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Hox genes, Arms and the Man

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Homeodomain transcription factors come in a plethora of flavours, but among the most remarkable are the clustered Hox genes, famous for their control of anterior-posterior patterning during the embryonic development of flies, mice and worms. *Hox* mutations can lead to the development of legs in lieu of antennae in flies, to vertebral transformations in mammals, and, as demonstrated by Mortlock and Innis in this issue (page 179), to syndactyly and uterine abnormalities in humans¹. The 39 mammalian Hox genes, organized in four rather similar clusters that may be partly redundant, are arranged just as Ed Lewis found the fly genes: genes that work in anterior plus posterior regions are at one end of the cluster and genes that work only in more posterior regions are at the other. Hox genes provided the first of many dramatic cases of developmental gene conservation across vast spans of the animal kingdom.

With so many Hox genes, one might have expected a wealth of human and mouse diseases to be associated with their mutation. A rival homeobox gene class, the PAX genes, stepped out ahead with a remarkable array of disease connections² and Hox enthusiasts were left mumbling about redundancy and the obvious, if obscured, importance of genes that are so vital that they become tetraploid. Now HOX disease connections are attempting to catch up³. Human synpolydactyly has previously been linked to a mutation in

*HOXD13*⁴ and Mortlock and Innis have pushed HOX past the second post in demonstrating that mutations in *HOXA13*, the closest relative of *HOXD13*, are responsible for

Examples of polydactyly from *Materials for the Study of Variation Treated with Especial Regard to Discontinuity in the Origin of Species* by William Bateson (MacMillan & Co., London and New York, 1894).

IMAGE
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REASONS

Hand-foot-genital (HFG) syndrome. Limbs of HFG patients have reduced anterior and posterior digits; females often have a defect in Mullerian duct fusion, while males may have a ventrally displaced urethra. Other HOX genes may back up *HOXA13* in some tissues but not (adequately) in the two most affected tissues: limb and uterus. HFG was foreshadowed in earlier work by the same

authors on hypodactyly (Hd) in mice, which have a mutant *Hoxa13* and a similar phenotype to HFG in people⁵.

Mouse mutations have provided substantial information about Hox function. The first Hox phenotype observed in mice, megacolon, was a gain-of-function effect caused by overexpression of *Hoxa4* (ref. 6). Additional misexpression studies have been conducted in mice, frogs and chickens. *Hoxa13*, the HFG villain, dramatically inhibits the growth of proximal (large) limb bones when expressed ubiquitously with a retrovirus⁷. This was interpreted as a homeotic transformation of proximal bones into distal (small) bones but may equally indicate a role in growth control. Loss-of-function mutations, like the *Hox* knockouts constructed in mice, provide substantial support for parallels with the fly, in which Hox genes are active in nested overlapping domains, with more genes working in posterior tissues than in anterior ones. A consequence is that loss of Hox function often leads to transformations of posterior to anterior structures, while inappropriate *Hox* expression in anterior regions causes more posterior structures to develop there^{8–10}. Sometimes a structure is lost rather than homeotically transformed, implying that normal Hox functions are needed to stimulate growth. In the limb, the growth effects of Hox mutations are much more in evidence than are homeotic transformations.

Ever since Bateson pointed out the rele-

vance of polydactyly to evolutionary considerations in 1894¹¹ (by interesting coincidence, G. Bernard Shaw published *Arms and the Man* in the same year), the limb's pattern and polarity have made it a focus for biologists and others (see refs 12, 13). Limb phenotypes are common among Hox mutants. In vertebrates a basic repeating structure is modified into diverse shapes by Hox gene action, but in the limb, growth of the digits occurs as the pattern is formed so that Hox genes may affect growth^{8,14,15}, placement of joints, and polarization of structures. Because limb growth is experimentally difficult to separate from pattern, contributions of Hox genes to patterning are obscured. *Hoxd13* mutant mice have limb defects that appear to be due to altered cell proliferation. Similarly mice lacking both *Hoxd11* and *Hoxa11* or *Hoxa11* and *Hoxd12*—the genes are partly redundant—have a reduced radius and ulna^{6,13}. If the only effect of Hox proteins is to control growth, they could all regulate the same target genes — an attractive simplification compared with ideas involving coordination of many targets regulated with great specificity. Activation of *HOXA9* by retroviral insertion can lead to myeloid leukaemia^{17,18}, another indication of a link between Hox function and growth.

