HHS Public Access

Author manuscript

Semin Cell Dev Biol. Author manuscript; available in PMC 2025 January 01.

Published in final edited form as:

Semin Cell Dev Biol. 2024 ; 152-153: 44–57. doi:10.1016/j.semcdb.2023.03.014.

Establishing and maintaining *Hox* profiles during spinal cord development

Alexander Miller[a,*], **Jeremy S. Dasen**[a,*]

[a]NYU Neuroscience Institute and Developmental Genetics Programs, Department of Neuroscience and Physiology, NYU School of Medicine, New York, NY 10016, USA

Abstract

The chromosomally-arrayed *Hox* gene family plays central roles in embryonic patterning and the specification of cell identities throughout the animal kingdom. In vertebrates, the relatively large number of *Hox* genes and pervasive expression throughout the body has hindered understanding of their biological roles during differentiation. Studies on the subtype diversification of spinal motor neurons (MNs) have provided a tractable system to explore the function of *Hox* genes during differentiation, and have provided an entry point to explore how neuronal fate determinants contribute to motor circuit assembly. Recent work, using both *in vitro* and *in vivo* models of MN subtype differentiation, have revealed how patterning morphogens and regulation of chromatin structure determine cell-type specific programs of gene expression. These studies have not only shed light on basic mechanisms of rostrocaudal patterning in vertebrates, but also have illuminated mechanistic principles of gene regulation that likely operate in the development and maintenance of terminal fates in other systems.

Keywords

Hox gene; neural development; spinal cord; transcription factor; motor neuron

1. Introduction

Our ability to coordinately move and interact with the environment relies on the activity of neural circuits within the spinal cord. A key step in the assembly of motor circuits is the establishment of synaptic connections between spinal motor neurons (MNs) and their peripheral targets. The ability of mammalian nervous systems to coordinate movement depends on the generation of dozens of anatomically and functionally distinct MN subtypes. In vertebrates, spinal MN subtypes are housed in motor columns longitudinally arrayed along the rostrocaudal axis. Each motor column is further subdivided into motor pools,

*Corresponding authors: alexander.miller@nyulangone.org (A. Miller); jeremy.dasen@nyulangone.org (J.S. Dasen).

Declarations of interest

None.

clustered groups of MNs that target a single muscle. Motor columns and pools are positionally-defined along the rostrocaudal axis, and their location is largely stereotyped between animals of the same species. Over the past decade significant progress has been made in define the genetic programs that determine the molecular profiles and connectivity of diverse spinal MN subtypes (Dasen., 2022; Stifani., 2014; Osseward and Pfaff., 2019).

The selective and regulated expression of transcription factors (TFs) is integral during MN differentiation, with cell-type specific TF activity driving expression of effector genes that confer MN molecular identities and synaptic specificity. Central to spinal MN diversification is the large family of TFs encoded by chromosomally arrayed *Homeobox* (*Hox*) genes. *Hox* genes determine key features of MN fates including subtype-specific molecular profiles, somatotopic organization, and postsynaptic specificity (Phillipidou and Dasen, 2013; Carpenter, 2002; Dasen and Jessell., 2009). *Hox* genes are conserved in all metazoans, and their activity confers the rostrocaudal positional identity of neural and non-neuronal tissues in all organisms that have been examined (Ferrier and Holland., 2001; Hobert., 2021; Venkatasubramanian and Mann., 2019).

In addition to MNs, *Hox* genes function in the diversification of multiple classes of spinal neurons, operating both during early development and after terminal differentiation. Recent studies have shown *Hox* genes are involved in the subtype diversification of proprioceptive sensory neurons, spinal projection neurons, and locally-connected spinal interneurons (Sweeney et al., 2018; Shin et al., 2020; Baek et al., 2019). After differentiation, *Hox* genes are also required to maintain expression of genes that define terminal fates (Catela et al., 2022). Understanding the mechanisms by which *Hox* genes are regulated and maintained could provide insights into how spinal circuits are assembled during development and contribute to mature functional features of MNs.

This review outlines the mechanisms of *Hox* gene regulation during spinal cord development, focusing on the process of MN subtype differentiation. We will categorize these regulatory mechanisms in relation to the relative stages of neural differentiation. Namely, the early silencing of *Hox* expression preceding gastrulation, the establishment and further refinement of *Hox* expression domains during axis extension, and the postmitotic maintenance of *Hox* pattern. We outline the known and emerging mechanisms of *Hox* gene regulation, focusing on the regulatory mechanism operating within the developing spinal cord. In doing so, we hope to summarize the current knowledge on the regulation of *Hox* genes during neural development and provide a background for further study.

2. *Hox* gene organization and the subtype diversification of spinal MNs

Each *Hox* gene is characterized by a shared ~180 bp sequence element, encoding a 60 amino acid DNA-binding motif termed the Homeodomain (HD) (McGinnis et al., 1984; Scott and Weiner., 1984). Possessing a HD allows Hox proteins to associate with targets and directly regulate gene expression. Comparative genetic studies have found that *Hox* genes are conserved in vertebrates and other Animalia, from protostomes to humans (Carrasco et al., 1984; McGinnis et al., 1984; Burglin and Affolter, 2016; Gaunt, SJ. 2018; Pascual-Anaya J, et al. 2013; Ferrier and Holland, 2001). Analyses of *Hox* gene organization has led

to the understanding that *Hox* clusters were generated during repeated cycles of tandem gene duplications, combined with slow acquisition of new *Hox* regulatory functions (Gehring et al., 2009; Deschamps and Duboule, 2017; Krumlauf, 2018).

2.1 Vertebrate Hox clusters and early neural expression pattern

In tetrapods, *Hox* genes reside within four chromosomally-arrayed *Hox* clusters termed *HoxA, HoxB, HoxC*, and *HoxD*. Due to an additional round of genome duplication, zebrafish, salmon, pufferfish and medaka, have an average of 7 clusters (Woltering and Durston, 2006). The ordering of individual *Hox* genes within a cluster is linked to the timing of their induction and spatial domains of expression. This correlation is termed temporal and spatial collinearity, with early- and rostrally-expressed *Hox* genes positioned 3' in the cluster, while late- and caudal-expressed *Hox* genes existing 5' (Izpisua-Belmont et al., 1991; Gaunt et al., 2018). A prevailing model is that *Hox* gene clustering arose from the necessity to sequentially activate individual genes along the chromosome, reflecting the progressive opening of repressive chromatin structure (Gaunt and Gaunt, 2016; Soshnikova and Duboule, 2009). Interestingly, in some species where *Hox* genes lack a clustered organization, such as in the larvacean tunicate *Oikopleura dioica*, some aspects of temporal and spatial collinearity are retained (Gaunt, 2018; Pascual-Anaya et al., 2013).

The function of *Hox* genes during embryonic development has been intensely studied in both vertebrates and invertebrates, with mutation of individual *Hox* genes often having a profound impact on the formation of spatially resolved structures (Philippidou and Dasen., 2013; Mallo et al., 2010; Mallo and Alonso, 2013). The majority of *Hox* genes are expressed within the developing CNS, and the activity of over a dozen *Hox* genes are involved in the diversification and synaptic specificity of spinal MN subtypes (Philippidou and Dasen, 2013). *Hox1-Hox4* paralogs are expressed in the developing hindbrain, where their activity patterns the development of transiently-segmented structures called rhombomeres (Parker and Krumlauf, 2020). In regions of the neural tube that eventually give rise to the spinal cord, *Hox4-Hox13* genes are expressed.

2.2 Hox function in spinal MN diversification and connectivity

In the spinal cord, *Hox* genes determine the subtype identity and connectivity of segmentally-restricted MN subtypes (Dasen., 2020). Here we provide a brief summary of the known functions of Hox proteins during two key steps in MN diversification – the formation of motor columns and motor pools. Motor columns are longitudinally arrayed groups of MNs that project their axons to a specific region (e.g. limb or axial muscle) (Fig. 1 A,B). Within each motor column, MNs further segregate into motor pools, each pool innervating a single muscle. Motor columns and pools are generated within defined rostrocaudal positions, and multiple columns and pools can occupy a single segment. While most MNs rely on *Hox* function in tetrapods, MNs targeting axial muscle (HMC and MMC neurons) do not appear to rely on Hox function for their specification (Fig. 1C).

Hox proteins promote MN diversification through regulating expression of other fate determinants and can act in concert with additional TF classes, including Lim-, Mnx-, and Pbx homeodomain factors. A key direct target of Hox proteins in MNs is the transcription

factor *Foxp1*, which is induced at high levels by multiple Hox proteins expressed by limb-innervating LMC neurons, and at reduced levels by *Hoxc9* in thoracic preganglionic column (PGC) neurons (Fig. 1A,B) (Jung et al., 2014, Jung et al., 2010, Lacombe et al., 2013, Dasen et al., 2003). Mutation of *Foxp1* in mice leads to a loss of molecular signature for Hox-dependent MN columnar and pool subtypes, and reversion of MN identities to the more ancestral axial MN fate (Dasen et al., 2008; Rousso et al., 2008). Subsequent to its induction by Hox proteins, Foxp1 also acts act in conjunction with Hox proteins in LMC neurons to promote motor pools fates (Fig. 1E).

In forelimb LMC neurons, multiple genes in the *Hox4-Hox8* paralog group are involved in specifying motor pools. For example, *Hoxc8* is essential for establishing the molecular identities and connectivity of MN pools targeting distal forelimb muscles. *Hoxc8* contributes to motor pool fates by regulating expression of *Ret* and *GFRα* surface receptor genes that are essential for proper forelimb innervation (Fig. 1D, E) (Catela et al., 2016). Individual *Hox* genes and paralog groups also play more restricted roles in MN diversification. For example, two *Hox5* paralogs (*Hoxa5* and *Hoxc5*) are essential for the development of phrenic MNs targeting respiratory muscle (Philippidou et al., 2012). Recent studies indicate Hox5 proteins regulate expression of cadherin proteins involved in phrenic MN clustering and connectivity to premotor respiratory networks (Fig. 1B) (Vagnozzi et al., 2020). As described later, loss of *Hox* function also can also lead to MN fate transformations through derepression of *Hox* genes normally expressed in adjacent segments.

Hox proteins also act in conjunction with more broadly expressed co-factors promote fate MN specification. Members of the Pbx family are well known cofactors for Hox proteins, which and enhance the affinity and specificity of Hox proteins to target sites (Bobola and Sagerström., 2022). After deletion of *Pbx* genes (*Pbx1* and *Pbx3*) from MNs in mice, Hox-dependent subtype features are lost (Hanley et al., 2016). *Pbx* mutants are therefore similar to *Foxp1* mutants, but appear to affect a broader range of MN subtypes, including phrenic MNs. Pbx TFs also have Hox-independent functions that promote the organization of axial MNs, as *Pbx* mutants are characterized an erosion in axial MN molecular signatures and somatotopic organization (Hanley et al., 2016).

