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Meeting Review

Hox en Provence

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In the nearly 25 years since the cloning of the first *Hox* genes, the broad brushstrokes of their functions in axial patterning have become familiar motifs in developmental biology. The October 2007 Fondation des Treilles workshop on "*Hox* Genes in Development and Evolution" in Les Treilles, France, highlighted some of the finer details regarding the function of these genes in shaping animal morphology.

If any group of genes involved in animal development can be said to have celebrity status, then surely it would be the *Hox* genes. Mutations in these genes can result in spectacular transformations of one structure into another—the conversion of mouthparts to legs in the *Drosophila proboscipedia* mutant was even featured in an episode of "The X-Files." Because of these dramatic effects on animal morphology, *Hox* genes are also considered prime targets for evolutionary change. Over evolutionary timescales, changes in the function or expression of these genes are associated with the diversification of segmental structures along the animal anterior-posterior axis. "*Hox* Genes in Development and Evolution," organized by Walter Gehring and Marie Kmita, explored recent work on the organization of Hox clusters, the regulation of their complex expression patterns, the mechanisms underlying target specificity, and the effects of *Hox* genes on morphology.

Organization of Hox Clusters: Cleaning Up the Clutter?

Perhaps the most striking feature of *Hox* genes is their clustered arrangement and the remarkable colinearity of expression pattern along the anterior-posterior axis and position within a cluster (Figure 1A). *Drosophila* possesses eight *Hox* genes, split into the Antennapedia Complex (ANT-C) and the Bithorax Complex (BX-C). Generated from a single ancestral gene by duplication, each of the related paralogs is expressed in a distinct domain along the head-tail body axis that corresponds to gene order on the chromosome. Vertebrates have a greater number of *Hox* genes, owing to expansion of the posterior group and duplication of entire clusters—mice, chickens, and humans have 39 genes, representing 13 different paralogy groups, in four clusters, and zebrafish have 48 genes in seven clusters. In addition, vertebrate clusters exhibit temporal colinearity—the *Hox* genes are activated in a progressive sequence, with anterior genes expressed earlier than posterior genes. Whereas the *Drosophila* cluster is split, has genes transcribed in both orientations, and has genes with non-Hox functions interspersed within it, the vertebrate clusters are compact, transcribed in a uniform orientation, and are not peppered with "foreign" genes. Denis Duboule (University of Geneva, Switzerland) and Walter Gehring (University of Basel, Switzerland) presented contrasting models for the evolution of the clustered arrangements. Duboule suggested that the highly organized, compact clusters of vertebrates are derived from ancestral clusters that were less compact and less well organized (Duboule, 2007). If the ancestral cluster was highly organized, there must have been many losses of this arrangement and retention only within the vertebrates; if the ancestral cluster was disorganized, then we only need postulate a single consolidation within the lineage leading to vertebrates—is it more parsimonious to consider evolutionary erosion or a tendency toward organization? Consolidation from a disorganized arrangement to a tightly organized arrangement seems counterintuitive, so Duboule proposed that the recruitment of global control regions (GCRs) outside of the cluster would selectively favor compaction. The duplications that generated additional Hox clusters in vertebrates would release constraints on the clusters and allow the emergence of global regulatory schemes along with the removal of maladapted genes, rather than simply passive *Hox* gene loss due to redundancy. Walter Gehring suggested that unequal crossing over expanded a simple cluster consisting of only *Hox1* and *Hox9* genes into more gene-rich clusters, and that the original clusters must have been organized, not disorganized, based on conservation of spatial colinearity. Based on his model, the ancestral, or "*UrHox*," gene sequence is most closely preserved in the middle of the cluster (*Hox6/7*) by the unequal recombination events, whereas outer genes untouched by the homogenizing effects of this recombination will have accumulated more mutations and thus be more divergent. The developmental ground state of a tissue is generally considered to be the fate of that tissue in the absence of selector gene function. However, Gehring argued against this conception, and proposed that the developmental ground state in the fly actually corresponds to the fate of the region in which the *UrHox/Antennapedia/Hox6* gene is expressed—the second thoracic segment, consonant with the original model of Ed Lewis for Hox function. Gehring supported his proposal with the observation that the phenotype associated with *Hox* loss of function, regardless of whether anterior or posterior *Hox* genes are lost, always causes transformations toward this ground state, whereas

