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Review

Hox genes and regional patterning of the vertebrate body plan

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ABSTRACT

Several decades have passed since the discovery of *Hox* genes in the fruit fly *Drosophila melanogaster*. Their unique ability to regulate morphologies along the anteroposterior (AP) axis (Lewis, 1978) earned them well-deserved attention as important regulators of embryonic development. Phenotypes due to loss- and gain-of-function mutations in mouse *Hox* genes have revealed that the spatio-temporally controlled expression of these genes is critical for the correct morphogenesis of embryonic axial structures. Here, we review recent novel insight into the modalities of Hox protein function in imparting specific identity to anatomical regions of the vertebral column, and in controlling the emergence of these tissues concomitantly with providing them with axial identity. The control of these functions must have been intimately linked to the shaping of the body plan during evolution.

Anterior to posterior expression and function of individual *Hox* genes

Hox genes encode transcriptional regulatory proteins that control axial patterning in all bilaterians (Garcia-Fernandez, 2005). While these genes have been organized in a cluster since the ancestral situation (Duboule, 2007), genome duplications during evolution have given rise to four *Hox* clusters in most vertebrates, *HoxA*, *B*, *C* and *D*, the zebrafish possessing 7 clusters as a result of an additional genome duplication and subsequent loss of one of the clusters (Woltering and Durston, 2006). At corresponding positions within the four clusters, therefore, are genes with particular sequence similarity, called paralogous genes. The 39 mammalian *Hox* gene family members are thus subdivided into 13 paralogous groups (PGs).

In general, *Hox* genes within a cluster are expressed from 3′ to 5′, with the earliest genes expressed in the primitive streak at pre-somite stages, and more 5′ genes expressed in the posterior part of the growing embryo at progressively later time points (Deschamps and van Nes, 2005; Dressler and Gruss, 1989; Duboule and Dolle, 1989; Gaunt, 1991; Gaunt and Strachan, 1996; Graham et al., 1989; Iimura and Pourquie, 2006; Izpisua-Belmonte et al., 1991). The spatio-temporal features of *Hox* gene expression onset are thus to a large extent coupled to the growth and elongation of the embryonic axis (Duboule, 1994), resulting in posteriorly overlapping *Hox* expression domains with spatially staggered anterior boundaries of expression. 3′ genes (*Hox1* through *Hox4*) display rostral expression limits in the hindbrain region of the embryo and more 5′ genes exhibit increasingly posteriorly restricted expression domains along the AP axis. The most 5′ genes are activated in the tail bud around mid-gestation.

Vertebrate *Hox* genes confer axial positional information to emerging embryonic tissues from the three germ layers. Loss-of-function mutations in individual mouse *Hox* genes have been found to alter the identity of tissues located within the expression domain of the genes, most often in the rostral part of that domain. Historically, two models were proposed to account for the *Hox* patterning along the mouse AP axis. Kessel and Gruss postulated the existence of a Hox code whereby varying combination of *Hox* genes functioning at any given axial level resulted in the specific morphologies along the AP axis (Kessel and Gruss, 1990). On the other hand, a mechanism of posterior prevalence was proposed to account for the observation that despite the broad, overlapping patterns of axial *Hox* expression, single loss-of-function *Hox* mutants only displayed phenotypes in the most anterior regions of their expression domains (Duboule and Morata, 1994). This concept, based on a mechanism of interference called "phenotypic suppression" in *Drosophila*, proposed that more posterior *Hox* genes are functionally dominant over anterior genes. While a thorough evaluation of these models is beyond the scope of the review, none of the models can fully account for all phenotypes produced by gain-of-function of individual *Hox* genes, or by loss-of-function of whole *Hox* paralogous groups of genes (*HoxPGs*). Clearly,

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only a deeper molecular understanding of the mechanism of *Hox* gene activity will provide the keys to better account for and predict *Hox* patterning along the AP axis.

Patterning alterations due to *Hox* mutations have been reported to affect neural tissues, neural crest, endodermal or mesodermal derivatives, depending on the gene(s) mutated (Krumlauf, 1993; Mallo et al., 2009; Manley and Capecchi, 1998; Trainor and Krumlauf, 2000; Wellik, 2009; Wellik et al., 2002). However, the most abundantly studied phenotypic modifications in single *Hox* mutants have been changes in axial identity of particular derivatives of the segmented paraxial mesoderm (Iimura et al., 2009; Wellik, 2009). Somites provide the metameric pattern that prefigures the axial skeleton (reviewed in Hirsinger et al., 2000). During development, somites differentiate progressively into distinct compartments, each of them closely related to the production of specific tissues. The dermo-myotome of each somite gives rise to the dermal layer of the skin and the muscles of the body and limbs. The sclerotomal region of the somites differentiates into the axial skeleton, and the tendons that connect muscle and skeletal tissues derive from the somitic syndetome (Brent and Tabin, 2002; Christ et al., 2007).

All tetrapod vertebrates possess a number of occipital somites generating the base of the skull, followed by cervical, thoracic, lumbar, sacral and caudal somites. Over the 350 million year tetrapod vertebrate history, the total number of segments and their distribution among the different regions have diverged among species, but common morphological traits for each region have been preserved. The vertebra that forms an articulation with the skull, the atlas, is the first of the elements of the cervical region that constitutes the skeleton of the neck. Just posterior to this area, rib-bearing thoracic vertebrae form the rib cage. A variable number of these vertebrae grow ribs around the body that join at the sternum, while the ribs associated to the rest of the thoracic vertebrae remain as floating ribs. Immediately posterior to the rib cage we find the lumbar vertebrae, which in land vertebrates constitute the load bearing skeleton and are generally the largest and densest of the vertebrae. They are followed by the sacral vertebrae, which grow lateral protrusions that fuse and are the site of pelvic attachment. The caudal vertebrae constitute the most posterior part of the axial skeleton, and their number varies widely between tetrapods, from the long, prehensile tail of some monkeys that have more than 30 caudal vertebrae (Schultz and Straus, 1945) to the set of 3 to 5 fused vertebrae of the human coccyx. The typical axial formula of mice consists of 7 cervical, 13 thoracic (with 7 sternal and 6 floating ribs), 5 or 6 lumbar (depending on the genetic background), 4 sacral and approximately 28 caudal vertebrae. Squamate reptiles, such as snakes, exhibit a much-diverged vertebral formula with an enormously elongated thorax (sometimes containing more than 200 thoracic vertebrae), and extremely reduced cervical, lumbosacral and caudal regions. A larger total number of somites in these animals was suggested to result from a faster segmentation process compared to the developmental rate (Gomez et al., 2008).