3. Establishing presumptive *Hox* boundaries in neural progenitors

In order to specify MN fates, *Hox* expression must be precisely regulated during development. Immediately following gastrulation, *Hox1-Hox3* paralogs are expressed in the mesoderm and weakly expressed in the epiblast at the caudal section of the primitive streak (Deschamps and Wijgerde, 1993; Young and Deschamps, 2009). As the embryo lengthens, expression of these early *Hox* genes extends rostrally, eventually reaching their definitive boundaries in the neuroectoderm, mesoderm, and endoderm. The initial induction of *Hox* genes, and therefore the axial patterning of the embryo, appears to occur prior to neurogenesis (Metzis et al., 2018). The exact rostral limit of *Hox* expression is more rostral for 3’ genes than 5’. This rostral extension of the *Hox* domains results in an overlapping pattern of expression which is further refined, to produce demarcated *Hox* expression domains (Fig. 2A). In the following subsections, we will discuss how signaling

from extrinsic morphogens, regulation of chromatin structure, and intrinsic factors establish presumptive *Hox* domains in the neural tube during axial elongation.

3.1 Regulation of axial positional identities by morphogens during neurogenesis

In neural progenitors, retinoic acid (RA) signaling functions to promote the expression of *Hox1-Hox5* paralogs, while fibroblast growth factor signaling (FGF) initiates caudal *Hox* expression in conjunction with Wnt signaling. The synthesis of RA during development is regulated by Retinaldehyde Dehydrogenase 2 (RALDH2), which is expressed in trunk somites and presomitic mesoderm (Niederreither et al., 1997). In addition, RA is degraded by Cytochrome P450 26 proteins (CYP26A1/B1/C1) expressed in the tailbud, creating a rostral-caudal gradient of RA activity along the neural tube (Bernheim et al., 2020). *Fgf8* is transcribed in the tail bud and generates a caudal-rostral gradient through mRNA decay (Dubrulle and Pourquié, 2004). FGF signaling also restricts RA synthesis by activating *CYP26A1* expression (Niederreither et al., 2002), preventing RA signaling in regions of high FGF (Boulet et al., 2012; Diez del Corral., 2003). In rostral regions, RA inhibits the expression of *FGF8* through binding of retinoic acid receptors (RARs) at regulatory sequence upstream of the gene (Kumar and Duester, 2014, Diez del Corral et al., 2003). RA and FGF therefore sets up rostrocaudal patterning through reciprocal cross-regulatory interactions (Diez del Corral and Storey, 2004), providing the necessary inductive signals to establish the early pattern of *Hox* expressions (Fig. 2A).

3.2 Retinoic acid and rostral patterning of Hox expression in spinal progenitors

The initial rostral expansion of *Hox* expression in the neural tube is driven by the activity of RA, which is essential for many developmental processes including rostrocaudal patterning, somitogenesis, and neural development (Iimura et al., 2019; Janesick et al., 2019). Following synthesis of RA and diffusion to neighboring cells, RA acts as a ligand for RARs which heterodimerize with Retinoic X Receptors (RXRs), both of which are widely expressed in many tissues (Kastner et al., 1997; Diez del Corral et al., 2002; Mahoney et al., 2011). In the presence of RA, RAR-RXR heterodimers interact with co-activator complexes at Retinoic Acid Response Elements (RAREs) to activate gene expression. These RAREs are exemplified by direct repeats of the hexameric “(A/G)G(G/T)TCA”, often found in close proximity to promoter regions of target genes (Lalevée et al., 2011; Mahoney et al., 2011). Once activated by RA and bound to RAREs, RAR/RXRs can initiate transcription through the interactions with general coactivators and RNA Polymerase II machinery.

Genes within *Hox* clusters are differentially susceptible to RA concentration, with genes such as *Hoxa1* rapidly induced following RA induction and more caudal *Hox* genes responding more slowly. RAREs have been found proximal to *Hox1-Hox5* genes (Nolte and Krumlauf., 2013; Mazzoni et al., 2013, Nolte et al., 2019). They primarily function to promote the expression of nearby *Hox* genes, but in the developing heart and gut RAREs flanking the *HoxB* cluster have been found to distally regulate *Hox* expression over longer genomic distances (Nolte et al., 2013; Qian et al., 2018).

The mechanisms of gene activation following RA induction is varied. During hindbrain patterning, RARs associated with the promoter of *Hoxa1* and in the absence of RA pause

RNA PolII and prevent transcription (Gaertner et al., 2012). Activation of RARs by RA results in the unpausing of RNA PolII and the fast release of repression. Contrasting this, RARs are only recruited to the RARE proximal to *Hoxb1* in the presence of RA, thereby resulting in a slower onset of transcription compared to *Hoxa1* (Lin et al., 2011). Preliminary evidence suggests that regions flanking the core RARE sequence dictate enhancer strength, and thus the differential sensitivity of *Hox* groups to RA concentrations.

3.3 Roles of RA and TAD boundaries in regulating Hox expression in MN progenitors

The induction of *Hox1-Hox5* genes by RA in ESC-derived MNs (ESC-MNs) is associated with the clearance of Polycomb-associated repressive H3K27me3 histone marks and presence of Trithorax complex-associated activation histone marks (H3K4me) (Fig. 2B) (Mazzoni et al., 2013; Narendra et al., 2015). The extent of clearance of PRC repressive marks is constrained by the position of binding sites for CTCF (CCCTC-binding factor). CTCF is an evolutionarily conserved DNA binding protein that localizes to borders between topologically-associated domains (TADs), mega-base regions of localized chromatin interactions (Dixon et al., 2012). Although the precise functions of TADs in regulating gene expression are unclear, CTCF binding at TAD boundaries appears to act as an insulator that prevents interactions between enhancer and repressor elements in neighboring TADs (Batut et al., 2022). Studies in the developing mouse limb bud support an essential role for CTCF function and its binding sites in regulating the pattern of *Hox* expression (Soshnikova et al., 2012; Rodríguez-Carballo et al., 2020).

Studies in ESC-MNs have examined the role of CTCF sites in the regulation of *Hox* expression during neural patterning. Deletion of a CTCF binding site between *Hoxa5* and *Hoxa6* (C5|6) results in the ectopic expression of *Hoxa7* following RA induction. Combined deletion of the *Hoxa5|6* site and a CTCF site located between *Hoxa7* and *Hoxa9* (C7|C9) leads to the loss of the C5|6 TAD boundary and ectopic expression of *Hoxa7-Hoxa10* genes (Fig. 2C) (Narendra et al., 2015, Narendra et al., 2016). Similar deletion of CTCF sites from the *HoxC* cluster result in ectopic expression of *Hoxc6* and *Foxp1* in ESC-derived MNs (Narendra et al., 2016).

Studies performed *in vivo* also support a role for CTCF sites in *Hox* gene regulation. Mutation of CTCF binding sites in the *HoxA* and *HoxC* clusters leads to a homeotic transformation of axial skeletal elements, confirming that CTCF is required for *Hox*-dependent body patterning (Narendra et al., 2016). A participating cofactor in the CTCF complex, Myc-Associated Z-Finger (MAZ) has also been found to directly interact with CTCF in ESC-MNs, and is required for the demarcation of active-inactive compartments of the *HoxA* cluster (Ortabozkoyun et al., 2022). These results indicate that transcriptional activation of *Hox* genes following RA treatment is constrained by the activity of CTCF and MAZ, preventing large-scale derepression of the *Hox* clusters.

3.4 FGFs induce caudal Hox genes in spinal progenitors via Cdx proteins

In addition to the regulation of *Hox1-Hox5* paralog groups by RA, FGFs provide a caudalizing signal for patterning *Hox* expression in the neural tube. FGFs are known to be involved in many aspects of early vertebrate development, including endoderm formation,

gastrulation, and neural induction (Böttcher and Neihrs, 2002). Early evidence supporting a role for FGF in caudalization of *Hox* patterning came from studies in *Xenopus*, where FGFs were shown to upregulate caudal *Hox* gene expression in a dose-dependent manner (Lamb and Harland., 1995). Higher concentrations of FGF results in the expression of progressively 5' *Hox* genes (Pownall et al., 1996; Kengaku and Okamoto., 1993; Cox and Hemmati-Brivanlou., 1995). Furthermore, expression of a dominant-negative FGFR results in the reduction of *Hoxb9* expression and defects in caudal CNS development (Godsave and Durston., 1997). In mice, hypomorphic mutants of FGFR1 results in caudal shifts in expression of *Hoxd4* and *Hoxb9* (Partanen et al., 1998).

In explants of chick neural progenitors, addition of FGF8 is sufficient to promote expression of *Hoxc6, Hoxc8*, and *Hoxc9* in MNs in a dose dependent manner (Liu et al., 2001), indicating that *Hox* genes are sensitive to graded level of FGF. Similarly, adding FGFs to cultured chicken embryos results in a rostral shift of *Hoxb9* expression (Bel-Vialar et al., 2002). Elevation of FGF8 signaling *in vivo* extends the rostral limits of *Hoxc9* expression into in brachial-level MN progenitors, which in turn inhibits the expression of *Hoxc6* (Dasen et al., 2003). The transformation of *Hox* pattern after FGF elevation also leads to a switch in MNs fates in brachial segments, causing a loss of the LMC marker *Raldh2* and a gain of the PGC marker *Bmp5* (Dasen et al., 2003). Thus, elevation of FGF8 expression results in the transformation of brachial spinal MNs into a thoracic identity.

The induction of caudal *Hox* paralogs by FGF is mediated through the activity of Cdx homeodomain proteins. FGF signaling promotes expression of *Cdx* genes in chick and *Xenopus* through cooperation with Wnt and MAP kinase pathways (Bel-Vialar et al., 2002; Keenan et al., 2006; Nothrop and Kimmelman., 1994; Young et al., 2009). Overexpression of an activated form of the *Xenopus* homolog of *Cdx, XcadVP16*, results in an upregulation of *Hoxb9* and rostral extension of its expression domain (Bel-Vialar et al., 2002). By contrast, expression of a dominant negative allele of *Xcad3* abrogate activation of *Hoxb9* following FGF treatment, showing the role of the *Cdx* family downstream of FGF (Isaacs et al., 1998).

