Developmental Biology Select

Cell

The Hox genes encode homeodomain transcription factors that are crucial for body patterning during development. Hox genes exist in clusters and their chromosomal order mimics their spatial and temporal order of expression during embryonic development. This issue's Developmental Biology Select examines recent studies that shed further light on the key roles of Hox genes and their relatives during development and evolution.

Sealing a Whisker's Fate Map

The mouse trigeminal nerve collects sensory information from different parts of the face—such as the whiskers and lower jaw. These different branches of the trigeminal nerve provide inputs to nuclei in the brain stem from which the information is relayed to the thalamus and, ultimately, the somatosensory cortex. Oury et al. (2006) now report that the early segmentation of the mouse embryonic hindbrain correlates with the final topographical map of the neurons in the principal sensory (PrV) nucleus that receive input from the trigeminal nerve. During development, the vertebrate hindbrain is divided into segments called rhombomeres along the anterior/posterior axis. By genetically labeling specific rhombomeres to trace their fate, the authors found that certain regions in the PrV nucleus are derived from specific rhombomeres. For example, rhombomere 3 sets up the network of neurons in the PrV nucleus that receive sensory input from the whiskers. Given that Hox genes determine rhombomere identity, do these genes also contribute to the formation of this intricate pattern of neurons in the PrV nucleus? The authors homed in on *Hoxa2*, which is expressed in the second and third rhombomere and highly expressed in the region of PrV derived from rhombomere 3 during early development. Absence of *Hoxa2* resulted in a defect in the ability of the trigeminal ganglion neurons to find their way to the PrV, and instead they made aberrant projections into the cerebellum. During later stages of development, *Hoxa2* is required for arborization of "whisker" axons that project from the PrV nucleus to the thalamus. Lack of arborization of "whisker" axons in *Hoxa2*-deficient animals may be due to reduced expression in the PrV of Eph receptors, which are known to be important in axon guidance. Furthermore, during prenatal development, axons from the PrV nucleus in *Hox2a*-deficient mice project into the VPM nucleus but form aberrant patterns. The authors conclude that the correct "whisker map" is set up by *Hoxa2* expression in rhombomere 3. These results suggest that the complex neuronal circuitry in the face is established early in development and can be traced to specific to *Hox* gene expression in specific rhombomeres.

F. Oury et al. (2006). Science. Published online, August 10, 2006. 10.1126/science.1130042.

Hox Genes Don't Mind Trading Spaces

In mammals, there are 13 different Hox gene groups, each containing two to four Hox paralogs. Determining the function of these numerous Hox paralogs is an intense area of study. Tvrdik and Capecchi (2006) demonstrate that the two Hox paralogs, *Hoxa1* and *Hoxb1*, which are both involved in hindbrain segmentation, can be swapped in the mouse embryo with minimal interference to development. The proteins encoded by these genes have overlapping but not identical functions. Although these proteins are only 49% identical, their gene structure is conserved, and amino acid homology in the homeodomains is high. The authors expressed the Hox-A1 protein from the *Hoxb1* locus and vice versa. This exchange remarkably had little impact on mouse development in homozygous animals. Interestingly, hemizygous mice were not normal and exhibited some facial paralysis. The authors attributed this phenotype to reduced activity of the facial motor nerve as a result of decreased transcriptional activity of Hox-A1 when expressed from the *Hoxb1* locus. These findings indicate that when highly expressed (as in homozygotes), Hox-A1 can substitute for Hox-B1 and vice versa. However, when the amounts of these proteins are reduced (as in hemizygotes), Hox-A1 cannot maintain the level of expression of target genes that are normally under the control of Hox-B1. Insertion of the *Hoxb1* regulatory region upstream of *Hoxa1*—which allows this engineered *Hoxa1* locus to have the regulatory elements of both the *Hoxa1* and *Hoxb1* gene—results in normal development of the facial motor nerve even in the absence of Hox-B1. This engineered *Hoxa1* locus may recapitulate the structure of the ancestral Hox gene locus that existed prior to multiple gene duplications. Thus, the regulatory elements that control Hox gene expression may be crucial for the diversification of their function, which in some instances are interchangeable.

P. Tvrdik and M. R. Capecchi (2006). Dev. Cell ***11,*** *239–250.*

Stargazing at *homeobox* Sequences

Tracing the evolutionary origins of the Hox gene cluster is one way to infer the evolution of body plans given that these genes are key drivers of body patterning during development. Chourrout et al. (2006) examined Hox genes in two cnidarian species, *Nematostella vectensis* (sea anemone) and *Hydra magnipapillata* (freshwater polyp). Cnidaria predominantly display radial symmetry and are the sister group to the Bilateria. Using available genome shotgun sequences of these two cnidarians and BAC library screening, the authors identified their Hox genes and compared them to each other and to those of several basal bilaterians including the amphioxus *Branchiostoma floridae*. Hox genes are grouped based on the regions in the embryo that they specify, for example, the anterior and posterior Hox gene groups. Several Hox genes in *Nematostella* seem related to the anterior Hox genes of bilaterians and to the anterior ParaHox gene *gsx*. The ParaHox gene cluster is thought to have arisen by an early duplication of