Inactivation of single *Hox* genes often resulted in transformations in the identity of specific vertebral elements. Those affecting anatomical boundaries have been the most commonly studied, maybe because they usually contain the clearest morphological changes. From these studies, it was clear that several adjacent *Hox* genes were often found to contribute to the identity of vertebrae at particular axial level (Mallo et al., 2009; Wellik, 2007, 2009). Analysis of compound *Hox* mutants, including several members of the same paralogous group, invariably resulted in stronger phenotypes, which became extreme when the whole paralogous group was simultaneously inactivated (Horan et al., 1995; McIntyre et al., 2007; Wellik and Capecchi, 2003). These studies confirmed the functional redundancy among members of a given paralogous group, an idea that had been already suggested on the basis of similarities in their expression domains along the AP axis (Burke, 2000; Burke et al., 1995; Gaunt et al., 1989; Gruss and Kessel, 1991; Kessel and Gruss, 1990; McGinnis and Krumlauf, 1992; McIntyre et al., 2007). These studies on mutants of complete *Hox* paralogous groups also revealed an additional level of complexity in the function of *Hox* genes in patterning the vertebrate body plan. They indicated that particular *HoxPGs* control patterning of full anatomical axial domains, and they associated specific functions to individual paralogous groups, although the mechanisms by which this patterning occurs is only starting to be understood.

The control of regional patterns in the axial skeleton by *Hox* genes

HoxPG10 genes and the lumbar column

Recent data suggest that *Hox* paralogous groups control the specification of characteristic morphologies along regions of the vertebral column. This concept has shed new light on the patterning mechanisms by *Hox* genes. It has been shown that discrete paralogous groups of *Hox* genes play dominant roles in the morphogenesis of specific anatomical regions in the vertebral column. The first data that demonstrated that *Hox* activity controls regional patterning along the AP axis was provided by the analysis of compound mutants of *HoxPG10* (Wellik and Capecchi, 2003). Simultaneous inactivation of all three *HoxPG10* genes resulted in mice in which the prospective lumbosacral region had acquired thoracic-like characteristics, as these mutant vertebrae displayed associated ribs all along the thoraco-lumbar region (Fig. 1). While these phenotypes could also be classified as identity changes, they differed from previously described phenotypes for single or compound *Hox* mutants in two ways: they affected a larger number of segments, and all the segments affected belonged to a distinct anatomical region. The rib-suppressing activity of the *HoxPG10* genes was further demonstrated by ectopic expression of one of the *Hox10* genes in the paraxial mesoderm at axial levels that included the thoracic segments, resulting in completely rib-less animals (Carapuco et al., 2005) (Fig. 1).

A recent paper on the expression patterns of *Hox* genes in snakes and caecilians raised a question regarding the role of *HoxPG10* genes in the inhibition of rib development in vertebrates (Woltering et al., 2009). In this report it was shown that expression of *Hoxc10* in both "snake-like" animals extended well into the rib-forming domain of the paraxial mesoderm, which is at odds with the genetic data obtained in mice (Carapuco et al., 2005; Wellik and Capecchi, 2003). This unexpected expression was also shown in a recent report for *Hoxa10* but not for *Hoxd10* in squamate reptiles (Di-Poi et al., 2010). A possible molecular explanation to this enigma was proposed on the basis of the strong relaxation in the Hoxa10 and Hoxc10 coding sequences of snakes, which was not shared by the Hoxd10 protein (Di-Poi et al., 2010), suggesting that the former two proteins might have lost their rib-suppressing activity and contributed to an extended rib cage. Functional analysis of HoxPG10 proteins of squamate reptiles and other vertebrates will surely help to understand the role that these *Hox* genes played in the evolution of the vertebrate body plan.

HoxPG11 and the sacrum and tail

Formation of the sacrum absolutely depends on *HoxPG11* genes, as demonstrated by the complete absence of this structure in mutants missing *HoxPG11* (Wellik and Capecchi, 2003). In these animals the morphology of the vertebrae normally forming the sacrum was identical to that of the anterior lumbar vertebrae. The ability of *HoxPG11* to generate sacral characteristics has also been shown in overexpression experiments. In particular, transgenic embryos expressing *Hoxa11* in the presomitic mesoderm (PSM) throughout development, exhibit fusions between adjacent vertebrae that could be interpreted as a "sacralization" phenotype (Carapuco et al., 2005). However, proper formation of the sacrum also requires the activity other *Hox* genes, most particularly those of the *HoxPG10*. This

