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Intimations of a Creature

Minireview

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"It is impossible to say how the idea first entered my brain; but once conceived, it haunted me day and night. . . . I think it was his eye! yes, it was this! He had the eye of a vulture—" —Edgar Allan Poe, "The Tell-Tale Heart"

Strikingly different animals have more in common than meets the eye. Animal architecture is guided by many conserved regulators, among them homeobox genes that have related functions in mammals, insects, and worms. The surprising conservation of the regulators stands in stark contrast to the diversity of animal form. Our curiosity is drawn to the ancestral creatures that first used homeobox genes for pattern formation. Hints of the properties of these creatures lie in the genome. How were the master regulators retained as the creatures diverged? How were the earliest patterns of animal development controlled?

The genetic pathways that direct body patterning are remarkably durable, more enduring than the seemingly ancient geological structures around us. In some cases, a set of signaling components, such as the Ras entourage of signal transduction components, is used for more than one purpose in the same organism and for different purposes in different organisms. In other cases, a function seems conserved among diverse organisms. Homeobox genes offer three dramatic examples of functional conservation that serve to raise new questions about how evolution occurs.

The Hox *Gene Clusters*

Hox genes are a specific subset of the homeobox gene family, common to most or all animals and arranged in clusters on the chromosomes (reviewed by McGinnis and Krumlauf, 1992; Kenyon, 1994; Manak and Scott, 1994). Each member of the *Hox* gene cluster is expressed in a different domain along the anterior–posterior axis. Mutations in *Hox* genes lead to transformations of one part of the body into a copy of another, named homeosis by Bateson (1894), or to deletions of parts of the body pattern. In animals with repeating metameres, *Hox* genes direct differential development of metameres. A century ago the importance of metameric body organization and its evolutionary implications were clearly recognized: ". . . the resemblance between individual members of a series of Repeated Parts has led to the belief that they must originally have been alike, and that they have been formed by differentiation of members originally similar" (Bateson, 1894). Bateson saw homeotic changes affecting metameres as a key kind of variation, although metameric body plans are not necessary for Hox gene function. Pioneering studies by E. Lewis, A. Garcia-Bellido, T. Kaufman, and others clarified the ability of the *Hox* genes to control differentiation of segments in flies. Despite apparent differences in body plan among the nematodes, insects, and mammals, the patterning principles derived from studies of fly *Hox* genes are remarkably universal.

What Changes as Animals Evolve?

From the perspective of *Hox* regulators, central body is central body whether that happens to mean growing insect wings, a nematode vulva, or human ribs. Changes in the actions of *Hox* and other master regulators might be the basis for many kinds of evolutionary change in animal development. However, if *Hox* genes are conserved, what is not conserved? What makes animals different, the regulation of *Hox* genes or their effects on the genes they control?

Comparative studies of *Hox* gene expression and vertebral development show how changes in the regulation of *Hox* genes can be steps in evolution. It is an old question: "Which vertebra of a Pigeon, which has 15 cervical vertebrae, is homologous with the first dorsal vertebra of a Swan, which has 26 cervicals?" (Bateson, 1894). In modern terms, is cervical development guided by one *Hox* gene while the different shape of a thoracic vertebra depends on another? In this case, different birds would still display a correspondence between *Hox* gene expression and vertebral type. Or does a counting system, with reference to other vertebrae or body parts, decide where the transition from one vertebral type to the next will occur? In this case, *Hox* gene expression would be invariant in birds with different numbers of cervical vertebrae, but the response to a *Hox* gene—cervical or thoracic development—would change owing to other factors. A good correlation is observed between *Hox* gene expression and vertebral morphology (Gaunt, 1994; Burke et al., 1995). The particular transcription factor made determines the shape of the bone, working in the context of other factors regulating bone patterning. In making swans rather than pigeons, *Hox* genes needed for cervical vertebrae are expressed in more vertebrae. Similarly, differences in regulation of *Hox* genes in fly versus locust or butterfly abdomens correlate with morphological differences (Kelsh et al., 1993; Warren et al., 1994). The ability of a human *Antp*-like *Hox* gene ubiquitously expressed in flies to behave like fly *Antp* expressed in the same way also argues for the importance of regulatory changes with retention of downstream responses (Malicki et al., 1990).

