Organizing Axes in Time and Space; 25 Years of Colinear Tinkering

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During vertebrate development, clustered genes from the Hox family of transcription factors are activated in a precise temporal and spatial sequence that follows their chromosomal order (the "Hox clock"). Recent advances in the knowledge of the underlying mechanisms reveal that the embryo uses a variety of strategies to implement this colinear process, depending on both the type and the evolutionary history of axial structures. The search for a universal mechanism has likely hampered our understanding of this enigmatic phenomenon, which may be caused by various and unrelated regulatory processes, as long as the final distribution of proteins (the Hox code) is preserved.

In the course of animal embryogenesis, distinct morphological identities are established along the body axes. For example, mammals have thoracic vertebrae that bear ribs, whereas cervical vertebrae do not, and digits are eventually positioned at the distal ends of our limbs, rather than elsewhere. The genetic mechanism under-lying this patterning system was uncovered by studying mutations in Drosophila where correct structures were wrongly positioned. Drastic alterations, such as the outgrowth of a limb instead of an antenna or of a wing in place of a haltere, are associated with the misexpression of gene members of the Hox family of transcription factors.

Because Hox proteins are at work in animals displaying a variety of morphologies, they likely act as developmental switches, rather than as specific stonework of the body architecture. Twenty-five years ago, Lewis (1) showed that Hox genes were clustered along the chromosome, colinear with their domains of action in the thorax and abdomen of flies. This observation was subsequently extended to vertebrates and other animals (2). In many instances, these pro-

Decoding the Hox Code; Posterior Prevalence at Work

In flies, eight Hox genes belong to the Antennapedia and Bithorax clusters [the HOM complex (6)]. In many instances, these pro-

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In vertebrates, successively more caudal body levels tend to show an increasing amount and diversity of Hox products, resulting from the expression strategy (Fig. 1). Yet segmented structures do not become more elaborate toward the caudal end of the embryo, nor do they display a greater potential for variation after gene-inactivation experiments, thus excluding a strict combinatorial input. The patterning information delivered at one particular body level primarily relies on one Hox protein (or a group of paralogs), rather than on a combination of proteins (Fig. 1). The most posteriorly expressed gene usually imposes its function over that of more anterior genes through a suppressive mechanism that does not involve transcriptional repression (13). This posterior prevalence (14, 15) explains why the phenotypes induced by vertebrate Hox mutations are restricted either to a few body segments or to the upper morphological window in which a given group of paralogs is at work (16, 17). Large overlapping expression domains are merely another way to produce discrete functional domains (Fig. 1D).

Posterior prevalence is an interesting property for morphological evolution, given that an anterior shift in the expression of a caudal gene would lead to the functional inactivation of more rostral components (such as genes A and M in Fig. 1D). Therefore, the functional interplay between Hox proteins is the result of their colinear dis-

The Hox Clock

Vertebrate development follows a rostral-to-caudal temporal progression, best exemplified by somitogenesis (18). Initially, Hox genes are activated along and in the neigh-

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Spatial expression of a given Hox gene does not seem to depend critically on its location either within the cluster or as a randomly integrated transgene (20–22), even though a time delay was often scored in the latter situation. However, whereas targeted rearrangements of a cluster in vivo have uncovered a cluster-intrinsic timing device (23, 24), the deregulation of the posterior gene Hoxd13 observed after the positioning of an anterior gene nearby is at odds with this view (25). Taken together, these results suggest that the colinearity in temporal activation of Hox genes may not be the sole cause of their future spatial transcript distribution. If so, what is the rationale of this Hox clock, and how does it work? Unlike the situation in flies, where the activation mechanism depends on factors unequally distributed by the segmentation process, three classes of mechanisms have been evoked to implement colinearity in vertebrates, alone or in combination (Fig. 2).

The End Justifies the Means

The first mechanism relies on the progressive transcriptional availability of Hox genes, from one end of the cluster to the other, a process that may or may not be independent of their own transcription. For example, repressive or silencing factors (26, 27) could be released through a passive transition in chromatin states (24). Alternatively, transcription of the genes themselves could help remodel chromatin to allow the next gene to be accessed. The failure of the posterior HoxD cluster to efficiently repress the early expressed Hoxb1/lacz transgene (25) supports the latter possibility and suggests that an early gene can still recruit the necessary factors to be activated in a timely manner, even when positioned within a “closed” domain. In this view, a chromatin-dependent colinear process would involve a transcriptional entry point at one side of the cluster that triggers the processing from a closed to an open configuration through a proximity effect. This would allow for progressively more genes to be transcribed toward the other extremity of the cluster (Fig. 2B). The comparison between the transcriptional availability of genes within and outside their cluster (28) supports this view.