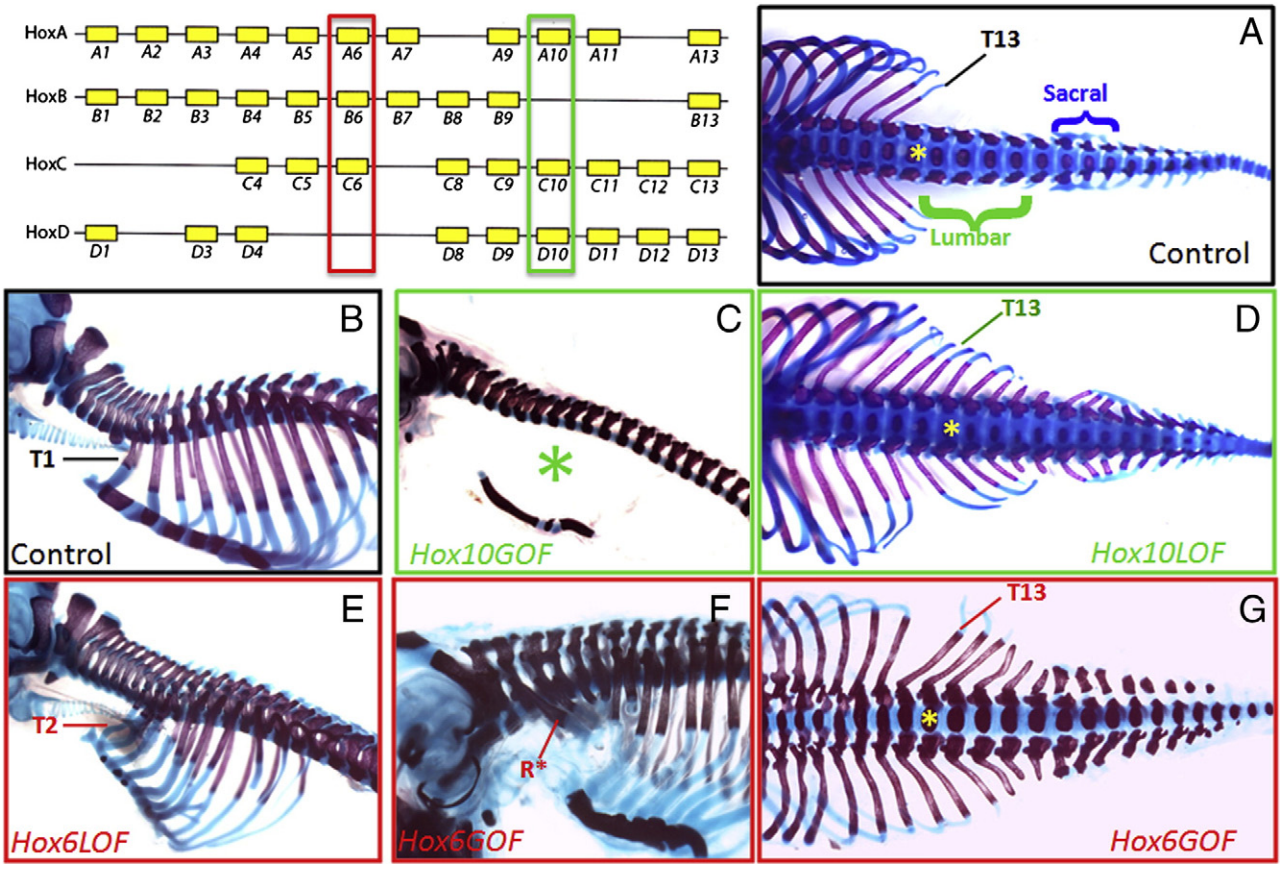

Fig. 1. Regional patterning of the axial skeleton by *Hox* paralogous groups 6 and 10. Skeletal preparations showing the effects of global inactivation (LOF) or of ectopic activation (GOF) of genes in the paralogous *Hox* groups 6 (red labels) and 10 (green labels). *HoxPG10* genes have rib blocking activity as shown by the ectopic ribs observed in the lumbar area of mice with complete inactivation of this paralog group (D) and by the complete absence of ribs (green asterisk) after precocious activation of *Hoxa10* in the presomitic mesoderm (C). *HoxPG6* genes are able to induce ectopic ribs at cervical (R* in F) and lumbar (G) levels. Complete inactivation of *HoxPG6* genes (E) results in smaller rib cages and loss of specific ribs (first rib in this specimen is attached to the second thoracic vertebra, T2), indicating that other genes must also cooperate in the rib-inducing process. A schematic representation of the *Hox* clusters is shown for reference. Skeletons of wild type embryos are shown in A and B. The lumbar and sacral domains, the position of the first thoracic (T1), last thoracic (T13), and first lumbar (yellow asterisks) vertebrae are indicated for reference.

conclusion stems from the observation that in the absence of *HoxPG10* genes sacral vertebrae still fuse laterally but modify their morphology to appear as rib-like appendages (Wellik and Capecchi, 2003). This suggests that formation of the sacrum requires both inhibition of the rib-forming activity provided by *HoxPG10* genes and an additional activity of *HoxPG11* genes to promote generation and fusion of lateral outgrowths from adjacent vertebrae to form the sacral wing. It is noteworthy that *HoxPG11* genes are also required for proper patterning the first several caudal vertebrae (Wellik and Capecchi, 2003). What makes *HoxPG11* genes produce sacral or anterior caudal vertebrae at different axial levels is still unknown. Transgenic experiments suggest that specific characteristics of the tissues in which the *HoxPG11* genes operate might play a role in this process, as somitic expression of *Hoxa11* resulted in caudal-like phenotypes instead of the "sacralization" resulting from *Hoxa11* expression in the PSM (Carapuco et al., 2005). Whether this is the case during physiological development of these areas, and which molecular mechanism underlies this specificity, remains to be determined.

HoxPG5–9 and the ribcage

HoxPG9 genes also have a regional patterning function. Genetic experiments indicate that the production of sternal versus floating ribs is under the control of *HoxPG9* (McIntyre et al., 2007). In particular, the activity of these genes seems to be required to produce floating ribs as the complete inactivation of this paralogous group resulted in rib cages with 13–14 ribs attached to the sternum instead of the typical 7. Expression patterns of genes in this paralogous group suggest that gene activity is likely to be required in the lateral plate mesoderm rather than in the somites (McIntyre et al., 2007). It has been shown that development of the distal rib requires migration of somitic mesoderm into the somatopleura (Sudo et al., 2001), possibly requiring the interaction of rib primordia with sternal precursors. The control of this migration seems to depend on a variety of signals from the surface ectoderm and the somatopleura itself (Aoyama et al., 2005; Sudo et al., 2001). Thus, it is possible that *HoxPG9* modulates the response of the lateral plate mesoderm to the migration signals or the production of the signals themselves, so that penetration of rib precursors into the somatopleura is properly organized at the different rostro-caudal levels of the rib cage.

Regarding rib-inducing activity, no single *Hox* paralogous group inactivation so far resulted in rib loss in the entire rib cage, suggesting that more than one *Hox* paralogous group is involved in promoting rib formation (or that the process is controlled by as yet undiscovered genes). However, defects in the rib cage have been noted in mutants for *Hox* genes from *HoxPG5–9*. More recently, it has been shown that transgenic mice expressing a *HoxPG6* gene in the PSM throughout axial extension develop ribs associated to vertebrae in the prospective neck and lumbar areas, in addition to those of the thoracic region (Vinagre et al., 2010) (Fig. 1), indicating that *Hox* genes in this paralogous group are able to confer a thoracic character to vertebral elements anterior and posterior to the thorax.

