of the iris in heterozygotes. A similar defect is seen in mice that are heterozygous for the *Small eye* mutation. Mice homozygous for *Small eye* completely lack eyes and die *in utero*.

Molecular analysis revealed that the same gene, *Pax6*, was affected in both the *Aniridia* and the *Small eye* syndromes. Pax6 belongs to a paired box/homeodomain family of transcriptional regulators (Fig. 2B). As expected, the Pax6 protein is abundantly expressed in the eye from the earliest stages until the end of eye morphogenesis: initially, in the optic sulcus, and subsequently in the eye vesicle, lens, retina, and finally in the cornea (53,58,59). In *Drosophila*, the genes *eyeless* (*ey*) and *twin of eyeless* (*toy*) encode proteins that are homologs of Pax6 (the *eyeless* gene has undergone duplication during insect evolution, placing *eyeless* under a direct control of *toy* (see 60 for details). Both *ey* and *toy* are expressed at high levels in the cells that will form a photoreceptor field of the *Drosophila* eye, as well as in some other regions of the developing nervous system. Weak mutations in *eyeless* lead to the reduction or complete loss of compound eyes, whereas strong ones are lethal when homozygous (35,36)—phenotypes mimicking the defects observed in mice. Even more striking was the observation that targeted expression of the *Drosophila eyeless* or mouse *Pax6* genes in various fly tissues led to the formation of ectopic eyes on wings, legs, and antennae (36,60). Recently, misexpression of *Pax6* has been shown to cause ectopic eye formation in vertebrates (61). These results demonstrate that *Pax6/eyeless* genes are not only required but are sufficient to promote eye development, and therefore have been called master control genes for eye morphogenesis (Fig. 2B).

A traditional view maintained by generations of morphologists, based on the drastic differences observed in eye development and structure in mammals, insects, and mollusks, holds that the eye organ evolved independently in different phyla (6). And indeed this is partly true, as the organization of the organ has diverged extensively in different animal lineages. However, the current evidence indicates that a variety of modern animals specify fields of photoreceptor cells using the same *Pax6* controls that triggered the development of the ancestral "eye." Recently, *Pax6* homologs have been also identified in other triploblastic animals (e.g., flatworms, nematodes), and even in Cnidarians (see 62 and references therein). Deep conservation in the visual system is further supported by the fact that all animals use opsins as photoreceptor proteins (63).

As was mentioned for *tinman/NKX2-5*, patterning genes do not work in isolation, and additional genetic circuitry beyond *Pax6* appears to be conserved in different animals. In the fly, Ey activates the expression of the genes for the nuclear proteins Sine oculis (So), Eyes absent (Eya), and Dachsund (Dac), all of which are also essential for eye development. Vertebrate homologs of these proteins have been identified (several *Six*, *Eya*, and *Dach* genes, respectively). Remarkably, their expression patterns, activation by Pax6, molecular interactions, and their role in eye and retinal development have also been conserved, further supporting the existence of a common pathway initiating the development of the visual system. *Dach* maps to human chromosome 13q21.3–22 and is a candidate gene for postaxial polydactyly type A2 (PAPA2), consistent with its additional expression in the limb primordia in both mice and flies (see 64 and references therein).

Limitations of space prevent us from describing other apparent examples of genetic conservation of animal patterning systems, such as a common mechanism for dorsal/ventral (D/V) patterning involving TGF-β family members Dpp/BMP-4 and their interacting ligands Sog/Chordin (65,66); recruitment of the *achaete-scute* genes for the establishment of neuronal precursor cells (67); expression of the *Distalless* (*Dlx*) genes in appendage primordia of many metazoans (19); periodic expression of *engrailed*-related genes, suggesting that the bilateral ancestor of vertebrates and insects might have used a common genetic system to control metamerization (68); conservation of genetic determinants for the anterior (*orthodenticle/Otx* and *empty spiracles/Emx*) and posterior (*caudal/Cdx*) ends of the body (69–72); deployment of the FGF pathway at multiple stages of tracheal and lung branching (73); and others. The existence of common genetic pathways between distantly related organisms suggests that the Urbilaterian (a common ancestor of all bilaterally symmetrical animals) was a sophisticated creature, with many architectural and organ-specifying genetic systems already in place (65,74). Figure 3 shows a proposed diagram of that ancestral worm-like creature.

Fascinating Questions Concerning the Function of Hox Genes in Humans

In every organism, architectural patterning genes are part of a complex developmental program encoded in that animal's genome. They have to be expressed in the right place at the right time, and they have to exert specific and precise control over their downstream target genes. Disrupting key interactions at any of these levels can lead to abnormal developmental decisions and ultimately result in mutant phenotypes. The remainder of this paper is devoted to analysis of several fascinating unsolved problems that reside at different levels in the Hox regulatory hierarchy, with an emphasis on implications for human pathology.

