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Genomic Regulation of *Hox* Collinearity

During vertebrate limb development, *Hoxd* genes are transcribed in two temporal phases; an early wave controls growth and polarity up to the forearm and a late wave patterns the digits. In this issue of *Developmental Cell*, Tarchini and Duboule (2006) report that two opposite regulatory modules direct early collinear expression of *Hoxd* genes.

The vertebrate limb is a complex structure. The human forelimb, for example, contains 29 bones that are polarized along three axes: proximodistal (shoulder to fingertips), anteroposterior (thumb to small finger) and dorsoventral (back of hand to palm). The limb skeleton develops from a simple bud of undifferentiated mesenchyme that belies its ultimate complexity. Transformation of a homogeneous population of cells into this elaborate network of structures involves establishment of positional information and translation of these coordinates into differentiation programs by cells in different parts of the bud (Wolpert, 1996). Paramount to the orchestration of this process are the *Hox* genes, clustered transcription factors whose primary role in animal evolution has been to pattern the head-to-tail axis. The origin of fins (the forerunners to tetrapod limbs) involved cooption of this axial patterning system to establish polarity and regulate growth of the appendages.

Hoxd genes are activated in two transcriptional waves that are associated with distinct early and late phases of limb development, when, respectively, proximal and distal structures are laid down (Nelson et al., 1996). They are deployed sequentially, in both time and space, with genes situated at the telomeric (3′) end of the cluster (e.g., *Hoxd9*) being expressed before, and anterior to, their centromeric (5′) neighbors (e.g., *Hoxd13*). Over the course of limb development, these domains become dynamic, with different phases of expression corresponding to specific regions of the emerging limb skeleton. *Hox* gene expression adheres to the rules of collinear gene regulation, first described by Ed Lewis for the *Drosophila* Bithorax complex (Lewis, 1978). The phenomena of spatial and temporal collinearity have been recognized in vertebrate *Hox* genes for nearly two decades (Gaunt, 1988), but only recently have the genomic mechanisms underlying collinear transcription of the *Hoxd* complex been revealed. The late phase of *Hoxd* expression in the distal part of the limb controls development of the digits, and Duboule and colleagues have shown that a single enhancer (the "digit enhancer"), embedded within a *g*lobal *c*ontrol *r*egion (GCR), regulates the timing, spatial position, and quantitative levels of transcriptional activity (Kmita et al., 2002; Spitz et al., 2001; Zákány et al., 2004). In this issue of *Developmental Cell*, Tarchini and Duboule (2006) report on the identification of two regulatory elements that direct the first wave of *Hoxd* transcription, which controls development up to the forearm.

By generating an extensive series of mouse lines carrying LoxP sites between each of the *Hoxd* genes, the Duboule laboratory has engineered an elegant series of duplications and deletions within the *Hoxd* cluster to determine the effects of these rearrangements on transcription. Previously, this approach uncovered the existence of regulatory sequences located outside the cluster itself (Spitz et al., 2001). The picture was refined when an inversion of the *Hox* cluster demonstrated that this regulatory domain, termed the *e*arly *l*imb *c*ontrol *r*egion (ELCR), is located on the telomeric side of the cluster. The ELCR can act on any of the *Hoxd* promoters and establishes the anteroposterior polarity of the 5′ *Hoxd* genes, which is required for positioning the *Sonic hedgehog* domain posteriorly (Zákány et al., 2004). The position at which a given *Hoxd* gene is expressed in the limb may simply reflect the gene's proximity to this telomeric enhancer (Zákány et al., 2004).

Now, Tarchini and Duboule (2006) have engineered a series of "nested deletions," in which they excised DNA between *Hoxd13* and either *Hoxd8*, *Hoxd9*, *Hoxd10*, or *Hoxd11* to investigate the effect of bringing a gene closer to the telomereric side of the cluster. In each of these deletion experiments, they observed a systematic premature activation of genes located 5′ to the deletion breakpoints, consistent with the existence of telomerically positioned regulatory sequences. They also observed a disruption of spatial collinearity, as genes located 5′ to the deletion breakpoints were expressed more anteriorly. The authors then generated a series of "nested internal deletions," in which one to three genes between *Hoxd8* and *Hoxd13* were removed, leaving intact the native genes on either side of the deletion, and these excisions caused similar changes to the transcriptional timing and spatial localization of the remaining genes.

In a complementary experiment, the authors produced internal duplications within the *Hoxd* cluster, by generating a tandem repeat of the region containing *Hoxd8* and either *Hoxd9* or *Hoxd10*. This resulted in an opposite effect on the temporal and spatial collinearity; genes situated 5′ to the duplicated fragments experienced delayed transcription and were expressed more posteriorly in the limb buds. Together, their experiments demonstrate the importance of genomic distance between the transcriptional units and the ELCR to the timing and pattern of *Hoxd* gene expression in the early limb bud. If transcriptional patterns of *Hoxd* genes simply reflect the spatial relationship of a *Hoxd* gene to the ELCR, then genes located 3′ to the deletions should not be affected. However, Tarchini and Duboule found that expression of these genes is posteriorized in the presumptive forearm, leading them to conclude that regulatory regions on the other (5′) side of the cluster may have an opposite effect on collinearity by restricting *Hoxd* expression to the posterior part of the limb.

Interestingly, when the authors examined later stages of limb development, they found that internal duplications did not alter *Hoxd* patterns in the handplate. In the presumptive forearm, by contrast, *Hoxd* expression domains were weaker and anteriorly truncated. These observations support the idea that early/proximal and late/distal *Hoxd* expression in the limb buds is regulated by an independent set of mechanisms. The authors propose that collinear expression of *Hoxd* genes along the proximodistal axis of the limb may not result from mechanistic linkage, but instead may be an artifact of the proximal and distal domains being under separate regulatory control ("virtual collinearity").

This independence may reflect the different evolutionary histories of the proximal and distal parts of the limb (Shubin et al., 1997; Sordino et al., 1995). The transition from fish fins to tetrapod limbs involved development of a new set of structures, the digits, and the regulatory independence revealed here makes it unlikely that modulation of the ELCR played a role in this evolutionary innovation. Rather, the second wave of *Hoxd* transcription that controls digit formation may have been facilitated by evolution of a novel regulatory element, the digit enhancer (Spitz et al., 2001). Resolution of this evolutionary question will require a more detailed understanding of Hox gene expression, and the underlying regulation and genomic organization, in the fins of fishes at key phylogenetic positions, such as sharks and lungfishes.

Perhaps the most important implication of this work for our understanding of evolution is the discovery that the genomic distance between a transcriptional unit and its regulatory element can affect the temporal and spatial pattern of gene expression. Modulation of these distances can be achieved by gain or loss of nucleotides in intergenic DNA, regions traditionally viewed as inconsequential to morphological evolution. This mechanism for introducing subtle alterations to the spatial and temporal patterns of *Hox* gene expression (and the resultant morphology) provides an appealing alternative to evolution by accumulation of mutations in regulatory elements or coding regions. Comparative genomic and developmental analyses will reveal the extent to which the distances across the genomic landscape have shaped the evolution of development.

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