The possibility that *HoxPG6* genes are involved in the specification of the boundary between cervical and thoracic regions was initially suggested by studies showing that their anterior limits of expression in the paraxial mesoderm correlated with the cervical to thoracic transition rather than to absolute somite number in vertebrate species with a different number of cervical vertebrae (Burke et al., 1995; Gaunt, 1994). In the mouse, *HoxPG6* rostral expression limit is at the level of somite 13, corresponding to the cervico-thoracic transition at vertebra 8, while the anterior level of *Hoxc6* expression in chicken was mapped to somite 20, fitting the rostral border of the thoracic region at vertebra 15 (Burke et al., 1995). The phenotype of the transgenic embryos expressing *Hoxb6* in the mouse PSM at all axial levels seems to confirm that *HoxPG6* can indeed promote formation of ribs from the developing somites and could thus be an integral part of the genetic network controlling formation of the rib cage (Vinagre et al., 2010). However, inactivation of *HoxPG6* did not produce mice without a rib cage, although the mutant rib cages were smaller in size than those in wild type embryos and displayed abnormal rib phenotypes, including the absence of the first rib and distal fusions of ribs associated to thoracic vertebrae 2 to 4 (McIntyre et al., 2007) (Fig. 1). This indicates that additional factors must operate in this process. *HoxPG5* genes are among the candidates for an additional role in rib induction, since their expression domains in the paraxial mesoderm are similar to those of *HoxPG6* genes (Burke et al., 1995), and their regional inactivation also has phenotypic impact on the anterior rib cage (McIntyre et al., 2007). Proof for a redundant function for *HoxPG5* and *PG6* in rib induction processes would require simultaneous complete inactivation of both paralogous groups, which has not been performed to date.

Hox genes and the patterning of the neck

Much less is known about the mechanisms of regional patterning by *Hox* genes leading to proper morphogenesis of the cervical vertebrae. Loss-of-function experiments of genes of the *HoxPG3*, *PG4* and *PG5* have demonstrated a role for these genes in establishing morphologies in the cervical skeleton. Horan et al. showed that the *HoxPG4* genes are critical for establishing the appropriate morphology of the cervical skeleton (Horan et al., 1995). With loss of three of the four *HoxPG4* genes, the vertebrae in the cervical region were transformed into morphologies typical of the first (atlas) and second (axis) cervical vertebrae. Loss of *HoxPG5* resulted in defects in both the cervical and thoracic skeleton, including a transformation of cervical vertebrae 3 to 7 towards axis morphology (McIntyre et al., 2007), and *Hoxa3*/*Hoxd3* control the generation of the atlas (Condie and Capecchi, 1994). However, complete transformation of the cervical region into a different vertebral domain has not yet been reported for any combination of loss-of-function *Hox* mutants.

In summary, it is clear that *Hox* genes functionally define regional anatomical domains along the axial skeleton. It is likely that *Hox* function in axial patterning results from an inherent ability to promote the generation of different structures from tissue progenitors, and that it is the combination of inputs from the different *HoxPG* genes that leads to the properly regionalized vertebral column.

Molecular mechanisms of Hox gene control of rib development

While the involvement of *Hox* genes in axial development has been extensively documented, relatively little is known regarding the mechanisms underlying their activity. A recent report has shed light on how *HoxPG6* and *PG10* modulate somite differentiation to produce rib-bearing and rib-less areas in the vertebral column (Vinagre et al., 2010). Although ribs derive mostly from the sclerotome (Burke and Nowicki, 2003; Huang et al., 2000), this compartment may not to be the primary target of *Hox* genes in their control of rib development. Instead, the activity of these *Hox* genes seems to be mediated to a large extent by their ability to modulate expression of *Myf5* and *Myf6* (previously known as *MRF4*) in a specific area of the myotome, the hypaxial (lateral) compartment (Vinagre et al., 2010). In particular, *HoxPG6* genes promote this expression in interlimb somites, which are those generating the thoracic region, and *HoxPG10* blocks it in somites adjacent to the hindlimb bud, which are those generating lumbosacral vertebrae. Hypaxial modulation of *Myf5* and *Myf6* expression seemed to be also responsible for the "all-rib" or completely rib-less phenotypes resulting from ectopic activation of *Hoxb6* and *Hoxa10*, respectively. The *Hox* patterning information gathered in the hypaxial myotome by *Myf5*/*Myf6* seems to be transmitted to the adjacent sclerotome by PDGF and FGF signals to promote skeletogenesis. This paracrine mechanism is consistent with the correlated myotomal expression of *Pdgfa* and *Fgf4* with that of the *Myf5*/*Myf6* genes and with genetic data showing the involvement of the FGF and PDGF signaling pathways in rib formation (Grass et al., 1996; Huang et al., 2003; Soriano, 1997; Tallquist et al., 2000).

Rib-less phenotypes closely resembling those of the transgenics ectopically expressing *Hoxa10* resulted from mutations in the *Myf5* and *Myf6* genes, which seem to have redundant roles in rib formation (Braun and Arnold, 1995; Braun et al., 1992; Kassar-Duchossoy et al., 2004; Patapoutian et al., 1995; Tajbakhsh et al., 1996; Yoon et al., 1997; Zhang et al., 1995). *Myf6* expression using an hypaxial promoter proved quite active in rescuing the *Hoxa10*-mediated rib-less phenotype (Vinagre et al., 2010), revealing that regulation of *Myf5*/*Myf6* expression accounts for the rib-modulating *Hox* activity to a very large extent. This indicates that, while the involvement of additional factors cannot be ruled out, explaining *Hox* activity to control rib-containing versus rib-less areas in the skeleton mostly requires understanding how *Hox* genes regulate expression of *Myf5* and *Myf6* in the hypaxial myotome. Some evidence indicates that Hox proteins bind to an enhancer involved in the control of hypaxial expression of *Myf5* (Buchberger et al., 2007) (and probably *Myf6*), both *in vivo* and *in vitro* (Vinagre et al., 2010), suggesting that the control of *Myf* expression by Hox proteins may be direct. Confoundingly, there is an apparent spatial and temporal gap between when and where *Hox* gene activity is required for vertebral patterning, and the effects on *Myf5*/*Myf6* transcription. In transgenic experiments, the rib-inducing or repressing activities of *HoxPG6* and *PG10* were only observed when these genes were ectopically expressed using an enhancer active in the presomitic mesoderm, but not using a driver that was activated only later in the somites (Carapuco et al., 2005). However, the regulated *Myf5*/*Myf6* expression is only observed at later stages, in the developing somites. Whether this results from the carryover of Hox proteins expressed in the PSM into the developing somites or whether modulation of the *Myf* enhancer requires sequential input from Hox proteins in the PSM and later from additional factors in the developing somite remains to be determined.