***Nematostella vectensis*. Image courtesy of T. Nuechter.**

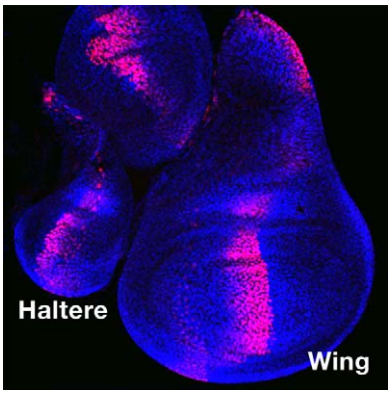
the "ProtoHox" cluster. However, the authors did not find strong candidates for posterior or central Hox genes in the two cnidarians that have been fully sequenced. As the Hox genes in bilaterians are clustered, the authors next examined the arrangement of homeobox genes in their two cnidarians. Several Hox genes in *Nematostella* are indeed clustered, yet mostly as a result of independent tandem gene duplication and rearrangement events. In contrast, no clustering was observed in *Hydra*, which seems to have lost many homeobox genes. The authors propose a model in which two anterior-like ProtoHox genes gave rise to a simple Hox and ParaHox gene cluster during evolution with the Hox gene cluster then expanding by gene duplications. Posterior and central Hox genes may never have existed in cnidarians. It is currently debated whether cnidarians could simply be bilaterians that lost their central and posterior Hox genes. Although the authors do not rule out this possibility, they favor the idea of a simple ProtoHox cluster with only anterior genes in the common ancestor of cnidarians and bilaterians. Accordingly, all nonanterior genes evolved independently in both lineages and a Hox cluster consisting of anterior, central and posterior genes, evolved only in the Bilateria after the split from the Cnidarians. One might speculate that this step was crucial for the evolution of diverse bilaterian body plans.

In a related study, Mulley et al. (2006) used sequence-based phylogenetics to understand why certain gene clusters such as the paraHox gene clusters (*Cdx*, *Xlox*, and *Gsx*) are maintained or lost in certain organisms. They show that the paraHox cluster was lost in the evolution of ray-finned fish (teleosts). The authors searched the available genome sequences of the zebrafish and two puffer fish (all teleosts) and determined that the paraHox genes are not clustered in these organisms though they are in the mouse, frog, human, and protochordate amphioxus. They then examined the paraHox cluster in teleost ancestors: the bichir (*Polypterus senegalus*) and the bowfin (*Amia calva).* Interestingly, these ancient organisms possess paraHox clusters. The authors propose that genome duplication, which occurred at the base of the teleost lineage, resulted in the breakup of the paraHox gene cluster in ray-finned fish. Given that the total number of paraHox genes is the same in teleosts, mouse and humans, the breakup of the paraHox gene cluster in ray-finned fish is probably due to gene loss.

*D. Chourrout et al. (2006) Nature **442**, 684–687.*
*J. F. Mulley et al. (2006) Proc. Natl. Acad. Sci U S A **103**, 10369-72. Published online June 26, 2006. 10.1073/pnas.0600341103*.

Hox genes take flight

To tackle the question of how organ size is controlled, Crickmore and Mann (2006) examine the flight machinery of the fruitfly: the wing and the haltere. The haltere is a small structure adjacent to the wing that is important for balance. The two structures are homologous but drastically different in size; the haltere has five times fewer cells than the wing. The differences between the haltere and the wing can be attributed to the Hox gene, *Ultrabithorax* (*Ubx*), which is expressed in the imaginal discs of the haltere, but not those of the wing. The absence of *Ubx* results in bigger haltere imaginal discs. Given that the long-range morphogen Decapentaplegic (Dpp) is important in determining wing size and is secreted from a specific band of cells in both the wing and the haltere, the authors focused on this morphogen. Interestingly, even in wild-type flies, the authors noticed a reduction in *dpp* expression in the haltere implying that Dpp may play a role in specifying the size of this organ. In fact, reducing the amount of Dpp in wings led to smaller wings. Additionally, the diffusion of Dpp is decreased in the haltere. The authors show that the decreased diffusion of Dpp is due to an increase in expression of Dpp's receptor, *tkv*, in the haltere. Increased expression of *tkv* in the wing reduced its size, whereas reduced expression of *tkv* in the haltere increased its size. How does Ubx fit into the picture? The authors propose that in haltere cells expressing *Ubx*, *tkv* is upregulated, an inhibitor of *tkv* is downregulated, and Dpp production and diffusion are limited resulting in the small size of these flight appendages. Although the mechanistic details of this pathway still need to be elucidated, this study reveals that by affecting morphogen gradients, a single Hox gene can orchestrate remarkable variation in two closely related appendages.



When compared with the wing, *dpp* expression (red) and the activation of the Dpp pathway (blue) are restricted in the developing haltere. Image courtesy of M. Crickmore.

*M. A. Crickmore and R. S. Mann (2006). Science **313**, 63–68. Published online June 1, 2006. 10.1126/science.1128650*.

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