Studies in ESC-MNs indicate that *Cdx2* directly binds at caudal *Hox* genes and its binding is associated with the removal of H3K27me3 repressive marks from *Hox6-Hox9* genes in the presence of FGF (Fig. 2B) (Neijts et al., 2017; Mazzoni et al., 2013). Cdx/FGF signaling also decompacts *Hox* clusters and enables the deposition of the activating H3K27ac signal (Mazzoni et al., 2014; Neijts et al., 2016; Neijts et al., 2017). Complete removal of H3K27me3, and thus full activation of more caudal *Hox* paralogs, likely requires additional patterning signals. These signals include *Gdf11*, which has been shown to be necessary for activation of *Hox10* genes chick explants, mouse embryos, and human ESC-MNs (Shi and Liu., 2011; Liu., 2006; Mouilleau et al., 2021). This work indicates that extrinsic signals and *Cdx* proteins regulate the chromatin landscape of *Hox* clusters, removing inhibitory marks and making *Hox* targets available for transcriptional activation.

In addition to binding of Cdx and RAR within *Hox* clusters, distal regulatory control regions, and topological DNA organization have been implicated in the regulation *Hox* expression (Darbellay and Duboule., 2016). Defining the specific contributions of local

and long-range regulatory elements in regulating *Hox* expression and chromatin structure has been challenging to disentangle. To tease apart the functional requirement for specific genomic elements, a synthetic construct of the rat *HoxA* cluster has recently been developed for fine control of *cis*-regulatory elements within the cluster (Pinglay et al., 2022). Consistent with previous studies, deletion of RAREs within the 3'-region *HoxA* cluster prevented removal of H3K27me3 marks after RA treatment, and *Hox1-Hox5* failed to be activated. By contrast, the absence of distal enhancers had no effect on chromatin remodeling in response to RA, but were required for high levels of *Hox* gene expression. These studies support a model in which internal elements within the *Hox* clusters, such as local RAR, Cdx, and CTCF binding sites, are necessary to establish appropriate patterns of *Hox* gene expression, while distal enhancers are required for robust levels of gene expression.

4. Hox cross-repressive interactions and establishment of postmitotic MN positional identities

Following the morphogen-induced patterning of *Hox* expression in neural progenitors, there is initially considerable overlap between the domains of *Hox* expression in caudal segments of the neural tube. In order to correctly specify neuronal subtype identities, *Hox* gene expression is further restricted along the rostrocaudal axis and within a single segment. This pattern of refinement is driven through direct cross-regulatory interactions between Hox proteins and *Hox* genes and appears to occur in postmitotic neurons.

Pairs of *Hox* genes display mutually-exclusive patterns of expression in MNs along the rostrocaudal axis, similar to the boundaries of TF expression established in spinal progenitors along the dorsoventral axis (Dasen et al., 2003; Dasen et al., 2005; Briscoe et al., 2000). For example, the boundary between brachial and thoracic segments is established through cross-repressive interactions between *Hoxc6* and *Hoxc9*, which demarcates the positional boundary between limb-innervating LMC and thoracic PGC neurons (Fig. 3A). Ectopic postmitotic expression of *Hoxc9* in brachial segments can inhibit expression of *Hoxc6* and other brachial-expressed *Hox* genes, while mutation in *Hoxc9* in mice results in derepression of brachial *Hox* genes in thoracic segments (Jung et al., 2010). Depression of brachial *Hox* genes in *Hoxc9* mutants leads to an extension of forelimb-innervating MN subtypes into thoracic segments. Interestingly, in vertebrates which normally lack brachial limb MN subtypes, such as in limbless snakes, the pattern of *Hoxc9* extends throughout the spinal cord and is associated with an absence of *Hox4-Hox8* paralog expression by MNs (Jung et al., 2014). Although the precise mechanisms that determine the expanded domain of *Hoxc9* in snakes is unclear, these observations suggest that changes in *Hox* gene expression contributed to the evolutionary of spinal MN organization. This idea is further supported by studies in the little skate *Leucoraja erinacea*, which lack the *Hoxc9* gene, and display an expanded domain of fin-innervating LMC neurons along the spinal cord (Jung et al., 2018).

Cross-repressive interactions can also occur within a single segmental level of the spinal cord. For example, cross-repressive interactions between *Hox4-Hox8* genes determine identity of motor pools within the brachial LMC (Dasen et al., 2005; Catela et al., 2016).

However, not all intrasegmental Hox cross-repressive interactions give rise to mutually exclusive expression of *Hox* genes. For example, interactions between Hoxc6, Hoxc8, and Hoxc9 allow for the specification of a subtype of LMC neuron that innervate forelimb digits (Fig. 1E) (Mendelsohn et al., 2017). These MNs lack the canonical limb MN determinant Hoxc6, and express low levels of Hoxc9 and Hoxc8, possibly reflecting differential weighting in the repressive activity of Hoxc9 towards the *Hoxc8* and *Hoxc6* genes.

Studies in ESC-MNs indicate that cross-repressive activities are mediated through direct interactions of Hox proteins on *Hox* genes. Chip-seq studies in ESC-MNs show that Hoxc9 binds to genomic regions near *Hox4-Hox8* paralogs (Jung et al., 2010; Bulajić et al., 2020). Misexpression of more caudally-expressed *Hox* genes such as *Hoxc10* or *Hoxc13* results in the repression of a larger group *Hox* genes. For example, misexpression of Hoxc13 in chick represses *Hox4-Hox10* paralogs, and binds at additional 5'-sites within *Hox* clusters (Sawai et al., 2022; Bujalic et al., 2020). The extent to which *Hox* genes are repressed therefore appears to correlate with cluster position, with proteins encoded by 5'-*Hox* genes repressing larger number of 3'-*Hox* genes. This ability of caudal *Hox* genes to repress more rostral *Hox* genes is reminiscent of a phenomena first described in *Drosophila* called termed posterior dominance or phenotype repression (Duboule and Morata, 1994).

5. Post-transcriptional regulation of *Hox* genes by non-coding RNAs

The pattern of *Hox* expression in MNs can also be fine-tuned by non-coding RNAs that post-transcriptionally regulate specific *Hox* genes. A major class of ncRNAs, long non-coding RNAs (lncRNAs), have been implicated in *Hox* gene regulation. One of the first lncRNAs reported to affect *Hox* expression in vertebrates is *HOTAIR*, transcribed from the *HoxC* cluster (Rinn et al., 2007). Following deletion of the *HOTAIR* in fibroblasts, *HoxD* cluster are derepressed (Li et al., 2013). *HOTAIR* has been shown to associate with PRC2, suggesting this lncRNA functions to recruit repressors to *Hox* loci (Wu et al., 2013). Despite this, mouse mutants of *HOTAIR* display no detectable change in embryonic *HoxD* expression (Amândio et al., 2016), and *HOTAIR*-mediated repression can occur independently of PRC2 activity (Portoso et al., 2017), bringing into question the significance of *HOTAIR* for developmental patterning.

A lncRNA produced from the *Dlk1-Dio3* locus, *meg3*, is highly expressed in postmitotic brachial MNs (Yen et al., 2018). *Meg3* associates with the Jarid2/PRC2 complex to inhibit the expression of caudal *Hox* genes (Yen et al., 2018). Knockdown of *meg3* on ESC-MNs results in a reduction in PRC2-deposited H3K27me3 mark and an upregulation of *Hox8-Hox13* paralogs. In mice lacking *meg3* activity, there is a derepression of *Hoxc8* in rostral brachial segments, and an expansion in the motor pools normally located in caudal brachial segments (Fig. 3B). Interestingly, more caudal *Hox* genes, such as *Hox9* and *Hox10* genes, are not affected by *meg3* depletion (Yen et al., 2018), suggesting segment-restricted functions for lncRNAs in refining *Hox* pattern in MNs.

A second class of ncRNAs, microRNAs, are 22-bp single stranded RNA molecules which function to post-transcriptionally regulate gene expression. In *Drosophila* several miRNAs

that inhibit *Hox* expression have been identified, including *miR-iab-4-5p*, mapped upstream of the *Abd-A* gene in the *BX-C* cluster (Tanzer et al., 2005). Expression of miRNAs is often restricted to the same spatial domains as their coding *Hox* counterparts (Bae et al., 2002). *miR-iab-4-5p* has been found to be functionally conserved in vertebrates (Tanzer et al., 2005), and its overexpression in *Drosophila* phenotypically mirrors loss of *Ubx* (Ronshaugen et al., 2005). This pattern of *Hox*-repression by miRNA is consistent with other miRNA sequences found associated to the *BX-C* cluster in *Drosophila*, such as *miR-iab-9-5b*, mapped upstream of the *Abd-B* locus. Due to their close proximity to the coding sequence of the *Hox* homologs, it is thought that these miRNAs are regulated in tandem with their gene targets (Wang et al., 2008). *miR-iab-4-5p* and *miR-iab-9-5p* both inhibit the activity of rostrally-expressing *Ubx, Abd-A*, and *Antp*. This raises the possibility that the generation of miRNAs in caudal regions facilitates the posterior dominance and refinement of *Hox* domains (Singh and Mishra, 2008).

The posttranscriptional regulation of *Hox* genes by microRNAs is conserved in mammals. In mammalian cell lines overexpressing *miR-196* (analogous to *iab-4*), a miRNA associated with the *HOXB8* locus, show repression of the *HOXB8* transcript (Yekta et al., 2004). In ESC-MNs, loss of the miRNA synthesizing enzyme *Dicer* results in precocious and noisy expression of Hoxa5 protein and an erosion of the normal Hoxa5/Hoxc8 boundary within brachial segments, indicating miRNAs are involved in the tempering of *Hox* expression (Fig. 3B) (Li et al., 2017). Depleting the function of *miR-27* in both mESCs and chick embryos leads to ectopic Hoxa5 expression in the *Hoxc8* expression domain, mirroring the disrupted *Hoxa5-Hoxc8* boundary observed in *Dicer* mutants.

6. Polycomb group proteins and the regulation *Hox* expression

Due to their integral role in neuronal subtype diversification, the timing of *Hox* gene expression must be tightly regulated in both space and time. Prior to gastrulation, as well as in pluripotent embryonic stem cells (ESCs), *Hox* clusters are compacted and transcriptionally silent (Fig. 4A) (Montavan and Soshnikova, 2014, Boyer et al., 2006). The early silencing of *Hox* genes, and the eventual maintenance of *Hox* repression, is regulated by histone-associated proteins in the Polycomb group (PcG) family. In *Drosophila*, mutation of the *Polycomb* (*Pc*) PcG protein results in misexpression of *Bithorax* genes and homeotic transformation of body pattern, providing evidence that PcG proteins function to silence *Hox* expression (EB Lewis., 1978). Further characterization of PcG proteins in *Drosophila*, mouse, and other model systems solidified an evolutionary conserved role in the developmental regulation of *Hox* expression (Gentile and Kmita, 2020; Lanzuolo and Orlando, 2012).