Regardless of the mechanism that eventually explains the spatial offset and temporal gap in this *Hox*-mediated control, it is clear that *Hox* genes must interact with other factors to control *Myf5*/*Myf6* expression (Vinagre et al., 2010). *Pax3* and *Six1*/*Six4* are prime candidates for a cooperative role because they are also required for the activity of the hypaxial *Myf5* enhancer bound by Hox gene products (Bajard et al., 2006; Giordani et al., 2007) and abnormal rib phenotypes have been described in mutant mice for these genes (Grifone et al., 2005; Henderson et al., 1999). Interestingly, specific functional interactions between members of the *Hox* and *Pax* families have been described in other apparently unrelated processes in the developing embryo (Gong et al., 2007; Wellik et al., 2002; Yallowitz et al., 2009), indicating that interactions between these genes might belong to general developmental mechanisms. It should be noted that the interaction between Hox proteins with Pax3 and Six1/4 to modulate Myf5/Myf6 expression might follow some non-conventional mechanism because the latter are not present in the PSM, which is where *Hox* activity is required.

Hox control of rib formation and evolution of the body plan

The control of rib development through regulated *Myf5*/*Myf6* expression suggests a possible mechanism for coordinated evolution of the vertebrate body plan under the control of *Hox* genes, which would derive from a dual function of these Myf factors. While *Myf5* and *Myf6* seem to have a non-myogenic activity responsible for rib-inducing processes, they still exert their classical myogenic role in these tissues (Pownall et al., 2002). Wherever ribs are formed adjacent to the *Myf5*/*Myf6*-expressing hypaxial myotome, the corresponding set of intercostal muscles will be produced by the activity of the same genes in the corresponding myotome. Indeed, cell-tracing experiments have indicated that precursors of ribs and intercostal muscles are intimately associated in the developing somite (Evans, 2003). In addition, muscles with intercostal characteristics were observed associated to the ectopic ribs that developed upon *Hoxb6* ectopic expression, indicating that this mechanism can operate efficiently *in vivo* (Vinagre et al., 2010). Interestingly, formation of the turtle carapace, which derives from a distinct course of rib development, has been shown to correlate with both turtle-specific patterns of *Myf5* expression in the hypaxial myotome (Ohya et al., 2006) and turtle-specific muscle connections (Nagashima et al., 2009), further reinforcing the relevance of aspects of *Myf5* expression in the production of species-specific skeletal body plans.

A function for *Hox* and *caudal*-related genes in axial growth

Posterior axial elongation of the vertebrate trunk occurs from progenitors in the primitive streak area before somite formation and at early somite stages, and in the growth zone of the tailbud thereafter (Cambray and Wilson, 2002, 2007; Tzouanacou et al., 2009). The *Hox* genes and their relatives, the *Drosophila Caudal*-related *Cdx* genes are highly expressed in this posterior embryonic region (Fig. 2), and have been suggested to confer patterning information to tissues emerging from the growth zone (Deschamps and van Nes, 2005). It was recently shown that, in addition to this patterning function, *Cdx* and *Hox* genes also play a role in posterior tissue generation (Young et al., 2009).

Hox genes of the central paralogous groups have the potential to stimulate axial growth

Loss-of-function studies of the *ParaHox Cdx* genes have shown that these genes are required for posterior axial extension of both vertebrates and invertebrates (Chawengsaksophak et al., 2004; Copf et al., 2004; Faas and Isaacs, 2009; Isaacs et al., 1998; Shimizu et al., 2005; Shinmyo et al., 2005; van den Akker et al., 2002; Young et al., 2009; Savory et al., 2009). In the mouse the importance of *Cdx2* in this process was already clear from the analysis of embryos developing in the absence of one or both *Cdx2* alleles, as they exhibited mild or severe posterior truncations, respectively (Chawengsaksophak et al., 2004, 1997). The participation of the other two mouse *Cdx* genes, *Cdx1* and *Cdx4*, in posterior elongation of axial tissues was not clear from initial genetic studies as single or double inactivation of these genes did not have an effect on axial extension (Subramanian et al., 1995; van Nes et al., 2006), However, inactivation of either of these two genes was able to increase the severity of the axial growth arrest of *Cdx2* heterozygotes, indicating that these two genes also participate in posterior elongation of axial tissues (van den Akker et al., 2002; Young et al., 2009). The fact that *Hox* genes are evolutionarily closely related to the *Cdx* genes, and that *Hox* genes of the anterior and central paralogous groups are expressed similarly to *Cdx* genes in the posterior embryonic growth zone throughout axis elongation of the trunk (Young and Deschamps, 2009) incited Young and colleagues to test whether these *Hox* genes could compensate for missing *Cdx* alleles in compound mutant embryos. Direct experimental tests of this hypothesis showed that this was indeed the case as two "central" *Hox* genes, *Hoxa5* and *Hoxb8*, were found to rescue the posterior truncation phenotype of compound *Cdx* mutants when expressed in the spatio-temporal window of *Cdx* activity (Young et al., 2009) (Fig. 3). The length and morphology of the sacro-caudal part of the embryonic axis of *Cdx* mutants were significantly restored by each of these *Hox* transgenes, indicating that *Hox* genes are capable of stimulating well-balanced posterior axial growth. Whether these *Hox* genes act downstream or in parallel to *Cdx* when compensating for the compromised axial growth resulting from the loss of *Cdx* genes is not yet clear. Previous work showing the regulatory capacity of *Cdx* gene products on *Hox* promoters (Charite et al., 1998; Tabaries et al., 2005), and the shifts of some *Hox* expression domains observed in *Cdx*