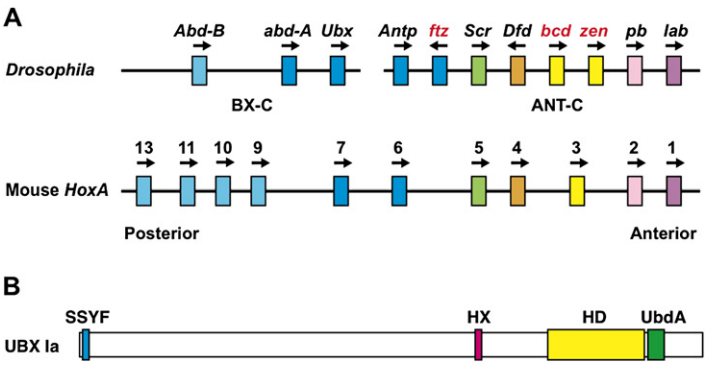

Figure 1. Hox Cluster Arrangement and Protein Motifs

(A) Schematic representation of *Drosophila* Hox complexes and a single vertebrate cluster. Colored boxes correspond to paralogy groups (genes derived from a common ancestral Hox gene), and arrows indicate direction of transcription. *zen*, *bcd*, and *ftz*, which are derived from Hox genes but now perform only non-Hox functions, are indicated in red. This depiction is a simplification that emphasizes the similarities between invertebrate and vertebrate Hox clusters, but in so doing fails to capture differences, including relative distances between genes.

(B) UBXIa protein isoform, indicating the N-terminal SSYF activation domain, the hexapeptide (HX) or YPWM motif, the DNA-binding homeodomain (HD), and the UbdA peptide. The linker region connecting the HX and HD motifs is of variable length between Hox proteins, and the UbdA peptide is specific to UBX and ABD-A.

gain-of-function results in transformations away from the ground state.

One characteristic of Duboule's disorganized clusters is that genes without Hox functions may be interspersed within the cluster. This situation is certainly the case in the *Drosophila* ANT-C, with genes like *fushi tarazu* (*ftz*), *zerknullt* (*zen*), and *bicoid* (*bcd*), which have lost homeotic function, nested within it. The *bcd* gene arose as a duplication of *zen*, the *Hox3* paralog, in the Cyclorrhapha group of dipteran flies. Though *bcd* does not have a conventional Hox function, it does act in anterior-posterior patterning. Thought to be the prototypical morphogen, a gradient of *bcd* activity patterns the *Drosophila* head region. Ulrike Löhr of Herbert Jäckle's laboratory (Max-Planck Institute for Biophysical Chemistry, Göttingen, Germany) described experiments that manipulated the *bcd* gradient, with intriguing results. Instead of localized increases in *bcd* activity, Löhr engineered a more consistent upregulation of *bcd* activity across the embryo, leading to a uniform distribution of the morphogen. This distribution would be expected to erase any positional information specified by that morphogen. However, the pattern of gene expression in these embryos revealed the persistence of positional information. Thus, while a *bcd* gradient can impart positional information, it is not necessary to establish anterior-posterior polarity within the embryo. Since other insects are capable of patterning the head even in the absence of a *bcd* gene, it should perhaps not be surprising that other systems for generating initial anterior-posterior polarity are retained in *Drosophila*, but the status of *bcd* as *the* morphogen in fly development may have suffered a blow.