A quite different answer comes from comparisons of insect wing morphology and gene expression. Flies have two wings on the second thoracic segment and two vestigial wings called halteres on the third thoracic segment. The difference is controlled by homeotic genes (Lewis, 1978). *Ubx* is expressed in halteres but not wings. Loss-of-function *Ubx* mutations convert halteres into T2 wings, while activation of *Ubx* in wing primordia causes halteres to develop (White and Akam, 1985). Butterflies have four wings; the straightforward correlation between morphology and *Hox* expression seen in bird vertebrae would predict the butterfly *Ubx* gene to be inactive in the butterfly wing primordia. The actual result is entirely different: a

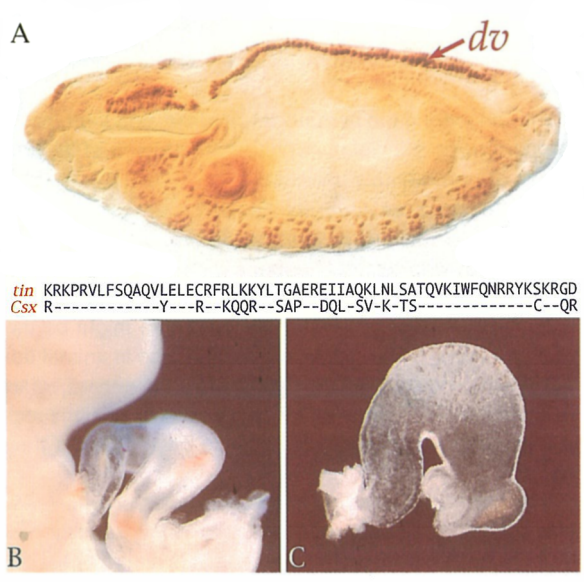

Figure 1. Two Types of Heart and Their Molecular Link

(A) The dorsal vessel (dv) of a Drosophila embryo stained to detect β-galactosidase using a fly line containing a *lacZ* gene governed by an unknown enhancer (figure provided by R. Bodmer).

(B and C) Embryonic chick hearts, in place and dissected. The developing eye is visible in the upper left of (B). The sequences of *tinman* and *Csx* homeodomains are shown aligned, with dashes representing identical residues. Circulation in both animals is from posterior to anterior (right to left in each panel).

high level of *Ubx* expression is observed in butterfly hindwing primordia (Warren et al., 1994). Instead of *Ubx* gene regulation having changed between butterflies and flies, the relationship with cofactors or target genes is likely to have changed. *Ubx* modifies the type of wing formed, into a haltere in flies and into a T3-type wing in butterflies.

As we celebrate the century since Bateson's insights, we also mark a decade of homeobox gene studies (McGinnis et al., 1984; Scott and Weiner, 1984). *Hox* genes no longer stand alone in demonstrating conservation of function. Other classes of homeobox genes provide examples of functional conservation in animal development nearly as dramatic as the retention of *Hox* cluster function.

The Tell-Tale Heart

Insect and mammalian hearts have very different final appearances but, surprisingly, the development of both hearts employs at least two common regulators (Bodmer, 1995) (Figure 1). The earliest known markers of vertebrate heart development are two genes known as *MEF2C* (Martin et al., 1993; McDermott et al., 1993) and *Csx* (for cardiac-specific homeobox) (Komuro and Izumo, 1993), also known as *Nkx-2.5* (Lints et al., 1993). Both proteins are expressed in 7.5-day mouse embryos, at a stage when the primordial heart is simply a region of thickened splanchnic mesoderm that forms endothelial tubes. Expression of *Csx* continues as the initial two endothelial tubes fuse to form the heart tube. Csx might work in parallel with the MEF2C protein, which is a member of the MADS box family of transcription factors. MCM1 of yeast is also a MADS box protein and is known to associate with the homeodomain yeast repressor protein MATα2 (reviewed by Johnson, 1992). Whether Csx protein associates with MEF2C is unknown. A mutation in *Nkx-2.5* causes abnormal heart development (described by Bodmer, 1995). Binding sites for MEF2 proteins are found in many muscle-specific genes, and the binding sites are in many cases important for proper expression, so MEF2C may play a role in muscle differentiation.