What are the mechanisms responsible for the establishment and maintenance of HOX gene expression in humans?

As mentioned before, persistent expression of Hox proteins is required to maintain the identity of cells along the A/P axis. From the studies in *Drosophila*, it has been known for some time that generation of stable Hox expression domains is a two-step process. The initiation phase is controlled by the products of the coordinate, gap and pair-rule genes that establish initial boundaries of Hox expression. In mammals, little is known about the upstream mechanisms for initiating Hox expression patterns. A few documented examples include the requirement of a zinc-finger transcription factor Krox20 for the activation of *Hoxb-2* in the hindbrain of developing mice (75), involvement of the Maf/b-zip protein Kreisler in *Hoxb-3* activation (76), and the role of retinoic acid receptors (RAR proteins) in controlling the boundaries of expression of multiple Hox genes (77). Homologs of such Hox regulators in *Drosophila* are apparently not directly involved in Hox gene activation or repression.

Recent experiments have provided more evidence for conservation at the next, or maintenance, phase of Hox expression. In both flies and mice the initial zones of Hox expression are stabilized and maintained by a direct action of the proteins from the Trithorax and Polycomb groups (TrxG and PcG, respectively). Extensive characterization of PcG and TrxG functions in *Drosophila* have shown that PcG proteins are transcriptional repressors of a variety of genes, including Hox genes (Fig. 4). Conversely, TrxG proteins are transcriptional activators on Hox genes, as well as many other loci (reviewed in 78–82). Many of these proteins have been highly conserved in evolution, and a PcG protein has even been found in plants (83).

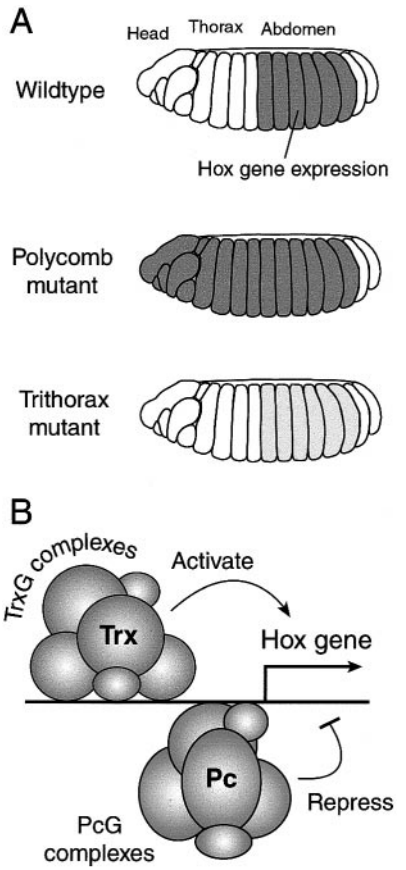

FIG. 4. The role of Polycomb (Pc) and Trithorax (Trx) group genes in the maintenance of Hox expression patterns. (A) Effects of Pc- and Trx-type mutations on domains of Hox gene expression. Upper panel shows a schematic expression domain of a Hox protein in a *Drosophila* embryo. In Polycomb group mutants (middle), the domain of expression of the Hox gene is expanded. Mutations in the Trithorax group genes (bottom) result in an opposite effect: the maintenance circuit is disrupted, which results in diminished levels of expression of the Hox gene. (B) The known molecular functions of TrxG and PcG proteins are accomplished in large multiprotein complexes that modify chromatin structure around Hox and other genes. PcG proteins (and their mammalian homologs, such as Eed, Bmi1, and others) are thought to be general repressors, whereas TrxG proteins (e.g., human Hrx) are general activators of Hox gene expression.

In mouse embryos that are mutants for PcG genes such as *Bmi1* or *eed*, Hox genes are expressed in more cells than in wild-type embryos, and such expanded expression domains can cause homeotic transformations (84–86) (Fig. 4A). Conversely, loss-of-function mutants in mouse TrxG genes have diminished levels of Hox gene products, with pheno-

types resembling mutations in the Hox genes themselves (87,88). The biochemical functions of TrxG and PcG members are achieved in multimeric protein complexes (Fig. 4B). In some cases, these complexes are known to maintain either an activated or repressed state of gene expression by regulating chromatin structure (89–91). In mammals, TrxG and PcG members are involved in developmental pathways such as hematopoiesis and cell proliferation in addition to their role in Hox gene transcription on the A/P body axis (84,92,93). For example, chromosomal rearrangements involving the human *HRX* gene (the homolog of *Drosophila trithorax*), known also as *MLL* or *ALL1*, often result in leukemias, which may be in part due to the deregulation of Hox genes in blood cells (reviewed in 94,95). The mutant defects that result from mutations in the Trx and Pc group genes have made them the subject of intensive clinical and genetic research.