PcG proteins associate to form two histone-modifying Polycomb Repressive Complexes (PRCs), PRC1 and PRC2. The activity of these complexes allows for the binding, methylation, and compaction of target histones, resulting the transcriptional silencing of genomic loci (Loubiere et al., 2019; Simon and Kingston, 2009). In vertebrates, PRC2 can be recruited to target histones by Polycomb-Like (PCL) proteins, often through the presence of unmethylated CpG islands (Ku et al., 2008; Li et al., 2017). Once associated with a locus, Ezh1/2 constituents of PRC2 methylate histone H3 at lysine 27 (H3K27me3). Mono-

and bi-methylation of H3K27 promote further methyltransferase activity through a positive-feedback loop driven by the core PRC2 subunits Eed and Suz12 (Ciferri et al., 2012). The H3K27me3 mark recruits PRC1 to a target locus, through interaction with the Cbx protein constituent of PRC1. Once bound, Ring1A/B proteins ubiquitinate Histone H2A at lysine119 (H2K119ub), while PHC polymerization contributes to spreading PRC1, thereby increasing the presence of the complex at a locus (Geng and Gao, 2020; Isono et al., 2005; Isono et al., 2013). The activities of PRC lead to the compaction of associated chromatin, the inhibition of RNA Pol II transcriptional initiation/elongation, and the transcriptional silencing of the resident genes (Stock et al., 2007).

6.1 Early silencing of Hox loci by PcG proteins

PRC activities are is essential for the controlled silencing of *Hox* clusters, until the correct timing of induction during development. Depletion of PRC2 activity in ESCs results in the loss of H3K27me3 marks in *Hox* clusters and ectopic *Hox* gene expression. Removal of PRC1 function during these early stages results in a similar decondensation of the *Hox* clusters, and loss of transcriptional silencing (Eskeland et al., 2010; Wang et al., 2004). Mouse mutants of core PRC1 and PRC2 components are embryonic lethal, often stalling development during early gastrulation (Faust et al., 1998, Voncken et al., 2003). Mutation of sub-stoichiometric PRC components display later developmental defects and homeotic transformations, suggesting a cell-type specific or temporal necessity of PRC constituents (Grijzenhout et al., 2016; Li et al., 2017; Golden and Dasen., 2013; Isono et al., 2005; Isono et al., 2013; Chawengsaksophak et al., 1997; Castelli-Gair et al.,1990). Core PRC subunits are therefore broadly required during early development, while specific interacting cofactors appear to enable cell-type specific control of gene expression in a variety of contexts

Following induction of *Hox* expression in neural progenitors, and refinement of rostrocaudal boundaries through cross-repression, the patterns of *Hox* expression are maintained in newly postmitotic neurons. As Hox proteins regulate key target effectors in postmitotic neurons, the profiles of *Hox* expression must be preserved through the transition from progenitors to terminal fates. Studies in *Drosophila* indicate the inheritance of positional identities ("epigenetic memory") are facilitated through PRC activities (Ciabrelli et al., 2017; Coleman and Struhl., 2017; Corley and Kroll., 2015). Below we discuss the mechanisms of PRC-mediated repression in vertebrates, their various accessory subunits, and the known functions of PRCs in the regulation of *Hox* gene expression in the CNS.

6.2 Mechanisms of PRC2 function in Hox regulation

The core of PRC2 consists of three subunits: Enhancer of Zeste 2 (Ezh2 or its paralog Ezh1), Suppressor of zeste 12 (Suz12), and Embryonic ectoderm development (Eed) (Loubiere et al., 2019). Ezh2 is the main catalytic constituent of PRC2, while its Ezh1 paralog has weaker activity and more restricted expression. Ezh2 functions as a histone methyl transferase (HMTase) to catalyze the methylation of H3K27, allowing for recruitment PRC1 to genomic loci. Both Suz12 and Eed positively regulate the HMTase activity of Ezh1/2. Suz12 serves a structural function, stabilizing the PRC2 complex and maintaining HMTase activity (Pasini et al., 2004; Hojfeldt et a., 2018). Eed promotes Ezh1/2 activity through

a positive-feedback loop, recognizing and binding to H3K27me3 signals and allosterically activating the HMTase activity of PRC2 (Margueron et al., 2009).

Additional cofactors can associate with PRC2 and modulate its activity (Fig. 4B) . PRC2.1 contains Polycomb-like proteins 1-3 (PCL1-3), which are thought to aid in the initial recruitment of PRC2 to loci (Bellare et al., 2012; Cai et al., 2013). Loss of PCL proteins in *Drosophila* and vertebrates results in homeotic transformations and *Hox* misexpression (Duncan., 1982; Li et al., 2011). PRC2.2 is defined by the presence of Jumonji, AT rich interactive domain 2 (JARID2) and adipocyte enhancer-binding protein 2 (AEBP2). JARID2 and AEBP2 both work to recruit PRC2 to chromatin and associate with PRC 1-deposited H2AK119ub, facilitating cross-talk between the PRC1 and PRC2 (Landeira et al., 2010; Cooper et al., 2016). Using separation-of-function mutants of SUZ12 in induced pluripotent stem cells (iPSCs), it has been found PRC2.2 associates with target genes at lower levels than PRC2.1, and enrichment of relative PRC2.2 abundance is paired with an upregulation of *Hox* target genes (Youmans et al., 2021). The exact functional relationship between PRC2.1 and PRC2.2 conformations is currently unknown, but they appear to act in both synergistic and independent fashions to deposit H3K27 methyl marks on target genes, and are both required for efficient silencing of *Hox* genes (Healy et al., 2019).

The role of PRC2 in the establishment and maintenance of neuronal identities has been explored in vertebrates through deletion of core subunit-encoding genes. Removal of PRC2 function can lead to defects in the temporal transition from neurogenesis to gliogeneis, neuronal subtype specification programs, and derepression of *Hox* genes (Wang et al., 2020; Yaghmaeian Salmani et al., 2018; Di Meglio et al., 2013; Schimmelmann et al., 2016; Hirabayashi et al., 2009). In spinal MNs, PRC2 function has been examined through deletion of *Eed* from neural progenitors and through combined deletion both *Ezh1/2* genes in MNs. Neural-specific deletion of these core PRC2 constituents in mice does not have a noticeable effect on embryonic *Hox* pattern or MN subtype diversification programs (Sawai et al., 2022). The absence of an observable phenotype PRC2 mutants may reflect residual H3K27me3 carried over from earlier stages, or a more pronounced reliance on PRC1 for maintaining postmitotic *Hox* expression.

6.2 PRC1 is essential to maintain Hox pattern in MNs

PRC1 conformations are also structurally and functionally diverse (Fig. 4B) . PRC1 conformations can be broadly classified as either canonical (cPRC1) or noncanonical PRC1 (ncPRC1), distinguished by their specific constituents. The core proteins of PRC1, which are shared in cPRC1 and ncPRC1, include Ring1A/B and one of six Polycomb Group ring fingers 1-6 (PCGF1-6). Ring1A/B is an E3 ubiquitin-ligase, which deposits H2AK119ub on target histones. There is uncertainty regarding the function of Ring1 ubiquitination activity in *Hox* gene regulation. Early silencing of *Hox* clusters appears independent of Ring1 ubiquitin-ligase activity, as enzymatically inactive mutants of Ring1B are capable of rescuing *Hox* silencing in *Ring1B*-null backgrounds (Eskeland et al., 2010; Tsuboi et al., 2018). Recent work has suggested that H2AK119ub is required in ESCs and neural progenitors to temporally repress neuronal genes, but is dispensable for long-term silencing

of *Hox* targets (Tsuboi et al., 2018). Other reports show that the ubiquitin-ligase activity of Ring1B is required for the maintenance of ESC identity (Endoh et al., 2012).

In canonical PRC1, Ring1A/B associates with a PCGF, one of three Polyhomeotic-like (PHC1-3) proteins, and a CBX protein (CBX2/4/6-8). CBX proteins contain a chromodomain which recognizes H3K27me3 marks and is involved in the recruitment of PRC1 to target loci (Kaustov et al., 2011). CBX proteins have also been found to possess chromatin-compaction activities and are necessary for *Hox* repression (Grau et al., 2011; Lau et al., 2017). Once bound to H3K27me3, polymerization of PRC1 subunits results in the spreading of PRC1 occupancy across a locus, the formation of discrete genomic domains of high interaction, and the generation of phase-separated PcG conglomerates (termed PcG bodies) (Isono et al., 2005; Isono et al. 2013; Robinson et al., 2012; Wani et al., 2016). Formation of PcG bodies involves polymerization of PHC proteins via its SAM-domain as well as through Cbx proteins (Isono et al., 2013; Piunti and Shilatifard, 2022). The formation of tightly-compacted loci are inaccessible to activating transcriptional machinery.

Due to compaction, genomic loci associated with PRCs become physically clustered and tightly associated. The linkage of genomically distant sites allows for increased coordinated regulation of the resident target genes. Through chromatin conformation analyses, it has been shown that sites enclosed within PcG bodies preferentially interact in discrete domains (Tolhuis et al., 2011; Batignies et al., 2011; Kundu et al., 2017). In mice, these domains demarcate active vs. inactive genomic regions, and following RA-differentiation they separate rostral and caudal-expressing *Hox* groups into two associated populations which are differentially regulated (Chambeyron and Bickmore, 2004).

Non-canonical PRC1 configurations contain RYBP (Ring1 and YY1 binding protein) or YAF2 (YY1 associated factor 2). RYBP can compete for the same binding pocket on Ring1B, and displace CBX from Ring1 (Wang et al., 2010). The presence of RYBP in ncPRC1 stimulates the E3 ubiquitin-ligase activity of Ring1A/B (Gao et al., 2012), and majority of the H2AK119ub genomic signals are thought to be deposited by ncPRC1 (Tavares et al., 2012). Despite this, the significance of H2AK119ub deposition and ncPRC1 activity for the regulation of *Hox* transcription during embryonic development is currently unknown. The function of ncPRC1 in specification of spinal MN subtypes also appears to be minimal. While global *Rybp* mutant mice are embryonic lethal (Pirity et al., 2005), combined conditional deletion of *Rybp* and *Yaf2* from MN progenitors show no obvious changes in *Hox* expression or MN subtype differentiation programs at embryonic stages (Sawai et al., 2022).