Regulation of *Hox* Gene Expression: The Story of Segments

Duboule suggested the consolidation of Hox clusters during evolution was propelled by their regulatory mechanisms—the imposed constraints of regulatory elements that control complex expression patterns of spatial and temporal colinearity favor organized clusters. Indeed, several layers of regulation, from global chromatin conformation to local *cis*-regulatory elements (CREs), control the expression patterns of *Hox* genes. Francois Karch (University of Geneva, Switzerland) described mechanisms acting within the *iab* regulatory domains that each control posterior *Hox* gene expression in a specific parasegment in *Drosophila*. These regulatory domains are separated from each other by boundary elements that prevent ectopic expression in inappropriate parasegments, but must also possess a bypass mechanism to allow initiation in the proper parasegment. In addition, maintenance elements preserve the active or inactive state. Karch used the Dam identification method (DamID) of targeting Dam methyltransferase to specific DNA sequences, and then monitoring methylation at distant regions to probe interactions between these sequences. He demonstrated physical interactions between the *Fab-7* boundary element, which separates *iab-6* and *iab-7*, and the *Abdominal-B (Abd-B)* promoter, 35 kb away, in tissues where *Abd-B* is inactive, but not in tissues where *Abd-B* is active (Cleard et al., 2006). He suggested that boundary elements in the BX-C act to tether chromatin loops, each harboring an *iab* domain, to the *Abd-B* promoter. As the boundary elements release in sequential segments, initiator sequences in the untethered *iab* domains are freed to activate expression. In addition, he described the introduction of *attP* integration sites (Bischof et al., 2007) into the BX-C that will allow testing of specific *iab* variants within their appropriate, native genomic context.

Dramatic remodeling of chromatin conformation is also observed during expression of the Hox cluster in mammalian cells. Beautiful fluorescence in situ hybridization experiments presented by Wendy Bickmore (MRC Human Genetics Unit, Edinburgh, United Kingdom) reveal that during differentiation—whether in mouse ES cells in culture or in vivo in the tail bud or rhombomeres of embryos—*HoxB* and *HoxD* loci loop out from compact chromosome territories, and loci within a cluster can be resolved from one another, indicating chromatin decondensation (Chambeyron and Bickmore, 2004; Chambeyron et al., 2005). Upon looping out from chromosome

territories, the *Hox* genes colocalize with foci of active, phosphorylated RNA polymerase II within nuclei of differentiating cells, suggesting that nuclear reorganization allows *Hox* genes greater access to transcription factories. However, in the developing limb bud, *HoxD* loci are as decondensed as in the tail bud, but do not move out from the chromosome territories (Morey et al., 2007), prompting Bickmore to suggest that the powerful enhancer elements operating in the limb may allow assembly of transcription factories within the chromosome territory and obviate the need to move the loci to the factories.

Early cellular choices to activate or repress particular *Hox* genes are retained through the action of the Polycomb group (PcG), which maintains a silenced state, or the Trithorax group (TrxG), which preserves an active state through modification of chromatin. Tomonori Katsuyama of Renato Paro's laboratory (ETH, Zurich, Switzerland) used chromatin immunoprecipitation to show that histone H3 methylation covers the entire transcriptional unit of inactive *Abd-B*, and components of the PcG are bound at all regulatory elements. In the active state, methylation is replaced by extensive histone H4 hyperacetylation, and Trx protein is bound at promoter sites (Beisel et al., 2007). More surprising, though, was their observation that Pleiohomeotic (PHO) protein, a PcG protein that can recruit the repression complex, is widely bound across the active *Abd-B* locus, implying that it may be important for both repression and activation. Though the mammalian homolog of PHO, YY1, can act as either a repressor or an activator, previous analysis of PHO had not detected a capacity to activate gene expression. Also exploring the role of the PcG in regulating differentiation, Shoichiro Kurata (Tohoku University, Sendai, Japan) described the genetic interactions of *winged eye* (*wge*), a chromatin-associated protein, with the PcG. WGE protein binds to specific sites on polytene chromosomes, including all sites that are bound by Posterior Sex Combs of the PcG (Katsuyama et al., 2005). In *wge* mutants additional sex combs appear in distal regions of the 1st leg (a distal-to-proximal transformation), as well as on the 2nd leg (a posterior-to-anterior transformation), but this phenotype is suppressed by both *Polycomb* and *trithorax* mutations. Though this result would suggest that *wge* may act in both complexes, and Kurata found that it did possess TrxG-like function in a transgenic assay of *Fab-7* activity, *wge* did not appear to have a PcG-like function in the same assay.