An insect heart also forms during the segregation of different types of mesoderm (Bodmer, 1995). Cells on the ventral side of the blastoderm fly embryo invaginate and spread along the interior walls of the ectoderm. The cells segregate into two layers, an outer somatopleure that will form body wall musculature and an inner splanchnopleure that will form visceral muscle. Mederm cells dorsal to the splanchnopleure, which may be a distinct type of mesoderm, form the tube-shaped dorsal vessel. The heart forms from cardioblast cells at the posterior part of the dorsal vessel tube, at the dorsal midline of the embryo. The heartbeat is independent of innervation, as in the early embryonic vertebrate heart.

The fly homologs of *Csx* and *MEF2*, called *tinman* and *D-mef2* (Bodmer, 1993; Azpiazu and Frasch, 1993; Lilly et al., 1994; other references in Bodmer, 1995) are both implicated in heart development. *tinman* mutants have no heart or visceral mesoderm. Early regulation of mesoderm differentiation in insects has some similarity to the pathway for vertebrates (Lilly et al., 1994). In insects, *tinman* and *D-mef2* are initiated in all mesoderm cells, but, after segregation of somatopleure and splanchnopleure, both genes remain active in the visceral mesoderm, while only *D-mef2* expression persists in somatopleure. When the splanchnopleure separates into gut mesoderm and dorsal vessel mesoderm, only *tinman* expression persists. Thus, astonishingly, two early markers of heart precursors are common to flies and mice.

The Creature's Eye

A third striking case of conservation of homeobox gene function occurs in eye development. The *Pax6* gene is required for eye development in mice, humans, and flies. The autosomal dominant disease aniridia, which is associated with mutations in the *Pax6* gene (Ton et al., 1991), affects humans by preventing development of the iris, an exceptional muscle derived from the ectoderm rather than mesoderm. In mice, the corresponding gene is associated with the small eye semidominant mutation, which when homozygous causes mice to develop without eyes or nose. The mouse gene is expressed in the neural tube, fetal eye, retina, lens, cerebellum, and olfactory bulbs. The expression of *Pax6* in both retina and lens suggests that it could be involved in differentiation of both and that it could also stimulate the signals involved in the mutual induction of the two tissues or induction of cornea.

Compound eyes are formed in flies from imaginal discs, where cell–cell signaling events organize small groups of cells into the primordia of individual ommatidia (Tomlinson, 1985). Insect and vertebrate eyes seem almost completely distinct in structure, yet the Drosophila *eyeless* gene encodes the fly homolog of *Pax6* (Quiring et al., 1994). *eyeless* mutations are recessive; the most severe alleles are lethal. The gene is first transcribed in the central nervous system of embryos and then in the embryonic

primordia of the eye imaginal discs. At later stages, *eyeless* is transiently expressed early in compound eye differentiation, prior to the sorting of cells into ommatidia. In both insects and mammals, the timing of *Pax6* expression suggests early roles in forming eyes and later roles in their differentiation.

The Creature So Far: An Archetype?

These three examples, *Hox*, *Csx*, and *Pax6*, together with other conserved gene functions, imply a primeval creature ancestral to insects and mammals with the following characteristics. The creature had a clear anterior–posterior axis with at least the four types of *Hox* gene present in nematodes, flies, and mammals. A *labial* class homeobox gene was used to define more anterior structures and an *abdominal-B* class gene to define more posterior structures. Central structures were governed by representatives of the *Deformed* and *Antp* classes and perhaps others. All of these *Hox* genes probably worked in multiple tissue types. *Orthodenticle*, *empty spiracles*, and *Distal-less* genes may have acted in head and brain much as the *Hox* genes act in the trunk (reviewed by Manak and Scott, 1994). A heart of sorts was built from mesodermal cells expressing a homeobox gene of the *Csx/tinman* class and possibly a *MEF2* MADS box gene. Some sort of light detection or related brain function was marked by cells making Pax6 protein.