In spinal MNs, cPRC1 plays a critical role in maintaining *Hox* patterns and ensuring the silencing of inappropriate gene programs. Depletion of the PCGF protein Bmi1 in chick embryos leads to ectopic expression of *Hoxc9* in brachial segments and a switch in the fate of brachial LMC neurons to a thoracic PGC fate (Golden and Dasen, 2013). In mice, mutation of *Ring1A/B* from MNs results in a widespread derepression of dozens of fate determinants, including caudal *Hox* genes (Sawai et al., 2022). Derepression of caudal *Hox* genes, in particular *Hox13* paralogs, leads to the suppression of *Hox4-Hox10* expression in MNs (through cross-repression), and a loss of all Hox-dependent columnar

and pool subtypes (Fig. 4C). This indicates that in postmitotic MNs, cPRC1 contributes to the maintained repression caudal *Hox* genes. The degree to which postmitotic MNs rely on PRC2 function and the specific contribution of PRC1 subunits CBX and PHC in local compaction and silencing of *Hox* genes remains to be fully explored.

8. Future directions and remaining questions

Spatial and temporal control of *Hox* expression is necessary for body patterning and cellular differentiation. Work over the last decades has provided a wealth of information on the dynamic regulation of *Hox* expression during neural development, from early progenitor stages to maintenance in postmitotic neurons. Recent studies have also raised important questions concerning how and when patterning signals operate to regulate *Hox* expression. A classic model for neural development is the “activation-transformation” model in which neural progenitors are initially specified with a rostral (brain) identity, which lack *Hox* gene expression, and are later transformed to a caudal (hindbrain/spinal cord) fate (Stern., 2001). *In vitro* and *in vivo* studies of regions of condensed and open chromatin in neural progenitors indicate that opening of *Hox* regions precedes neural induction, suggesting that early patterning signals may act initially on progenitor’s cells prior to neural fate specification (Metzis et al., 2018). Further studies *in vitro* support a model in which the timing of *Hox* expression is governed by extrinsic patterning cues, as opposed to an intrinsic “Hox clock”-based timer (Mouilleau et al., 2021). The precise relationships between the timing of *Hox* induction, regulation of local chromatin structure, and mechanisms through which morphogens regulate *Hox* pattern remain to be determined.

Early programming of *Hox* profiles in stem cell populations prior to neurogenesis may serve to coordinate positional identities among neural and non-neural tissues. Patterning morphogens such as RA and FGF could also act at later stages to control more unique *Hox* codes in neural tissues. After rostrocaudal positional identities are established, *Hox* gene expression is further shaped along the dorsoventral axis. For example, early studies indicated that while *Hox* genes are initially uniformly expressed in neural progenitors, at later postmitotic stages *HoxB* genes become restricted to the dorsal spinal cord, while *HoxC* genes localized to ventral types, including MNs (Graham et al., 1991; Liu et al., 2001). These later changes could reflect further modification to *Hox* clusters, mediated through developmental regulation of chromatin-modifying proteins. The abundances of sub-stoichiometric PRC components are dynamic during progressive stages of neural differentiation (Kloet et al., 2016), with PRC2 proteins becoming markedly downregulating during NPC differentiation, while PRC1 remains bound to H3K27me3 loci. The differential function of PRC conformations at various stages of neural differentiation has proved challenging to decipher, due to the wide variety of interacting cofactors and the embryonic lethality of core PRC mutants. With the advent and improvement of genomic analysis methods, our ability to observe the topographical organization and compaction of *Hox* clusters has significantly improved. Correctly utilizing these techniques to study *Hox* cluster compaction, PcG body polymerization, and TF binding will be integral to fully resolve the dynamic regulation of *Hox* genes during nervous system development.

Acknowledgements

This work was supported by NIH NICHD grant T32 HD007520 to A.M and NIH NINDS grant R35 NS116858 to J.D.

References

Amândio AR, Necsulea A, Joye E, Mascrez B, Duboule D. Hotair Is Dispensible for Mouse Development. PLoS Genet. 2016 Dec 15;12(12):e1006232. doi: 10.1371/journal.pgen.1006232. [PubMed: 27977683]

Carrasco Andrés E., McGinnis William, Gehring Walter J., De Robertis Eddy M.. Cloning of an X. laevis gene expressed during early embryogenesis coding for a peptide region homologous to Drosophila homeotic genes. Cell. Volume 37, Issue 2. 1984

Bae E, Calhoun VC, Levine M, Lewis EB, Drewell RA. 2002. Characterization of the intergenic RNA profile at abdominal-A and Abdominal-B in the Drosophila bithorax complex. Proc Natl Acad Sci USA 99:16847–16852. [PubMed: 12481037]

Baek M, Menon V, Jessell TM, Hantman AW, Dasen JS. Molecular Logic of Spinocerebellar Tract Neuron Diversity and Connectivity. Cell Rep. 2019 May 28;27(9):2620–2635.e4. doi: 10.1016/j.celrep.2019.04.113. [PubMed: 31141687]

Batut PJ, Bing XY, Sisco Z, Raimundo J, Levo M, Levine MS. Genome organization controls transcriptional dynamics during development. Science. 2022 Feb 4;375(6580):566–570. doi: 10.1126/science.abi7178. Epub 2022 Feb 3. [PubMed: 35113722]

Bel-Vialar S, Itasaki N, Krumlauf R. "Initiating HOX expression: in the early chick neural tube differential sensitive to FGF and RA signaling subdivides the HOXB genes into two distinct groups". Development. (2002)

Bernheim S, Meilhac SM. Mesoderm patterning by a dynamic gradient of retinoic acid signalling. Philos Trans R Soc Lond B Biol Sci. 2020 Oct 12;375(1809):20190556. doi: 10.1098/rstb.2019.0556. Epub 2020 Aug 24. [PubMed: 32829679]

Bobola N, Sagerström CG. TALE transcription factors: Cofactors no more. Semin Cell Dev Biol. 2022 Dec 9: S1084-9521(22)00358-5. doi: 10.1016/j.semcdb.2022.11.015. Epub ahead of print.

Böttcher RT, & Niehrs C Fibroblast Growth Factor Signaling during Early Vertebrate Development. (2005). Endocrine Reviews, 26(1), 63–77. 10.1210/er.2003-0040 [PubMed: 15689573]

Boulet AM, Capecchi MR. Signaling by FGF4 and FGF8 is required for axial elongation of the mouse embryo. Dev Biol. 2012 Nov 15;371 (2):235–45. doi: 10.1016/j.ydbio.2012.08.017. Epub 2012 Aug 30. [PubMed: 22954964]

Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK, Bell GW, Otte AP, Vidal M, Gifford DK, Young RA, Jaenisch R. Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature. 2006 May 18;441(7091):349–53. doi: 10.1038/nature04733. Epub 2006 Apr 19. [PubMed: 16625203]

Briscoe J, Pierani A, Jessell TM, Ericson J. A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. Cell. 2000 May 12;101(4):435–45. doi: 10.1016/s0092-8674(00)80853-3. [PubMed: 10830170]

Bulajić M, Srivastava D, Dasen JS, Wichterle H, Mahony S, Mazzoni EO. Differential abilities to engage inaccessible chromatin diversify vertebrate Hox binding patterns. Development. 2020 Nov 23;147(22):dev194761. doi: 10.1242/dev.194761. [PubMed: 33028607]

Cai L, Rothbart SB, Lu R, Xu B, Chen WY, Tripathy A, Rockowitz S, Zheng D, Patel DJ, Allis CD, Strahl BD, Song J, Wang GG. An H3K36 methylation-engaging Tudor motif of polycomb-like proteins mediates PRC2 complex targeting. Mol Cell. 2013 Feb 7;49(3):571–82. doi: 10.1016/j.molcel.2012.11.026. Epub 2012 Dec 27. [PubMed: 23273982]

Carpenter EM. Hox genes and spinal cord development. Dev Neurosci. 2002;24(1):24–34. doi:10.1159/000064943. [PubMed: 12145408]

Castelli-Gair JE, Micol JL, García-Bellido A. Transvection in the Drosophila Ultrabithorax gene: a Cbx1 mutant allele induces ectopic expression of a normal allele in trans. Genetics. 1990 Sep;126(1):177–84. doi: 10.1093/genetics/126.1.177. [PubMed: 2121595]

Catela C, Chen Y, Weng Y, Wen K, Kratsios P. Control of spinal motor neuron terminal differentiation through sustained *Hoxc8* gene activity. Elife. 2022 Mar 22;11:e70766. doi: 10.7554/eLife.70766. [PubMed: 35315772]

Catela C, Shin MM, Lee DH, Liu JP, Dasen JS. Hox Proteins Coordinate Motor Neuron Differentiation and Connectivity Programs through Ret/Gfrα Genes. Cell Rep. 2016 Mar 1;14(8):1901–15. doi: 10.1016/j.celrep.2016.01.067. Epub 2016 Feb 18. [PubMed: 26904955]

Chambeyron S, Bickmore WA. Chromatin decondensation and nuclear reorganization of the HoxB locus upon induction of transcription. Genes Dev. 2004 May 15;18(10):1119–30. doi: 10.1101/gad.292104. [PubMed: 15155579]

Chawengsaksophak K, James R, Hammond V et al. Homeosis and intestinal tumours in *Cdx2* mutant mice. Nature 386, 84–87 (1997). 10.1038/386084a0 [PubMed: 9052785]

Ciabrelli F, Comoglio F, Fellous S, Bonev B, Ninova M, Szabo Q, Xuereb A, Klopp C, Aravin A, Paro R, Bantignies F, Cavalli G. Stable Polycomb-dependent transgenerational inheritance of chromatin states in Drosophila. Nat Genet. 2017 Jun;49(6):876–886. doi: 10.1038/ng.3848. Epub 2017 Apr 24. [PubMed: 28436983]

Ciferri C, Lander GC, Maiolica A, Herzog F, Aebersold R, Nogales E. Molecular architecture of human polycomb repressive complex 2. Elife. 2012 Oct 30;1:e00005. doi: 10.7554/eLife.00005. [PubMed: 23110252]

Coleman RT, Struhl G. Causal role for inheritance of H3K27me3 in maintaining the OFF state of a *Drosophila* HOX gene. Science. 2017 Apr 7;356(6333):eaai8236. doi: 10.1126/science.aai8236. Epub 2017 Mar 16. [PubMed: 28302795]