Chromatin conformation alone does not account for spatial and temporal colinearity of *Hox* gene expression. Additional *cis*-acting controls are important for the observed patterns. In the developing vertebrate limb, colinear expression in the posterior group genes of the *HoxD* cluster has both a spatial and quantitative aspect. The 5′-most gene, *Hoxd13*, is expressed most distally and most robustly, whereas *Hoxd9* is absent from the distal digit region and expression is less robust (Kmita et al., 2002). Duboule described a two-step model for the regulatory interactions that generate these expression patterns. First, a GCR, which lies 200 kb outside the cluster (Spitz et al., 2003), assembles a protein complex facilitating promoter-promoter interactions, as assessed by the chromosome conformation capture (3C) technique (Dekker et al., 2002). Second, this protein complex can then scan for the local promoters of individual *HoxD* genes (T. Montavon and D. Duboule, personal communication). Colinearity, both positional and quantitative, is then due to the combined effects of physical proximity to the GCR and differences in promoter affinity.

Segmental expression of *Hox* genes in specific rhombomeres within the hindbrain is controlled by the combined input of several modular CREs. Robb Krumlauf (Stowers Institute for Medical Research, Kansas City, MO) described the regulatory element controlling expression of *Hoxa2* in rhombomere 4 (r4) (Tumpel et al., 2007), and the crosstalk between *Hox* genes to establish and then maintain expression. This *Hoxa2* r4 CRE is located within an intron, but the CRE that activates r4 expression of *Hoxb2* is 5′ of the gene, indicating that the modular nature of these CREs allows flexibility in their arrangement with respect to each other and the genes they regulate. To explore the evolutionary dynamics of CREs, Krumlauf analyzed regulation of the duplicated versions of *Hoxa2* in various fishes. In some taxa, the CREs of duplicate genes underwent subfunctionalization (Tumpel et al., 2006), but are still capable of driving shared expression patterns in other taxa. In the case of fugu *Hoxa2(a)* and *Hoxa2(b)*, it was possible to identify subtle differences in CRE sequence that contribute to the observed changes in expression. Finally, he described computational approaches to identify regions of conservation between Hox clusters and between species. To test the function of these conserved noncoding regions, his lab is using BAC recombineering to introduce different reporter tags into each gene of *HoxB5–B9*, and then to assess expression driven by the modified BACs in mice. In the context of a BAC, expression patterns more accurately resemble endogenous expression than expression driven by smaller plasmids does, so the consequences of deleting the conserved regions may be determined more effectively.

Defining *Hox* Targets: Specificity and Diversity

The expression of *Hox* genes in specific regions (their "Hox code," whether distinct or overlapping) does not immediately explain how they generate different developmental outcomes in these regions. Understanding *Hox* function at this step is a dual challenge in both specificity and diversity: since Hox proteins appear to have very similar DNA binding sequence specificities, how do these proteins act on distinct target gene sets? And how does a single Hox protein modulate different functions during development? One mechanism for generating a diversity of responses is the production of an assortment of protein isoforms from a single *Hox* gene. Ernesto Sanchez-Herrero (Universidad Autónoma de Madrid, Spain) tested the ability of different Ultrabithorax (UBX) isoforms to rescue defects in a *Ubx* mutant background, and found that the shorter UBXIVa isoform is less capable of rescuing than either UBXIa or UBXIIa. In addition, Sanchez-Herrero