In addition to TrxG and PcG control, the maintenance of Hox gene expression is facilitated by multiple auto- and cross-regulatory interactions. Thus, *Drosophila* proteins Lab and Dfd maintain their own transcription through autoactivation enhancers (96–99), and similar autoactivation control has been found in the murine homologs of these genes, Hoxb-1 and Hoxb-4 (13,100). Cross-regulatory relationships play an equally important role in determination of Hox transcription patterns (13).

What is the basis for the specificity of Hox function?

Molecular geneticists have been puzzled by an apparent paradox. On one hand, different Hox functions result in unique morphologies, which suggests a great deal of specificity in Hox action. On the other hand, Hox protein monomers bind very similar DNA sequences *in vitro,* and even when a slight preference in such binding is observed, the resulting sequence recognition variations are not sufficient to provide the necessary patterns of expression when tested *in vivo* (101–103). To reconcile these apparently contradictory observations, a hypothesis was put forward that other proteins, called modulators or cofactors, would assist Hox proteins in assembling specific activation or repression complexes on the regulatory elements of Hox target genes (104,105).

In recent years, ample experimental support has been provided for the cofactor theory. One of the best-studied examples is *Drosophila* Extradenticle (Exd), a protein with a highly divergent homeodomain (106,107). Interestingly, embryos lacking all *exd* function show loss of most segmental differentiation, without any apparent changes in the expression patterns of Hox genes. This suggests that the Exd protein works in parallel to or downstream of Hox proteins, and might directly contribute to their function (Fig. 5A). Indeed, Exd was found to form stable heterodimer complexes on DNA with a variety of Hox proteins, and recently a crystal structure of such a complex was determined (108–110). Moreover, Hox-Exd heterodimer binding sites have been found in the regulatory regions of some known Hox targets, and mutations in the target sequences that abolish Hox-Exd binding often result in a loss of reporter expression *in vivo.* Exd is highly homologous to mammalian Pbx1, originally identified as the chromosome 1 partner of the t(1;19) translocation in human preB-cell ALL (111,112). Heterodimeric Hox-Pbx1 complexes are very similar in structural and functional properties to the *Drosophila* Hox-Exd complexes, suggesting that Hox-Pbx interactions are evolutionarily ancient (113). Oncogenic effects of Pbx1 mutations have been attributed to alterations in the function of mammalian Hox proteins (112).

Cooperative binding of a Hox protein with Exd enhances both the strength of interaction and the specificity of interaction of the heterodimer with some DNA sites (105,114,115). Recent evidence suggests that Hox-Exd heterodimer binding is important, but not sufficient to explain the specificity of Hox function. First of all, other cofactors are involved, such as the divergent homeodomain protein Homothorax (Hth) that is related to mammalian Meis1 and Prep1 proteins (116–121). Hth controls nuclear localization of Exd, and also participates in formation of heterotrimeric Hox-Exd-Hth complexes on DNA (122,123). Also, recent analysis of several natural Hox response elements has shown that real enhancers are complex and contain multiple Hox and cofactor binding sites, all of which contribute to the overall output from that regulatory element (99,109,124–127). In addition to determining Hox binding specificity, cofactors can play a role in uncovering a covert activation potential of the Hox protein already bound to DNA (Fig. 5A) (109,125). Leukemogenic phenotypes of mutations in Pbx1, Meis1, and other cofactors suggest that precise control of Hox activity is required for making correct regulatory decisions in differentiating cells, such as those involved in hematopoiesis (111,128). There is