Cooper S, Grijzenhout A, Underwood E, Ancelin K, Zhang T, Nesterova TB, Anil-Kirmizitas B, Bassett A, Kooistra SM, Agger K, Helin K, Heard E, Brockdorff N. Jarid2 binds mono-ubiquitylated H2A lysine 119 to mediate crosstalk between Polycomb complexes PRC1 and PRC2. Nat Commun. 2016 Nov 28;7:13661. doi: 10.1038/ncomms13661. [PubMed: 27892467]

Corley M, Kroll KL. The roles and regulation of Polycomb complexes in neural development. Cell Tissue Res. 2015 Jan;359(1):65–85. doi: 10.1007/s00441-014-2011-9. Epub 2014 Nov 1. [PubMed: 25367430]

Cox WG, Hemmati-Brivanlou A. Caudalization of neural fate by tissue recombination and bFGF. Development. 1995 Dec;121(12):4349–58. doi: 10.1242/dev.121.12.4349. [PubMed: 8575335]

Darbellay F, Duboule D. Topological Domains, Metagenes, and the Emergence of Pleiotropic Regulations at Hox Loci. Curr Top Dev Biol. 2016;116:299–314. doi: 10.1016/bs.ctdb.2015.11.022. Epub 2016 Jan 27. [PubMed: 26970625]

Dasen JS, De Camilli A, Wang B, Tucker PW, Jessell TM. Hox repertoires for motor neuron diversity and connectivity gated by a single accessory factor, FoxP1. Cell. 2008 Jul 25;134(2):304–16. doi: 10.1016/j.cell.2008.06.019. [PubMed: 18662545]

Dasen JS, Jessell TM. Hox networks and the origins of motor neuron diversity. Curr Top Dev Biol. 2009;88:169–200. doi: 10.1016/S0070-2153(09)88006-X. [PubMed: 19651305]

Dasen JS, Liu JP, Jessell TM. Motor neuron columnar fate imposed by sequential phases of Hox-c activity. Nature. 2003 Oct 30;425(6961):926–33. doi: 10.1038/nature02051. [PubMed: 14586461]

Dasen JS, Tice BC, Brenner-Morton S, Jessell TM. A Hox regulatory network establishes motor neuron pool identity and target-muscle connectivity. Cell. 2005 Nov 4;123(3):477–91. doi: 10.1016/j.cell.2005.09.009. [PubMed: 16269338]

Dasen JS. Establishing the Molecular and Functional Diversity of Spinal Motoneurons. Adv Neurobiol. 2022;28:3–44. doi: 10.1007/978-3-031-07167-6_1. [PubMed: 36066819]

Deschamps J, Duboule D. Embryonic timing, axial stem cells, chromatin dynamics, and the Hox clock. Genes Dev. 2017 Jul 15;31(14):1406–1416. doi: 10.1101/gad.303123.117. [PubMed: 28860158]

Deschamps J, Wijgerde M. Two phases in the establishment of HOX expression domains. Dev Biol. 1993 Apr;156(2):473–80. doi: 10.1006/dbio.1993.1093. [PubMed: 8096483]

Di Meglio T, Kratochwil CF, Vilain N, Loche A, Vitobello A, Yonehara K, Hrycaj SM, Roska B, Peters AH, Eichmann A, Wellik D, Ducret S, Rijli FM. Ezh2 orchestrates topographic migration and connectivity of mouse precerebellar neurons. Science. 2013 Jan 11;339(6116):204–7. doi: 10.1126/science.1229326. [PubMed: 23307742]

Diez del Corral R, Olivera-Martinez I, Goriely A, Gale E, Maden M, Storey K. Opposing FGF and retinoid pathways control ventral neural pattern, neuronal differentiation, and segmentation during body axis extension. Neuron. 2003 Sep 25;40(1):65–79. doi: 10.1016/s0896-6273(03)00565-8. [PubMed: 14527434]

Diez del Corral R, Storey KG. Opposing FGF and retinoid pathways: a signaling switch that controls differentiation and patterning onset in the extending vertebrate axis. Bioessays. (2004)

Diez del Corral R, Breitkreuz DN & Storey KG Onset of neuronal differentiation is regulated by paraxial mesoderm and requires attenuation of FGF signalling. Development 129, 1681–1691 (2002) [PubMed: 11923204]

Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature. 2012 Apr 11;485(7398):376–80. doi: 10.1038/nature11082. [PubMed: 22495300]

Duboule D, Morata G. 1994. Colinearity and functional hierarchy among genes of the homeotic complexes. Trends Genet 10:358–364 [PubMed: 7985240]

Dubrulle J, Pourquié O. 2004, *fgf8* mRNA decay establishes a gradient that couples axial elongation to patterning in the vertebrate embryo. Nature 427, 419–422. (**doi:**10.1038/nature02216) [PubMed: 14749824] **doi:**

Duncan IM. Polycomblike: a gene that appears to be required for the normal expression of the bithorax and antennapedia gene complexes of Drosophila melanogaster. Genetics. 1982 Sep;102(1):49–70. doi: 10.1093/genetics/102.1.49. [PubMed: 6813190]

Endoh M, Endo TA, Endoh T, Isono K, Sharif J, Ohara O, Toyoda T, Ito T, Eskeland R, Bickmore WA, Vidal M, Bernstein BE, Koseki H. Histone H2A mono-ubiquitination is a crucial step to mediate PRC1-dependent repression of developmental genes to maintain ES cell identity. PLoS Genet. 2012;8(7):e1002774. doi: 10.1371/journal.pgen.1002774. Epub 2012 Jul 26. [PubMed: 22844243]

Eskeland R, Leeb M, Grimes GR, Kress C, Boyle S, Sproul D, Gilbert N, Fan Y, Skoultchi AI, Wutz A, Bickmore WA. Ring1B compacts chromatin structure and represses gene expression independent of histone ubiquitination. Mol Cell. 2010 May 14;38(3):452–64. doi: 10.1016/j.molcel.2010.02.032. [PubMed: 20471950]

Faust C, Lawson KA, Schork NJ, Thiel B, Magnuson T. The Polycomb-group gene eed is required for normal morphogenetic movements during gastrulation in the mouse embryo. Development. 1998 Nov;125(22):4495–506. doi: 10.1242/dev.125.22.4495. [PubMed: 9778508]

Ferrier DE, Holland PW. Ancient origin of the Hox gene cluster. Nat Rev Genet. 2001 Jan;2(1):33–8. doi: 10.1038/35047605. [PubMed: 11253066]

Gaertner B, Johnston J, Chen K, Wallaschek N, Paulson A, Garruss AS, … Zeitlinger J (2012). Poised RNA polymerase II changes over developmental time and prepares genes for future expression. Cell Reports, 2(6), 1670–1683. 10.1016/j.celrep.2012.11.024 [PubMed: 23260668]

Gao Z, Zhang J, Bonasio R, Strino F, Sawai A, Parisi F, Kluger Y, Reinberg D. PCGF homologs, CBX proteins, and RYBP define functionally distinct PRC1 family complexes. Mol Cell. 2012 Feb 10;45(3):344–56. doi: 10.1016/j.molcel.2012.01.002. [PubMed: 22325352]

Gaunt SJ, Gaunt AL. Possible rules for the ancestral origin of Hox gene collinearity. J Theor Biol. 2016 Dec 7;410:1–8. doi: 10.1016/j.jtbi.2016.09.009. Epub 2016 Sep 10. [PubMed: 27622537]

Gaunt Stephen. (2018). Hox cluster genes and collinearities throughout the tree of animal life. The International Journal of Developmental Biology. 62. 673–683. 10.1387/ijdb.180162sg. [PubMed: 30604837]

Gehring WJ, Kloter U, Suga H. Evolution of the Hox gene complex from an evolutionary ground state. Curr Top Dev Biol. 2009;88:35–61. doi: 10.1016/S0070-2153(09)88002-2. [PubMed: 19651301]

Geng Z, Gao Z. Mammalian PRC1 Complexes: Compositional Complexity and Diverse Molecular Mechanisms. Int J Mol Sci. 2020 Nov 14;21(22):8594. doi: 10.3390/ijms21228594. [PubMed: 33202645]

Gentile C, Kmita M. Polycomb Repressive Complexes in Hox Gene Regulation: Silencing and Beyond: The Functional Dynamics of Polycomb Repressive Complexes in Hox Gene Regulation. Bioessays. 2020 oct;42(10):e1900249. doi: 10.1002/bies.201900249. Epub 2020 Aug 2. [PubMed: 32743818]

Godsave SF, Durston AJ. Neural induction and patterning in embryos deficient in FGF signaling. Int J Dev Biol. 1997.

Golden MG, Dasen JS. Polycomb repressive complex 1 activities determine the columnar organization of motor neurons. Genes Dev. 2012 Oct 1;26(19):2236–50. doi: 10.1101/gad.199133.112. [PubMed: 23028147]

Graham A, Maden M, Krumlauf R. The murine Hox-2 genes display dynamic dorsoventral patterns of expression during central nervous system development. Development. 1991 May;112(1):255–64. doi: 10.1242/dev.112.1.255. [PubMed: 1685115]

Grau DJ, Chapman BA, Garlick JD, Borowsky M, Francis NJ, Kingston RE. Compaction of chromatin by diverse Polycomb group proteins requires localized regions of high charge. Genes Dev. 2011 Oct 15;25(20):2210–21. doi: 10.1101/gad.17288211.. [PubMed: 22012622]

Grijzenhout A, Godwin J, Koseki H, Gdula MR, Szumska D, McGouran JF, Bhattacharya S, Kessler BM, Brockdorff N, Cooper S. Functional analysis of AEBP2, a PRC2 Polycomb protein, reveals a Trithorax phenotype in embryonic development and in ESCs. Development. 2016 Aug 1;143(15):2716–23. doi: 10.1242/dev.123935. Epub 2016 Jun 17. [PubMed: 27317809]

Hanley O, Zewdu R, Cohen LJ, Jung H, Lacombe J, Philippidou P, Lee DH, Selleri L, Dasen JS. Parallel Pbx-Dependent Pathways Govern the Coalescence and Fate of Motor Columns. Neuron. 2016 Sep 7;91(5):1005–1020. doi: 10.1016/j.neuron.2016.07.043. Epub 2016 Aug 25. [PubMed: 27568519]

Healy E, Mucha M, Glancy E, Fitzpatrick DJ, Conway E, Neikes HK, Monger C, Van Mierlo G, Baltissen MP, Koseki Y, Vermeulen M, Koseki H, Bracken AP. PRC2.1 and PRC2.2 Synergize to Coordinate H3K27 Trimethylation. Mol Cell. 2019 Nov 7;76(3):437–452.e6. doi: 10.1016/j.molcel.2019.08.012. Epub 2019 Sep 11. [PubMed: 31521505]