showed how the posterior group gene *Abd-B* M and R isoforms perform different roles in the regulation of target genes *abdominal-A* and *Distalless* in the genital disc, and that the regulatory interactions between *Abd-B* and *abdominal-A* in the genital disc are the opposite of those in the embryo (Foronda et al., 2006). The different effects of these isoforms may involve modification of protein-protein interactions rather than modulation of DNA binding, based on a model for Hox functional diversity described by Yacine Graba (IBDML, Marseille, France). Graba found that the short UbdA motif in UBX (Figure 1B) can mediate an interaction with the well-characterized Hox cofactor, Extradenticle (EXD) (Merabet et al., 2007), challenging the view that all Hox/EXD interactions occur through the hexapeptide motif region. This result can account for previously observed hexapeptide-independent, but EXD-dependent, functions of UBX and ABD-A (Galant et al., 2002; Merabet et al., 2003). In addition, Graba suggested that the mode of Hox/EXD interaction depends on the particular target gene, and that qualitatively distinct interactions of Hox proteins with the same cofactor may result in different conformations of the protein complex, thereby providing a structural basis for distinct activities. One well-characterized function of the EXD cofactor is its enhancement of the DNA binding specificity of Hox proteins, and Richard Mann (Columbia University, New York, NY) described biophysical investigations of Hox/EXD/DNA complexes. Using a 37 bp regulatory element of the *fork head* gene that is bound by Sex Combs Reduced (SCR) (Ryoo and Mann, 1999), a crystal structure of the protein-DNA complex revealed that the linker region of SCR, between the hexapeptide motif and homeodomain, lies within the minor groove of DNA. Though specific amino acids in SCR are crucial for high-affinity interaction with the minor groove, the interaction appears to depend less on specific DNA sequences than on the distribution of electrostatic potential of the DNA. Mann suggested that this linker region may mediate Hox target specificity by recognition of the electrostatic shape of DNA, and that differences in the linker regions of Hox proteins may therefore contribute to differences in DNA target selection (Joshi et al., 2007). Since Hox proteins bind to short AT-rich sequences that are relatively common, additional DNA sequences are likely important for Hox proteins to properly select their target genes. Bradley Hersh (Clemson University, Clemson, SC) presented mutational analysis of a wing-specific CRE for the *knot* gene that is directly repressed by UBX in the *Drosophila* haltere, and analysis of a CRE for the *CG13222* gene that is directly activated by UBX in the haltere (Hersh and Carroll, 2005; Hersh et al., 2007). These studies identified additional nonidentical sequences beyond the UBX core binding site (TAAT) necessary for target regulation by UBX, suggesting that a variety of other factors, whether physically interacting cofactors or noninteracting DNA binding proteins, collaborate to generate context-specific output.

Such a context-specific combinatorial mechanism appears to operate in the generation of vertebrate motor neuron diversity, as described by Jeremy Dasen (New York University, New York, NY). Spinal motor neurons possess columnar identities that determine to which general region the neurons will project their axons (such as muscles of the limb). Within these columns, neuronal identities are further subdivided into pools that project to different muscles, with each pool occupying a stereotypical position along the rostrocaudal axis of the spinal cord. Dasen showed that *Hoxc6* in the forelimb and *Hoxd10* in the hindlimb specify lateral motor column (LMC) identity in the brachial and lumbar regions, whereas *Hoxc9* specifies a preganglionic column identity in the thoracic region (Dasen et al., 2003). The *Hox5* and *Hox8* genes further subdivide the LMC into pools along the rostrocaudal axis, while *Hox4*, *Hox6*, and *Hox7* genes contribute to motor pool diversity at a single rostrocaudal position (Dasen et al., 2005). Both columnar and pool identities are generated by the collaboration of Hox proteins with graded activity of the FoxP1 transcription factor, which appears to contribute as a contextual cofactor for multiple *Hox* genes during establishment of motor neuron subtype identities.