How do Hox genes fit in with other powerful limb building genes? The players in limb development are a familiar cast: fibroblast growth factors (Fgf), bone morphogenetic proteins (Bmp), hedgehogs, Wnt signals, retinoids and their receptors, Gli-class and Hox-class transcription factors and others¹⁹. Some fibroblast growth factors are capable of initiating limb bud outgrowth from a competent flank of a chick embryo²⁰. One effect of implanting Fgf-soaked beads is to activate *Sonic hedgehog* (*Shh*), a gene that encodes a signaling protein and has become as popular as its eponymous video game character. During normal limb development, *Shh* is transcriptionally activated in the posterior limb bud mesenchyme, the zone of polarizing activity (ZPA). *Shh* appears sufficient to provide the known functions of the ZPA and is also necessary for the persistent expression of Fgf and therefore the continued outgrowth of the limb bud^{21,22}. Indeed regulators of all three axes, distal-proximal, anterior-posterior and dorsal-ventral, interact to give coordinated growth and patterning²³.

Removing *Shh* function from the limb prevents formation of distal limbs²⁴. Providing ectopic *Shh* to anterior chick limb mesenchyme causes a dramatic outgrowth of tissue and persuades limbs to grow posteriorly at the front end of the autopod, resulting in the frequent formation of extra digits²⁵. Components of the *Shh* signaling pathway affect limb patterning, at least in

part through *Hox* genes. *Shh* works in a peculiar double negative pathway²⁶. It inhibits the function of its receptor, *Patched* (*Ptc*), which itself appears to be an inhibitor of the transcription of multiple target genes including Hox genes and *Bmp2*. Smoothed (*Smo*), a seven transmembrane protein, is associated with *Ptc* in the membrane²⁷. The current hypothesis has *Ptc* inhibiting *Smo* until *Shh* comes along and binds to *Ptc*. *Shh* binding is believed to somehow unleash *Smo* activity, resulting in a signal to the nucleus to activate *Shh* target genes. In the limb, therefore, *Ptc* is thought to successfully repress target genes (including Hox genes) in more anterior regions while this repression is relieved in the posterior by *Shh* signal. The complex patterns of Hox expression may rely upon differential sensitivity to *Shh* signal and to other regulators.

PTC has recently been identified as the guilty party in a rare inherited human disease called basal cell nevus syndrome (BCNS)^{28,29}. BCNS patients have a heightened frequency of basal cell carcinoma, medulloblastoma and developmental abnormalities. One feature of BCNS is a tendency toward polydactyly and syndactyly, as might be expected if insufficient *PTC* function allows SHH targets, such as HOX genes, to come on where they should be off. Targets of SHH in the limb bud include *GLI*, a gene sometimes amplified in human glioblastomas and encoding a zinc finger protein³⁰ and BMP2. *GLI* genes come as a set of three³¹, one of which, *GLI3*, has been identified as the relevant gene in the human syndrome Greig cephalopolysyndactyly³² and also *extratoes*, a mouse mutant with a number of skeletal abnormalities³³. *GLI* proteins may be the transcription-regulating output of the SHH signaling pathway^{34,35}.

In response to SHH and perhaps other regulators, Hox genes perform a remarkably complex ritual that has been described extensively in chick limbs³⁶. Early in limb bud development the whole bud expresses *Hoxd9* and *Hoxd10*. In the second phase of development, *Hoxd9*, *10*, *11*, and *12* are expressed in nested patterns in more proximal regions of the limb starting from the posterior bud and spreading forward; posterior cells (which have more *Shh*) activate all of these Hox genes and anterior cells, progressively fewer. A third wave of expression involves *Hoxa* and *Hoxd* genes in the distal limb, the presumptive autopod that will form the digits. Here *Hoxa13* (our villain of the hour) and *Hoxd13* are expressed in most of the autopod cells; *Hoxd13* with a somewhat more posteriorly restricted domain. Hox genes may be important mediators and refiners of *Shh* commands with distinct roles at different stages of limb development.

What lies downstream of HOX genes?

Two candidates are Indian hedgehog³⁷, which is involved in the control of chondrogenesis, and BMP proteins which govern skeletal patterning and the placement of joints^{38,39}. Many puzzles remain. How is growth related to patterning in the limb bud? How is HOX regulation integrated with other pattern regulators? Are SHH effects on BMP transcription direct or are they mediated by HOX genes? Why does *GLI* amplification cause glioblastomas and not, so far as is known, other tumor types? What genes are regulated by HOX genes and how do they affect growth and patterning? Why are the A13 and D13 genes but not, as yet, 37 others, implicated in human developmental diseases? When the remaining mysteries are mustered, it seems that we understand very little about how HOX genes work. The future holds great excitement — at the moment, we're just limbering up. □

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