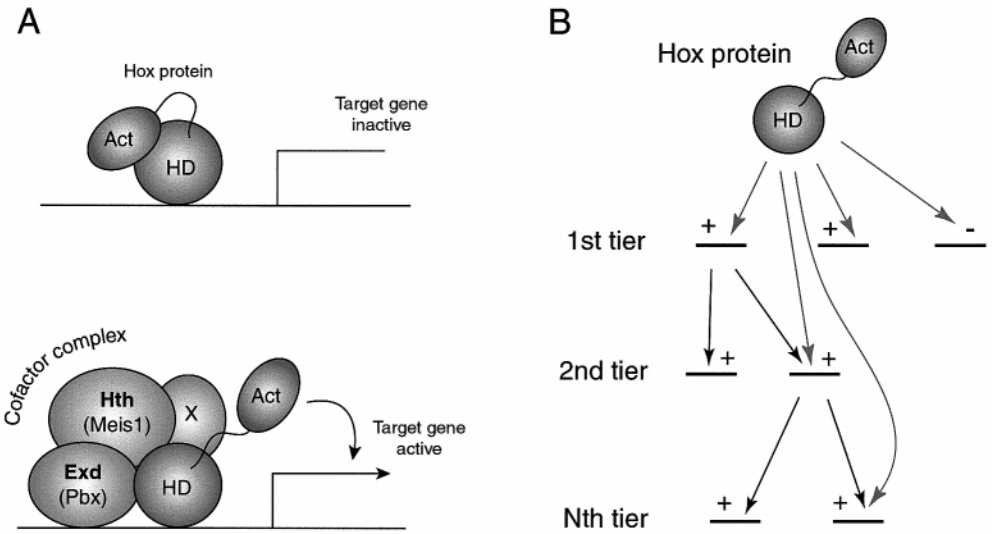

FIG. 5. Hox proteins function in association with cofactors and at multiple levels of their regulatory hierarchies. Monomer Hox proteins are capable of specifically binding DNA (A, top). However, such binding is probably neutral and has no effect on target gene expression. Multiple inputs from cofactor and modulator proteins are required to release the covert activation (or repression) potentials of the Hox proteins, as well as to stabilize their interactions with DNA (A, bottom). Examples of known *Drosophila* cofactors are shown in bold, and their mammalian homologs are given in parentheses. The protein labeled X indicates that there are likely to be other, as yet unidentified, cofactors and modulators. (B) According to several recent lines of evidence (136,138), Hox proteins are involved in target gene regulation at multiple levels in their hierarchical pathways (arrows). The first tier of Hox downstream genes includes immediate Hox targets, many of which are known to be transcription factors. These factors then activate or repress the genes at the second tier and further downstream, often in combination with the persistently expressed Hox proteins. This ultimately results in localized expression of the so-called "realizator genes." Realizators are the molecules involved in cell migration, adhesion, and differentiation, and their unique combinations determine the structural architecture of large fields of cells.

little doubt that the story of Hox cofactors and modulators will not be limited to interactions with Exd- and Hth-type proteins, and evidence for additional factors is gradually accumulating, primarily from genetic screens in the fly (129–132).

Hox targets: Dozens or thousands?

The functions of Hox proteins and their cofactors converge on Hox target genes. It has been recognized for some time that the morphological features that constitute the "identity" of a group of cells must be determined by a variety of proteins responsible for cell shape, movement, and differentiation. It is these "realizator" genes that are thought to be downstream of Hox hierarchical pathways (133). A variety of approaches, including testing candidate genes for Hox regulation, subtractive hybridization, and chromatin immunoprecipitation, have been employed in the search for Hox targets (reviewed in 134,135). The number of Hox targets has recently been proposed to be exceptionally numerous (136). However, only a limited number of candidate downstream genes have been determined to be directly under Hox control (137).

Recent experiments have provided clues for our understanding of the molecular logic of Hox target gene selection. It seems likely that Hox proteins can independently activate or repress many genes that function at different levels of the hierarchy leading from a Hox protein to a unique morphology (Fig. 5B). Thus, Hox proteins can directly control not only transcription factors that are still high in the regulatory pathway, but also genes for signaling proteins and other "realizator" functions (138). Moreover, many genes can apparently serve as direct targets for several Hox proteins (136,139). In order to understand how different Hox genes instruct one homologous structure to be different from another, we will have to know both the spectrum of their target genes and the architecture of their regulatory pathways.

Concluding Remarks

These are exciting times for developmental molecular genetics, particularly in the new genes and

insights that apply to human development. New discoveries have changed century-old paradigms in embryology and evolution and have allowed human medical genetics to become more sophisticated in its diagnostic and predictive power. In the race for understanding the molecular basis of disease, simple model organisms such as *Drosophila, C. elegans,* and others will continue to be an indispensable tool for providing answers relevant for human biology. At the functional genomic level, the research on these organisms will provide rich biological annotations when the human genomic sequence is finished, since fundamental body patterning mechanisms and the functions of key regulatory molecules have persisted through millions of years of evolutionary change. The recent technological breakthrough in gene expression profiling using DNA microarrays (140), combined with knowledge obtained from the *C. elegans*, *Drosophila,* and human genome sequences, will provide incredibly rapid advances in our understanding of developmental patterning genes under normal and pathological conditions.

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