Hox Regulation of Morphogenesis: Making the Right Moves

Given their dramatic effects on animal shape, *Hox* genes must ultimately regulate processes of morphogenesis, whether directly or indirectly. Olivier Pourquié (Stowers Institute for Medical Research, Kansas City, MO) described the role of *Hox* genes in vertebrates in one of the earliest and most important morphogenetic events—gastrulation (Iimura and Pourquié, 2006). In the chick embryo, *Hoxb* genes are activated in a collinear fashion in the paraxial mesoderm territories *prior* to ingression of these cells at the primitive streak, suggesting the possibility that the *Hoxb* genes act not merely as readouts of the anterior-posterior progression of gastrulation, but instead as regulators of cell ingression. This function was confirmed through grafting experiments, in which labeled cells that overexpress posterior *Hoxb* genes were observed to ingress at the primitive streak later than cells that overexpress anterior *Hoxb* genes, and to establish boundaries of expression that are more posterior. Cells cotransformed with *Hoxb9* and *Hoxb4* take up a boundary appropriate for *Hoxb9*, indicating that the posterior gene is functionally dominant over the anterior gene. However, Pourquié found that the initial collinear onset of *Hox* expression in the primitive streak positions the boundary level slightly more anteriorly than its final position. The balance of FGF and retinoic acid at the determination front, the signaling threshold at which the segmentation program is activated, maintains *Hox* expression caudally while switching off *Hox* transcription in the anterior presomitic mesoderm. Subsequent reactivation of *Hox* expression is accomplished in precursors located at the definitive boundary level immediately prior to their incorporation into the forming somite, leading to refinement of the final anterior boundary of expression to the appropriate level.

The process of vertebrate segmentation requires both the condensation of mesodermal tissue into somites and axial elongation to extend the length of the animal.

Jacqueline Deschamps (Hubrecht Institute, Utrecht, The Netherlands) described interactions between *Hox* genes and *Cdx* genes, members of the related ParaHox cluster, in mediating axial extension in the mouse. Knockouts of individual *Cdx* genes result in Hox-like transformations in the axial skeleton—for instance, extra cervical vertebrae or mild thoracic rib defects, and combinations of alleles give rise to additive effects on vertebral patterning (van den Akker et al., 2002). Deschamps shared additional results showing that a combination of either *Cdx1* or *Cdx4* mutations with a heterozygous *Cdx2* mutation results in dramatic posterior truncations of the axial skeleton. In these animals, expression of multiple *Hox* genes is reduced, suggesting that the *Cdx* genes may control axial elongation by acting on the *Hox* genes. In addition, overexpression of *Hoxa13* leads to axial truncations. She proposed that activation of the last Hox paralogy group may serve as the signal to end elongation. Supporting this model, Moisés Mallo (Instituto Gulbenkian de Ciência, Oeiras, Portugal) demonstrated that overexpression of *Hoxc13* in the presomitic mesoderm also leads to loss of caudal vertebrae and truncation of the lumbar region. Further testing the correlation of *Hox* expression patterns with morphological transitions in the axial skeleton, Mallo manipulated *Hox* expression in the mesoderm. Knockout of *Hox10* genes leads to transformation of lumbar and sacral vertebrae toward a thoracic identity (Wellik and Capecchi, 2003), but expression of *Hox10* genes in the somites does not match the thoracic-lumbar transition point. However, in the presomitic mesoderm, the expression boundary does match the transition point, and Mallo showed that overexpression of *Hoxa10* in the presomitic mesoderm led to a dramatic loss of ribs, whereas misexpression in the somites did not (Carapuco et al., 2005). Acting in the opposite direction, misexpression of *Hoxb6* induced rib structures at all positions along the axial skeleton. In both cases, Mallo suggested the effects of misexpression in the presomitic mesoderm altered the response to BMP signaling much later in the hypaxial myotome of the somite, suggesting that the *Hox* genes can modulate the response to external signals well in advance of the receipt of those signals.