Locking in a Dedicated Regulator: Seminal Regulatory Interactions

Could the observations of homeodomain protein functional conservation be misleading? Could convergent evolution explain the similarities in homeodomain function? Most homeobox genes are active in more than one tissue and do not appear to be dedicated to a single developmental process, so a focus on tissues expressing a gene both in mammals and insects is somewhat biased. Perhaps all the homeodomain protein classes existed in primitive organisms and were available for use in pattern formation as creatures became more complex. A convergent evolution model must then explain why particular types of homeodomain protein are especially well suited to early heart differentiation or early eye development and therefore were independently adopted for the purpose in already separated animal lineages. As unappealing as this idea may seem, it has not been firmly ruled out. In particular, a much clearer understanding of the relation between homeodomain proteins and their cofactors and regulated target genes is needed to understand how molecules could have become dedicated to particular developmental processes.

What might make a heart gene a heart gene? A key attribute of an organ or a specific axial part of the body may have required a specialized protein, a certain signal to be sent, a particular cellular architecture, or the proper timing of gene activation during development. The establishment of a regulatory interaction between a homeodomain transcription factor and such a target would constitute a founder event for a type of tissue or organ, together with transcription of the homeobox gene only in certain cells. The establishment of the relation between a particular homeodomain protein and a specialized target can be designated as a seminal regulatory interaction (SRI). The regulation of the same target gene by ancestors of *Csx/tinman* or of *Pax6/eyeless* in both mammals and insects would be a candidate SRI. Parts of the cytoskeleton required for tube formation (or innervation-independent contractile systems) might have been a target of the ancestor of *Csx*. Signal transduction systems involved in photodetection might have been early targets of *Pax6*. With time, other useful genes could come under the influence of the regulator, the evolutionary process occurring by random generation (or transposition) of enhancers. Acquisition of a Pax6 response element would give eye expression, allowing modifications of the eye. Useful constellations of targets would be retained, along with neutral targets that might constitute working material for further evolution. Multiple target genes under the control of one homeodomain protein would lock in the structure of both regulator and target, as neither could change without simultaneous compensatory changes in multiple other genes. The SRI is then the founding event in the evolution of a specialized tissue. A different sort of SRI is also possible: the evolution of homeodomain protein structures allowing useful protein–protein interactions.

An alternative to the SRI model could be referred to as the "because-it-is-there" model. If a homeodomain protein is produced only in certain tissues, it might be co-opted for controlling novel targets in those tissues. A gene might work both in mammalian and insect eye development because in a common ancestor the gene was expressed in cell types that evolved to form eyes, but the relationship between homeodomain protein and downstream targets would not be conserved. This model does not account in any simple way for why the homeodomain protein was expressed differentially in the ancestor, but accidental regulation might suffice. Whether our creature had established SRIs or localized homeodomain proteins ready to acquire distinct targets in distinct descendants, once a relationship between regulator and target was established the entire pathway could be co-opted for different developmental events simply by activation of the homeobox gene.

The three homeobox genes discussed here are only some of the genes whose functions seem conserved. *Prospero*-like homeobox genes act during neuronal differentiation (Doe et al., 1991; Vaessin et al., 1991; Oliver et al., 1993). *forkhead* class transcription factors, related to homeodomain proteins in structure, seem especially important for endoderm development (Weigel et al., 1989; Lai et al., 1993). The functions of *achaete–scute*, *MyoD*, *even-skipped*, and *hedgehog* genes provide intriguing parallels in diverse organisms. The discovery of additional cases of homeodomain proteins and other regulators largely dedicated to particular tissues or organs seems likely. Each case of functional conservation will provide new reasons to focus on the critical issue of how the action of a conserved regulator is interpreted distinctly to create the vast diversity of creatures.

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