Hirabayashi Y, Suzki N, Tsuboi M, Endo TA, Toyoda T, Shinga J, Koseki H, Vidal M, Gotoh Y. Polycomb limits the neurogenic competence of neural precursor cells to promote astrogenic fate transition. Neuron. 2009 Sep 10;63(5):600–13. doi: 10.1016/j.neuron.2009.08.021. [PubMed: 19755104]

Hobert O. Homeobox genes and the specification of neuronal identity. Nat Rev Neurosci. 2021 Oct;22(10):627–636. doi: 10.1038/s41583-021-00497-x. Epub 2021 Aug 26. [PubMed: 34446866]

Hojfeldt JW, Laugesen A, Willumsen BM, Damhofer H, Hedehus L, Tvardovskiy A, Mohammad F, Jensen ON, and Helin K (2018). Accurate H3K27 methylation can be established de novo by SUZ12-directed PRC2. Nat Struct Mol Biol 25, 225–232 [PubMed: 29483650]

Iimura T, Denans N, Pourquié O. Establishment of Hox vertebral identities in the embryonic spine precursors. Curr Top Dev Biol. 2009;88:201–34. doi: 10.1016/S0070-2153(09)88007-1. [PubMed: 19651306]

Isaacs HV, Pownall ME and Slack JM (1998), Regulation of Hox gene expression and posterior development by the *Xenopus* caudal homologue Xcad3. The EMBO Journal, 17: 3413–3427. 10.1093/emboj/17.12.3413 [PubMed: 9628877]

Isono K, Endo TA, Ku M, Yamada D, Suzuki R, Sharif J, Ishikura T, Toyoda T, Bernstein BE, Koseki H. SAM domain polymerization links subnuclear clustering of PRC1 to gene silencing. Dev Cell. 2013 Sep 30;26(6):565–77. doi: 10.1016/j.devcel.2013.08.016. [PubMed: 24091011]

Isono K, Fujimura Y, Shinga J, Yamaki M, O-Wang J, Takihara Y, Murahashi Y, Takada Y, Mizutani-Koseki Y, Koseki H. Mammalian polyhomeotic homologues Phc2 and Phc1 act in synergy to mediate polycomb repression of Hox genes. Mol Cell Biol. 2005 Aug;25(15):6694–706. doi: 10.1128/MCB.25.15.6694-6706.2005. Erratum in: Mol Cell Biol. 2014 Jul;34(14):2771. [PubMed: 16024804]

Izpisda-Belmonte J-C, Falkenstein H, Doll P&, bnucci A,, and Duboule D (1991) Murine genes related to the Dmmphilu AbdB homeotic gene are sequentially expressed during development of the posterior part of the body. EMBO J. 102279–2289.

Janesick A, Wu SC, Blumberg B. Retinoic acid signaling and neuronal differentiation. Cell Mol Life Sci. 2015 Apr;72(8):1559–76. doi: 10.1007/s00018-014-1815-9. Epub 2015 Jan 6. [PubMed: 25558812]

Jung H, Baek M, D'Elia KP, Boisvert C, Currie PD, Tay BH, Venkatesh B, Brown SM, Heguy A, Schoppik D, Dasen JS. The Ancient Origins of Neural Substrates for Land Walking. Cell. 2018 Feb 8;172(4):667–682.e15. doi: 10.1016/j.cell.2018.01.013. [PubMed: 29425489]

Jung H, Lacombe J, Mazzoni EO, Liem KF Jr, Grinstein J, Mahony S, Mukhopadhyay D, Gifford DK, Young RA, Anderson KV, Wichterle H, Dasen JS. Global control of motor neuron topography mediated by the repressive actions of a single hox gene. Neuron. 2010 Sep 9;67(5):781–96. doi: 10.1016/j.neuron.2010.08.008. [PubMed: 20826310]

Jung H, Mazzoni EO, Soshnikova N, Hanley O, Venkatesh B, Duboule D, Dasen JS. Evolving Hox activity profiles govern diversity in locomotor systems. Dev Cell. 2014 Apr 28;29(2):171–87. doi: 10.1016/j.devcel.2014.03.008. Epub 2014 Apr 17. [PubMed: 24746670]

Kastner P. et al. Genetic evidence that the retinoid signal is transduced by heterodimeric RXR/RAR functional units during mouse development. Development 124, 313–326 (1997) [PubMed: 9053308]

Kaustov L, Ouyang H, Amaya M, Lemak A, Nady N, Duan S, Wasney GA, Li Z, Vedadi M, Schapira M, Min J, Arrowsmith CH. Recognition and specificity determinants of the human cbx chromodomains. J Biol Chem. 2011 Jan 7;286(1):521–9. doi: 10.1074/jbc.M110.191411. Epub 2010 Nov 3. [PubMed: 21047797]

Keenan ID, Sharrard RM, Isaacs HV. FGF signal transduction and the regulation of Cdx gene expression. Dev Biol. 2006 Nov 15;299(2):478–88. doi: 10.1016/j.ydbio.2006.08.040. Epub 2006 Aug 24. [PubMed: 16982047]

Kengaku M, Okamoto H. Basic fibroblast growth factor induces differentiation of neural tube and neural crest lineages of cultured ectoderm cells from Xenopus gastrula. Development. 1993 Dec;119(4):1067–78. doi: 10.1242/dev.119.4.1067. [PubMed: 8306875]

Krumlauf R. Hox genes, clusters and collinearity. Int J Dev Biol. 2018;62(11-12):659–663. doi: 10.1387/ijdb.180330rr. [PubMed: 30604835]

Ku M, Koche RP, Rheinbay E, Mendenhall EM, Endoh M, Mikkelsen TS, Presser A, Nusbaum C, Xie X, Chi AS, Adli M, Kasif S, Ptaszek LM, Cowan CA, Lander ES, Koseki H, Bernstein BE. Genomewide analysis of PRC1 and PRC2 occupancy identifies two classes of bivalent domains. PLoS Genet. 2008 Oct;4(10):e1000242. doi: 10.1371/journal.pgen.1000242. Epub 2008 Oct 31. [PubMed: 18974828]

Kumar S, Duester G. Retinoic acid controls body axis extension by directly repressing Fgf8 transcription. Development. 2014 Aug;141(15):2972–7. doi: 10.1242/dev.112367. [PubMed: 25053430]

Kundu S, Ji F, Sunwoo H, Jain G, Lee JT, Sadreyev RI, Dekker J, Kingston RE. Polycomb Repressive Complex 1 Generates Discrete Compacted Domains that Change during Differentiation. Mol Cell. 2017 Feb 2;65(3):432–446.e5. doi: 10.1016/j.molcel.2017.01.009. Erratum in: Mol Cell. 2018 Jul 5;71(1):191. [PubMed: 28157505]

Lacombe J, Hanley O, Jung H, Philippidou P, Surmeli G, Grinstein J, Dasen JS. Genetic and functional modularity of Hox activities in the specification of limb-innervating motor neurons. PLoS Genet. 2013;9(1):e1003184. doi: 10.1371/journal.pgen.1003184. Epub 2013 Jan 24. [PubMed: 23359544]

Lalevée S, Anno YN, Chatagnon A, Samarut E, Poch O, Laudet V, Benoit G, Lecompte O, Rochette-Egly C. Genome-wide in silico identification of new conserved and functional retinoic acid receptor response elements (direct repeats separated by 5 bp). J Biol Chem. 2011 Sep 23;286(38):33322–34. doi: 10.1074/jbc.M111.263681. Epub 2011 Jul 29. [PubMed: 21803772]

Lamb TM, Harland RM. Fibroblast growth factor is a direct neural inducer, which combined with noggin generates anterior-posterior neural pattern. Development. (1995).

Landeira D, Sauer S, Poot R, Dvorkina M, Mazzarella L, Jorgensen HF, Pereira CF, Leleu M, Piccolo FM, Spivakov M, et al. (2010). Jarid2 is a PRC2 component in embryonic stem cells required for multi-lineage differentiation and recruitment of PRC1 and RNA Polymerase II to developmental regulators. Nat Cell Biol 12, 618–624. [PubMed: 20473294]

Lanzuolo C, Orlando V. Memories from the polycomb group proteins. Annu Rev Genet. 2012;46:561–89. doi: 10.1146/annurev-genet-110711-155603. Epub 2012 Sep 17. [PubMed: 22994356]

Lau MS, Schwartz MG, Kundu S, Savol AJ, Wang PI, Marr SK, Grau DJ, Schorderet P, Sadreyev RI, Tabin CJ, Kingston RE. Mutation of a nucleosome compaction region disrupts Polycomb-mediated

axial patterning. Science. 2017 Mar 10;355(6329):1081–1084. doi: 10.1126/science.aah5403. Epub 2017 Mar 9. [PubMed: 28280206]

Lewis EB. A gene complex controlling segmentation in Drosophila. Nature. 1978 Dec 7;276(5688):565–70. doi: 10.1038/276565a0. [PubMed: 103000]

Li CJ, Hong T, Tung YT, Yen YP, Hsu HC, Lu YL, Chang M, Nie Q, Chen JA. MicroRNA filters Hox temporal transcription noise to confer boundary formation in the spinal cord. Nat Commun. 2017 Mar 24;8:14685. doi: 10.1038/ncomms14685. [PubMed: 28337978]

Li H, Liefke R, Jiang J, Kurland JV, Tian W, Deng P, Zhang W, He Q, Patel DJ, Bulyk ML, Shi Y, Wang Z. Polycomb-like proteins link the PRC2 complex to CpG islands. Nature. 2017 Sep 14;549(7671):287–291. doi: 10.1038/nature23881. Epub 2017 Sep 6. [PubMed: 28869966]

Li L, Liu B, Wapinski OL, Tsai MC, Qu K, Zhang J, Carlson JC, Lin M, Fang F, Gupta RA, Helms JA, Chang HY. Targeted disruption of Hotair leads to homeotic transformation and gene derepression. Cell Rep. 2013 Oct 17;5(1):3–12. doi: 10.1016/j.celrep.2013.09.003. Epub 2013 Sep 26. [PubMed: 24075995]

Li X, Isono K, Yamada D, Endo TA, Endoh M, Shinga J, Mizutani-Koseki Y, Otte AP, Casanova M, Kitamura H, Kamijo T, Sharif J, Ohara O, Toyada T, Bernstein BE, Brockdorff N, Koseki H. Mammalian polycomb-like Pcl2/Mtf2 is a novel regulatory component of PRC2 that can differentially modulate polycomb activity both at the Hox gene cluster and at Cdkn2a genes. Mol Cell Biol. 2011 Jan;31(2):351–64. doi: 10.1128/MCB.00259-10. Epub 2010 Nov 8. Erratum in: Mol Cell Biol. 2014 Jul;34(14):2773. [PubMed: 21059868]