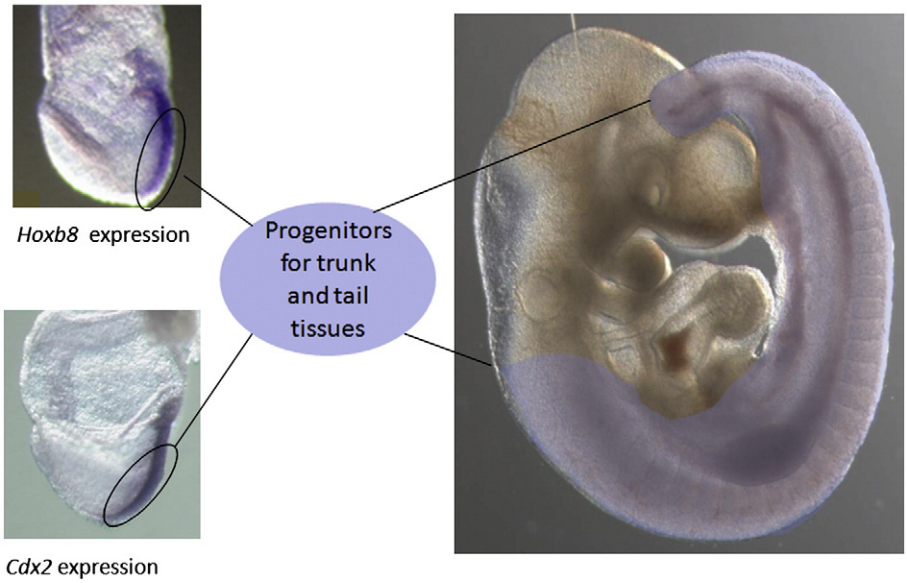

Fig. 2. *Cdx* and central *Hox* genes are expressed in the primitive streak area, where the progenitors for axial tissues for trunk and tail reside (see references in the text). Expression of *Cdx2* and *Hoxb8* is shown on the left at E7.5 (Head fold stage). Axial tissues generated by these areas is schematically indicated in purple on the E9.5 embryo on the right.

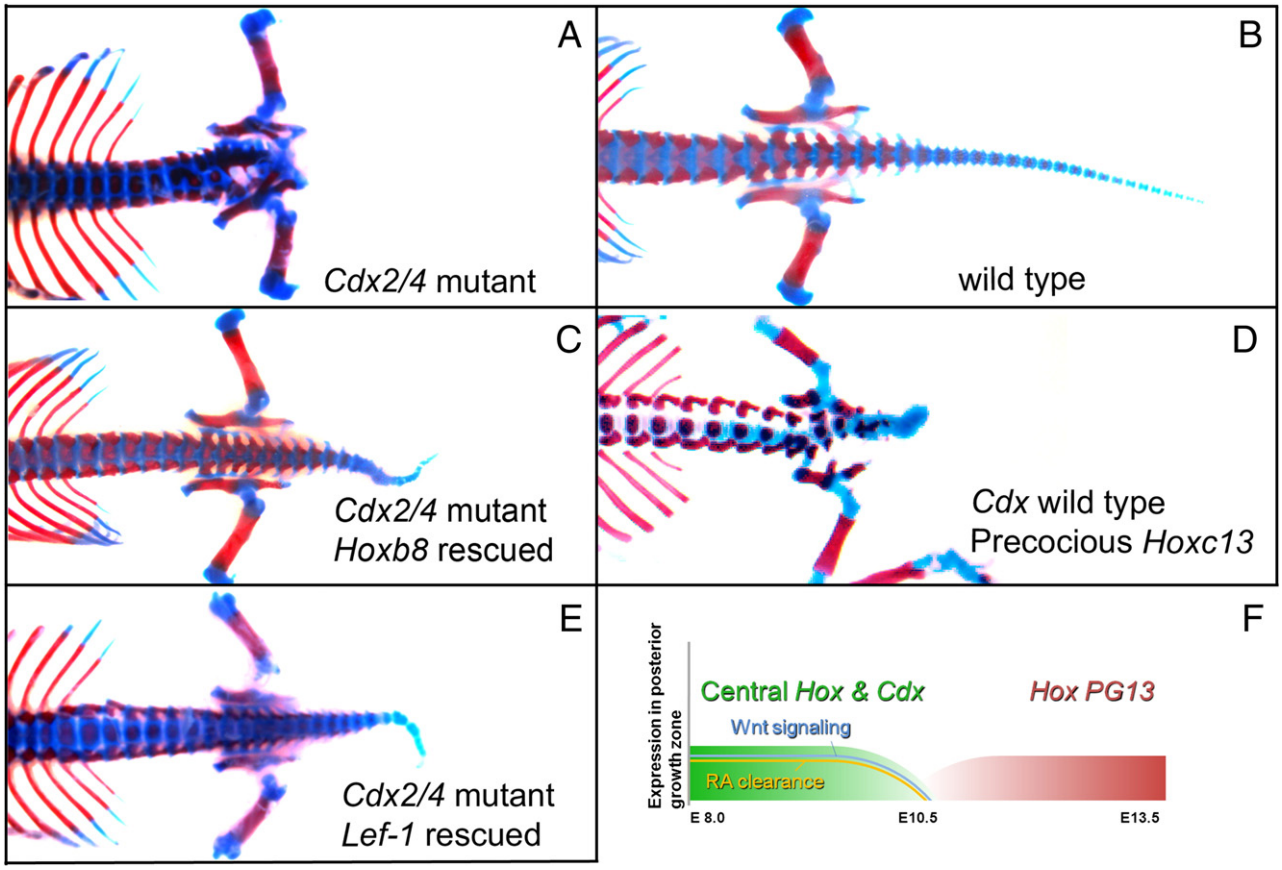

Fig. 3. Axial skeleton of newborn mice upon partial loss-of-function of *Cdx* genes and upon precocious *HoxPG13* expression (A–E) and schematic representation of positive and negative regulators of axial extension before and after the trunk–tail transition (F). A, *Cdx* loss-of-function mutations (loss of one allele of *Cdx2* and of both alleles of *Cdx4*) arrest axial extension prematurely. B, Wild type. C, Gain of function of the central *Hox* gene *Hoxb8* partially rescues axial extension in *Cdx* mutants. D, *Hoxc13* early gain of function leads to posterior axial truncation. E, Posterior activation of the canonical Wnt pathway by Lef-1 constitutive expression partially rescues axial elongation in *Cdx* mutants. The posterior axial skeleton of newborns is shown with anterior on the left. See text for more details. F, Schematic representation of axial extension of trunk and tail between early somite stage (E8.0, *X* axis) and the end of posterior extension by tissue addition (E13.5). Shown are positive ("Central" *Hox* genes, and *Cdx* genes), and negative (*HoxPG13*) regulators of axial extension, and some of the effectors of axial growth between developmental stages E8.0 and E13.5. Expression levels (not to scale) are schematically indicated in the *Y* dimension. In green, growth stimulatory central *Hox* and *Cdx* expression, with Wnt signaling (blue) and RA clearance (orange) showing a parallel course. In red, growth inhibitory *HoxPG13* expression. Decrease of axial growth stimulation and increase of inhibition correspond to the trunk–tail transition (around E10.5).