Hox genes also modulate FGF and Sonic Hedgehog signaling during morphogenesis of the vertebrate limb. Conditional deletion of the full *HoxA* and *HoxD* clusters in the limbs of mice leads to a highly truncated humerus and absence of all distal structures. Marie Kmita (Institut de Recherches Cliniques de Montréal, Québec, Canada) showed that *Shh* expression in these animals is lost in the limb bud, leading to disruption of FGF signaling, and that reduction in the size of the limb bud appears to be due to an increase in apoptotic death rather than a decrease in proliferation. Kmita also used serial deletions to restore different sets of *Hoxd* genes to the mutants, and showed that while each gene of *Hoxd10–13* can activate *Shh* expression, no individual *Hox* gene can recapitulate the full *Shh* pattern (Tarchini et al., 2006). Kmita proposed that emergence of the limb involved co-option of both the *Hox* genes and their ancestral mode of regulation, restricting expression of the *Hox10* to *Hox13* paralogs to the posterior of the limb bud, thereby restricting *Shh* to the posterior and establishing anterior-posterior polarity within the limb.

Filippo Rijli (IGBMC, Strasbourg, France) extended the morphogenetic role of *Hox* genes in the brain (Davenne et al., 1999) in his presentation about the role of the *Hoxa2* gene in mapping of neuronal inputs in the brainstem (Oury et al., 2006). Rijli showed that inputs from the lower jaw map specifically to the rhombomere 2 (r2)-derived dorsal portion of the principal sensory trigeminal nucleus (PrV) in the hindbrain, whereas the rhombomere 3 (r3)-derived ventral portion of PrV selectively receives inputs from whisker-related sensory neurons of the upper jaw. Though *Hoxa2* is expressed throughout r2 and r3 at early stages, by E14.5 *Hoxa2* is highly expressed in PrV in r3 descendants, but not in r2 descendants. Generating conditional knockouts of *Hoxa2* in r2 and r3 revealed multiple roles for *Hoxa2*. Between E10 and E12, *Hoxa2* is necessary in r2 to prevent the trigeminal nerve from migrating across the rhombomere 1 (r1)/r2 border. Later, between E13 and E16, afferent neurons fail to arborize in r3-derived dorsal PrV lacking *Hoxa2*, while arborization in r2-derived PrV is unaffected. In these mice, projections from the PrV to the ventral posterior medial (VPM) appear normal, but topographic mapping of these projections is lost. Rijli also shared experiments demonstrating that *Hoxa2* and *Hoxb2* affect migration of the pontine neurons by regulating expression of the repulsive cues *Slit* and *Robo*.

Perspectives

One danger with the perception of *Hox* genes as master control genes is the tendency to ascribe to them the ability to regulate any process in a developing animal. If every feature in an organism is explained simply by reference to *Hox* gene action, then the actual explanatory power of such an account is severely limited. The Les Treilles meeting demonstrated that *Hox* genes do control fundamental cellular processes, including proliferation, apoptosis, migration, and specification. However, far from falling into the trap of empty explanation, the detailed accounts presented at this meeting, generated through sophisticated use of novel cellular, molecular, and computational techniques, expand our mechanistic understanding of *Hox* gene action. The variety of ways in which *Hox* genes are themselves regulated and by which they, in turn, regulate morphogenetic processes suggests many layers of complexity yet to be unraveled. Both broad genomic approaches and continued analysis of specific cases will help characterize how factors that initially establish anterior-posterior polarity in animals interact with the regulatory elements of *Hox* genes to generate segmental identities, identify more complete *Hox* target gene networks, explore the evolution of those networks, and determine the specific effects of those *Hox* targets on morphology. We can appreciate the broad brushstrokes of our current understanding of *Hox* function, but there is still much of this canvas left to fill in.

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