The second scenario proposes that colinear activation in time and space is orchestrated by the integration of locally cis-acting control sequences. A gradient of signaling molecules [such as Fgf (29, 30)] could ultimately be read by a series of upstream sequences showing increasing or decreasing affinities for the effector molecule, all along the cluster. Because local enhancer functions are shared among subsets of neighboring genes, they ultimately provide distinct expression features through unequal partitioning of their activities on the genes they control (Fig. 2C) (31). Although this strategy accounts for the rather precise activation of randomly integrated transgenes, it may not be a key factor in tightly maintaining genes in clusters. Hence, it is likely not a primary mechanistic basis for the Hox clock.

The third possibility involves the existence of global enhancer sequences, located outside the clusters, which can regulate several genes in a relatively promoter-unspecific manner (Fig. 2D). The positions of these enhancers, close to either end of a Hox complex, introduce an intrinsic regulatory asymmetry that can be subsequently translated into a colinear mechanism. For example, the cycling expression of Hoxd genes in the presomitic mesoderm, in coordination with segmentation, involves a regulatory element located outside the cluster, which can act over several genes at different times (32). This regulatory element may be an outcome of the segmentation process, setting up the pace of the Hox clock and thereby keeping these two key aspects of patterning in phase with each other (32, 33).

Likewise, colinearity in developing limbs was recently shown to rely on the existence of a global digit enhancer element located far upstream on the other side of the cluster (34). Sequence-specific enhancer tropism, as well as promoter competition, eventually induces the terminal genes to be expressed in the most distal structures, the digits, with a progressively decreasing efficiency (35). In this case, colinearity is determined by the action of a global enhancer, and the necessity of gene clustering for obtaining the observed patterns can be readily understood.

Colinear Tinkering

It is also possible that gene clustering is required to maintain the colinear pattern, rather than to establish it. Indeed, correct transcriptional initiation must be followed by the persistence of the transcript domain to affect the morphology. Premature gene activation induced only subtle or transient variations in the AP level of the expression pattern (24). Conversely, a delay in gene acti-
remodeling. In this model, genes are initially silenced to become progres-

sively activated through a time-based activation process. This could set the stage for a subsequent wave of activation that would depend on cis-acting mechanisms, including auto-regulation and cross-regulation. This second phase could account for the colinear expression of randomly integrated genes, which would correctly execute the second phase of activation. The mechanisms described here are not exclusive. Instead, they may work in combination with each other, which might explain why a clear picture has not yet fully emerged. It is possible that these various processes reflect different phylogenetic histories. Perhaps an increase in both the number and complexity of structures to be patterned, as well as the design or improvement of original developmental strategies (such as for segmentation or appendages), triggered a novel colinear process and superimposed it on an older one. For instance, the design of a novel global enhancer–based colinear process, such as the one in limbs, may have been facilitated by the existence of a gene cluster already at work in a different context. From the perspective of the embryo, any mechanism that will ultimately generate the necessary distribution of Hox gene products, within a given axial structure, at the right time, should be acceptable. Thus, searching for a single and universal mechanistic explanation for colinearity may be a futile quest. Is this why we have experienced such difficulties in accommodating the large body of experimental data within a well-defined conceptual framework? It might be that the rational explanation of colinearity will not appear as aesthetically pleasing as the process itself. Instead, it may merely reflect a tinkering without any particular underlying logic other than that of the intended result.

Fig. 2. Models of colinear transcriptional activation along a Hox cluster. (A) Expression of three Hox genes in developing mouse embryos. Nested domains are visible in both the trunk (dotted lines) and limbs (flags). The anterior (proximal) limits of expression are colinear with gene order on the chromosome. The positions of the transcription domains in the trunk can vary between the neural tube (orange) and paraxial mesoderm (blue), even to a large extent (Hox9), highlighting tissue-specific mechanisms for colinear gene regulation. (B) to (D) Molecular mechanisms proposed for colinear activation. A time-based activation may derive from the gradual accessibility of genes to the transcription machinery (B), through progressive chromatin remodeling. In this model, genes are initially silenced to become progressively accessible. Alternatively, interspersed locally acting cis-regulatory sequences could implement colinear expression (C), for instance by displaying graded affinities (green or yellow arrows) or various specificities for upstream regulators [stars in (C)]. The sharing of these local enhancers between neighboring genes could also participate in directional gene activation. In model (D), global enhancer sequences located outside the cluster regulate the various genes differently, as a result of a distance effect, promoter competition, or sequence-specific recognition. This latter model is implemented during vertebrate limb development. Dark blue and light blue arrows depict regulatory inputs of 5' and 3' remote cis-acting elements, respectively. These models are not mutually exclusive. Instead, it is likely that colinear activation in various structures relies upon distinct mixtures of these mechanisms. Red arrows in (B), (C) and (D) indicate genes that are active transcriptionally.

References and Notes
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