mutants (van den Akker et al., 2002) suggested that *Hox* genes are downstream targets of Cdx transcription factors. However, study of the expression of endogenous *Hoxa5* and *Hoxb8* in time and space in the truncated *Cdx* mutants (Young et al., 2009), favors a mechanism whereby Cdx and transgenic Hox gene products would act in concert rather than hierarchically to stimulate axial growth. It will be important to understand the nature and functional relevance of the growth stimulating activity of central *Hox* genes. Does it represent an atavistic function from an ancestral time when Hox and Cdx gene products were even more closely related than they now are? And do mouse Hox gene products regulate the same transcriptional target program as Cdx proteins to promote axial growth? Future experimental work should shed light on these questions. It will also be interesting to evaluate the capacity of "*Hox* genes only" to sustain posterior axial growth and patterning in the absence of any active *Cdx* genes.

Regardless of the hierarchical relationship between *Cdx* and rescuing *Hox* genes in the experiments of Young et al., the fact that two "central" *Hox* genes of paralogous groups 5 and 8 can rescue the *Cdx* mutant defects in axial growth (Young et al., 2009) suggests that the potential to stimulate trunk axial extension may be a generic property of central *Hox* genes. This also remains to be fully investigated.

The mechanism through which *Cdx* mutations impede posterior body axis extension includes, at least in part, the maintenance of canonical Wnt signaling in the growth zone, as shown by the rescue of the truncation phenotype of *Cdx* mutants by posterior expression of an activated downstream effector of Wnt signaling, Lef-1 (Young et al., 2009) (Fig. 3). Cdx proteins also control the clearance of retinoic acid (RA) from the growth zone by the RA-degrading enzyme Cyp26a1, contributing to growth by protecting the axial progenitors from the differentiating effect of RA (Savory et al., 2009; Young et al., 2009).

HoxPG13 genes arrest posterior axis extension: posterior Hox genes as selective substrate for body length variation

Inactivation of *Hoxb13* in the mouse resulted in a small extension of the vertebral column (Economides et al., 2003), already suggesting that *Hox PG13* genes may exert a negative instead of positive control on posterior axial elongation. In agreement with this observation, transgenic animals expressing *Hoxa13*, *Hoxb13* or *Hoxc13* under the control of the *Cdx2* promoter prematurely terminated axial growth (Young et al., 2009) (Fig. 3). This termination was preceded by a premature down-regulation of Wnt signaling and of retinoic acid clearance, two events that also precede "natural" axial termination in wild type embryos, and truncation in *Cdx* mutants (Fig. 3). Interestingly, the premature axial termination by *HoxPG13* genes is prevented by co-expression of a *Cdx2*-driven central *Hox* gene, providing evidence that *HoxPG13* genes may control the same genetic pathways as *Cdx* genes during axial growth (Young et al., 2009). These results obtained with mouse mutants suggest that modulation of the *Cdx*/*Hox* pathways, either by prolonging the *Cdx*/central *Hox*-mediated maintenance of Wnt signaling in the growth zone, or by

delaying or weakening the inhibitory action of *HoxPG13* genes on posterior growth, can positively affect axial length. Interestingly, it has been recently shown that *Hoxa13* and *Hoxd13* are not expressed at post-cloacal levels in snake embryos (Di-Poi et al., 2010). The authors conclude from their findings that snakes possess a simplified posterior *Hox* code, having eliminated the contribution of two of the *HoxPG13* genes normally involved in axial termination. It is therefore possible that reduction of *HoxPG13* activity was a key event for the evolution of elongated body plans.

Given the strong evolutionary conservation of the AP patterning function of *Hox* genes, a question arises regarding whether central and posterior *Hox* genes of short germ band insects also participate in posterior axial growth. This question is becoming an exciting issue, as several components of the *Cdx*/*Hox*/*Wnt* pathway have been proven to play a crucial role in axial extension in these insects and in crustaceans that, like vertebrates, extend their axes by posterior addition of tissues (Bolognesi et al., 2008; Martin and Kimelman, 2009; McGregor et al., 2008). Therefore, conservation of *Hox* and *Cdx*-mediated functions between vertebrates and invertebrates could extend beyond the classical AP patterning to include a shared genetic network underlying posterior axial growth.

Is axial extension governed by a single genetic system from anterior trunk to the tip of the tail?

The analysis of an allelic series of *Cdx* mutants has demonstrated the requirement of all three *Cdx* genes for extension of the trunk and tail (van den Akker et al., 2002; van Nes et al., 2006; Young et al., 2009). Posterior axial truncation in the most severely impaired *Cdx* loss-of-function mutants studied so far occurs at a level beyond the forelimbs. The anterior trunk develops normally in these mutants. It will be necessary to await the analysis of *Cdx* null embryos to know whether these genes are responsible for driving extension of the complete trunk and tail axis, as it seems to be the case in intermediate and short germ band insects. Injection of *Caudal* RNAi in the cricket *Gryllus imaculatus* resulted in axial truncation at a very rostral level (Shinmyo et al., 2005). An alternative hypothesis is that invertebrate *Cdx* has a realm of action covering a more extensive part of the axis than vertebrate *Cdx* genes. In one possible scenario, vertebrate *Cdx* genes would control posterior trunk and tail extension whereas anterior trunk extension would be under separate genetic control. In this regard, it will be challenging to study the functional relationship between *Cdx* genes and *T*/*Brachyury*, a unique gene required for posterior axial growth in mice (Wilkinson et al., 1990; Wilson and Beddington, 1997). Homozygous mutant embryos for *T*/*Brachyury* still generate 6–8 somites, indicating that the gene is not required for the formation of the most anterior somites. At early developmental (primitive streak) stages *T*/*Brachyury* and *Cdx* work independently as shown by the fact that expression of *T*/*Brachyury* is unaffected in early *Cdx* mutants, and vice versa (Young et al., 2009). Whether the *T*/*Brachyury* and *Cdx* pathways are hierarchically linked at later stages of axial extension is still an unsolved question. Transgenic reporter experiments suggest that *T*/*Brachyury* is a *Cdx* target (Savory et al., 2009). However, *T*/*Brachyury* is expressed in the growth zone much longer than *Cdx* genes, and thus cannot work as a *Cdx*-dependent gene after E11.0. Regardless of how these genes interact, they must combine their effect in order to regulate posterior growth, since they both impact on the canonical Wnt signaling in a dosage-dependent way (Martin and Kimelman, 2008, 2009; Young et al., 2009).

Hox/*Cdx and the progenitors for the axis*

Recent work has localized long-term progenitors for the axis with self-renewing properties in the area between the node and the anterior primitive streak in early somite mouse embryos, and in the remnant of this region in the posterior growth zone at subsequent stages (Cambray and Wilson, 2002, 2007; Tzouanacou et al., 2009; Wilson et al., 2009). Evidence for bipotent mesoderm and neuroectoderm-generating progenitors had been acquired in the past from single cell labeling experiments in the mouse (Forlani et al., 2003; Lawson et al., 1991). The contribution of posterior stem cell-like cells to the notochord (Selleck and Stern, 1991; Stern et al., 1992), the somites (Selleck and Stern, 1991; Stern et al., 1992), and the neural tube (Mathis and Nicolas, 2000a,b, 2003) had also been reported.

The transcription factors Cdx, central Hox and T/Brachyury exert their function at least in part by maintaining growth-favoring signals in the posterior growth zone. Impaired *Cdx* activity can be compensated by constitutively active canonical Wnt signaling, similar to what was observed in zebrafish *T*/*Brachyury* mutants (Martin and Kimelman, 2008, 2009; Young et al., 2009). It is therefore likely that *Cdx*, *Hox* and *T*/*Brachyury* are required to provide the progenitors with an adequate niche. These transcription factor-encoding genes, as well as the signaling factors Wnt3a and Fgf8 are expressed throughout the growth zone (Young et al., 2009). While a molecular signature of the axial progenitor cell population is still missing, it is possible that these cells owe their self-renewing properties to their localization at a very precise position within the growth zone, relatively to the node and streak, and/or to the area undergoing continuing active transcription of *Fgf8* (Dubrulle and Pourquie, 2004).

The self-renewing progenitors in the growth zone represent a continuum of axial tissue delivery (Wilson et al., 2009) that gets exhausted when axial extension is terminated. Changes in *Cdx* and *Hox* dosage may affect the niche of the self-renewing axial progenitors for trunk and tail via a positive feedback loop on Wnt signaling.

Conclusion

The present overview of recent progress on the role of *Hox* genes in vertebrate embryonic morphogenesis (mostly the mouse) has focused on three major recent discoveries that shed new light on *Hox* function.

First, the recently uncovered concept that some *Hox* genes confer properties to a collection of structures within a given anatomical region has gained wider and wider support by both loss- and gain-of-function studies. In earlier studies, observations of local phenotypes, such as changes in the identity of an individual vertebra, were reported when a single *Hox* gene was inactivated. Work performed more recently, and reviewed above, has demonstrated that inactivation or overexpression of *Hox* genes can also result in the transformation of anatomical regions, not just individual elements. Accordingly, expression of *HoxPG6* induces rib morphogenesis, expression of *HoxPG10* inhibits rib formation, *HoxPG11* proteins are necessary for development of the sacrum, and no floating ribs are formed in the absence of *HoxPG9* genes. The regional patterning function of *Hox* genes is therefore dependent not only on the temporal and spatial regulation of their expression, but also on features unique to proteins of each particular *Hox* paralogous group. These parameters are key to understanding the molecular basis of body plan evolution.

A second important area of recent progress in the *Hox* field that we have covered in this review is an insight into the mechanism of action of regional patterning by *Hox* genes. The downstream program of *HoxPG6* in inducing rib formation involves the stimulation of a non-myogenic activity of the myogenic genes *Myf5*/*6* in the myotome that instructs the adjacent sclerotome, a program that is suppressed by *HoxPG10* genes to produce the rib-less domains caudal to the rib cage. Even if the road to fully understand *Hox* control of rib morphogenesis is still long, the discovery of the *Hox-Myf* link is surely a milestone.

The third recent advance in the molecular genetics of *Hox* gene function during vertebrate development that we review here concerns the involvement of these genes in controlling body axis length. *Hox* genes thus appear as regulators of both body length and

body shape. Evidence was gathered that *Hox* genes expressed in the trunk can participate in elongating the axis when *Cdx* (*ParaHox*) gene function is impaired by mutation and fails to allow completion of axial growth. Moreover, whilst these central *Hox* genes are capable of stimulating axial growth, genes of the last *Hox* paralogous group, *HoxPG13*, cause the arrest of axial extension. In a sense, this control activity of axial growth and termination are regional *Hox* activities as well, as they impart morphogenetic information to the anatomical regions rostral and caudal to the trunk–tail transition. Stimulation of axial growth by *Hox* genes of the central paralogous groups was found to rely on the maintenance of Wnt signaling in the embryonic growth zone, and on clearance of retinoic acid away from this area. Future work is expected to further elucidate how central *Hox* genes favor axial growth, and how *Hox* genes of the last paralogous group counteract this action. Here as well, regulation of *HoxPG13* expression in time and space appears to have been an important substrate for body length variation during evolution of animals. Recent work provides a nice demonstration that a decrease in the number of active alleles of *HoxPG13* in post-cloacal paraxial mesoderm is likely to have allowed the delay in axial growth arrest in squamate reptiles. These data, and the newly uncovered regional control of morphogenesis by *Hox* genes thus support the notion that *Hox* genes have been very important players in modifying the animal body plan during evolution.

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