Lin C, Garrett AS, De Kumar B, Smith ER, Gogol M, Seidel C, … Shilatifard A (2011). Dynamic transcriptional events in embryonic stem cells mediated by the super elongation complex (SEC). Genes & Development, 25(14), 1486–1498. 10.1101/gad.2059211 [PubMed: 21764852]

Liu JP. The function of growth/differentiation factor 11 (Gdf1) in rostrocaudal patterning of the developing spinal cord. Development. 2006 Aug;133(15):2865–74. doi: 10.1242/dev.02478. Epub 2006 Jun 21. [PubMed: 16790475]

Liu JP, Laufer E & Jessell TM Assigning the positional identity of spinal motor neurons: rostrocaudal patterning of Hox-c expression by FGFs, Gdf11, and retinoids. Neuron 32, 997–1012 (2001) [PubMed: 11754833]

Loubiere V, Martinez AM, Cavalli G. Cell Fate and Developmental Regulation Dynamics by Polycomb Proteins and 3D Genome Architecture. Bioessays. 2019 Mar;41(3):e1800222. doi: 10.1002/bies.201800222. Epub 2019 Feb 22. [PubMed: 30793782]

Mallo M, Alonso CR. The regulation of Hox gene expression during animal development. Development. 2013 Oct;140(19):3951–63. doi: 10.1242/dev.068346. [PubMed: 24046316]

Mallo M, Wellik DM, Deschamps J. Hox genes and regional patterning of the vertebrate body plan. Dev Biol. 2010 Aug 1;344(1):7–15. doi: 10.1016/j.ydbio.2010.04.024. Epub 2010 May 7. [PubMed: 20435029]

Mazzoni EO, Mahony S, Peljto M, Patel T, Thornton SR, McCuine S, Reeder C, Boyer LA, Young RA, Gifford DK, Wichterle H. Saltatory remodeling of Hox chromatin in response to rostrocaudal patterning signals. Nat Neurosci. 2013 Sep;16(9):1191–1198. doi: 10.1038/nn.3490. Epub 2013 Aug 18. [PubMed: 23955559]

McGinnis W, Levine M, Hafen E et al. A conserved DNA sequence in homoeotic genes of the *Drosophila* Antennapedia and bithorax complexes. Nature 308, 428–433 (1984). 10.1038/308428a0 [PubMed: 6323992]

Mendelsohn AI, Dasen JS, Jessell TM. Divergent Hox Coding and Evasion of Retinoid Signaling Specifies Motor Neurons Innervating Digit Muscles. Neuron. 2017 Feb 22;93(4):792–805.e4. doi: 10.1016/j.neuron.2017.01.017. Epub 2017 Feb 9. [PubMed: 28190640]

Metzis V, Steinhauser S, Pakanavicius E, Gouti M, Stamataki D, Ivanovitch K, Watson T, Rayon T, Mousavy Gharavy SN, Lovell-Badge R, Luscombe NM, Briscoe J. Nervous System Regionalization Entails Axial Allocation before Neural Differentiation. Cell. 2018 Nov 1;175(4):1105–1118.e17. doi: 10.1016/j.cell.2018.09.040. Epub 2018 Oct 18. [PubMed: 30343898]

Montavon T, Soshnikova N. Hox gene regulation and timing in embryogenesis. Semin Cell Dev Biol. 2014 Oct;34:76–84. doi: 10.1016/j.semcdb.2014.06.005. Epub 2014 Jun 12. [PubMed: 24930771]

Mouilleau V, Vaslin C, Robert R, Gribaudo S, Nicolas N, Jarrige M, Terray A, Lesueur L, Mathis MW, Croft G, Daynac M, Rouiller-Fabre V, Wichterle H, Ribes V, Martinat C, Nedelec S. Dynamic extrinsic pacing of the HOX clock in human axial progenitors controls motor neuron subtype specification. Development. 2021 Mar 29;148(6):dev194514. doi: 10.1242/dev.194514. [PubMed: 33782043]

Narendra V, Bulajić M, Dekker J, Mazzoni EO, Reinberg D. CTCF-mediated topological boundaries during development foster appropriate gene regulation. Genes Dev. 2016 Dec 15;30(24):2657–2662. doi: 10.1101/gad.288324.116. Erratum in: Genes Dev. 2017 Aug 15;31(16):1714. [PubMed: 28087711]

Narendra V, Rocha PP, An D, Raviram R, Skok JA, Mazzoni EO, Reinberg D. CTCF establishes discrete functional chromatin domains at the Hox clusters during differentiation. Science. 2015 Feb 27;347(6225):1017–21. doi: 10.1126/science.1262088. [PubMed: 25722416] Oh et al., “Retinoic Acid and CTCF Play Key Roles in Inducing the Collinear Expression of the Hoxa Cluster.”

Neijts R, Amin S, van Rooijen C, Deschamps J. Cdx is crucial for the timing mechanism driving colinear Hox activation and defines a trunk segment in the Hox cluster topology. Dev Biol. 2017 Feb 15;422(2):146–154. doi: 10.1016/j.ydbio.2016.12.024. Epub 2016 Dec 29. [PubMed: 28041967]

Neijts R, Amin S, van Rooijen C, Tan S, Creyghton MP, de Laat W, Deschamps J. Polarized regulatory landscape and Wnt responsiveness underlie Hox activation in embryos. Genes Dev. 2016 Sep 1;30(17):1937–42. doi: 10.1101/gad.285767.116. Epub 2016 Sep 15. [PubMed: 27633012]

Niederreither K, Abu-Abed S, Schuhbaur B, Petkovich M, Chambon P, Dolle P. Genetic evidence that oxidative derivatives of retinoic acid are not involved in retinoid signaling during mouse development. Nat Genet. 2002 May;31(1):84–8. doi: 10.1038/ng876. Epub 2002 Apr 15. [PubMed: 11953746]

Niederreither K, McCaffery P, Dräger UC, Chambon P, Dollé P. Restricted expression and retinoic acid-induced downregulation of the retinaldehyde dehydrogenase type 2 (RALDH-2) gene during mouse development. Mech Dev. 1997 Feb;62(1):67–78. doi: 10.1016/s0925-4773(96)00653-3. [PubMed: 9106168]

Nolte C, Jinks T, Wang X, Martinez Pastor MT, Krumlauf R. Shadow enhancers flanking the HoxB cluster direct dynamic Hox expression in early heart and endoderm development. Dev Biol. 2013 Nov 1;383(1):158–73. doi: 10.1016/j.ydbio.2013.09.016. Epub 2013 Sep 18. [PubMed: 24055171]

Nolte C, Krumlauf R. Expression of Hox Genes in the Nervous System of Vertebrates. In: Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience; 2000–2013. Available from: https://www.ncbi.nlm.nih.gov/books/NBK6519/

Nolte C, De Kumar B, Krumlauf R *Hox* genes: Downstream “effectors” of retinoic acid signaling in vertebrate embryogenesis. Genesis. 2019;57:e23306. 10.1002/dvg.23306 [PubMed: 31111645]

Northrop JL, Kimelman D. Dorsal-ventral differences in Xcad-3 expression in response to FGF-mediated induction in Xenopus. Dev Biol. 1994 Feb;161(2):490–503. doi: 10.1006/dbio.1994.1047. [PubMed: 7906234]

Ortabozkoyun H, Huang PY, Cho H, Narendra V, LeRoy G, Gonzalez-Buendia E, Skok JA, Tsirigos A, Mazzoni EO, Reinberg D. CRISPR and biochemical screens identify MAZ as a cofactor in CTCF-mediated insulation at Hox clusters. Nat Genet. 2022 Feb;54(2):202–212. doi: 10.1038/s41588-021-01008-5. Epub 2022 Feb 10. [PubMed: 35145304]

Osseward PJ 2nd , Pfaff SL. Cell type and circuit modules in the spinal cord. Curr Opin Neurobiol. 2019 Jun;56:175–184. doi: 10.1016/j.conb.2019.03.003. Epub 2019 Apr 5. [PubMed: 30954861]

Parker HJ, Krumlauf R. A Hox gene regulatory network for hindbrain segmentation. Curr Top Dev Biol. 2020;139:169–203. doi: 10.1016/bs.ctdb.2020.03.001. Epub 2020 Apr 9. [PubMed: 32450960]

Partanen J, Schwartz L, and Rossant J. Opposite phenotypes of hypomorphic and Y766 phosphorylation site mutations reveal a function for fgfr1 in anteroposterior patterning of mouse embryos. Genes Dev. (1998).

Pascual-Anaya J, D'Aniello S, Kuratani S et al. Evolution of *Hox* gene clusters in deuterostomes. BMC Dev Biol 13, 26 (2013). 10.1186/1471-213X-13-26 [PubMed: 23819519]

Pasini D, Bracken AP, Jensen MR, Denchi EL and Helin K (2004), Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. The EMBO Journal, 23: 4061–4071. 10.1038/sj.emboj.7600402 [PubMed: 15385962]

Philippidou P, Dasen JS. Hox genes: choreographers in neural development, architects of circuit organization. Neuron. 2013 Oct 2;80(1):12–34. doi: 10.1016/j.neuron.2013.09.020. Epub 2013 Oct 2. [PubMed: 24094100]

Philippidou P, Walsh CM, Aubin J, Jeannotte L, Dasen JS. Sustained Hox5 gene activity is required for respiratory motor neuron development. Nat Neurosci. 2012 Dec;15(12):1636–44. doi: 10.1038/nn.3242. Epub 2012 Oct 28. [PubMed: 23103965]

Pinglay S, Bulajić M, Rahe DP, Huang E, Brosh R, Mamrak NE, King BR, German S, Cadley JA, Rieber L, Easo N, Lionnet T, Mahony S, Maurano MT, Holt LJ, Mazzoni EO, Boeke JD. Synthetic regulatory reconstitution reveals principles of mammalian *Hox* cluster regulation. Science. 2022 Jul;377(6601):eabk2820. doi: 10.1126/science.abk2820. Epub 2022 Jul 1. [PubMed: 35771912]

Pirity MK, Locker J, Schreiber-Agus N. Rybp/DEDAF is required for early postimplantation and for central nervous system development. Mol Cell Biol. 2005 Aug;25(16):7193–202. doi: 10.1128/MCB.25.16.7193-7202.2005. [PubMed: 16055728]

Piunti A, Shilatifard A. Author Correction: The roles of Polycomb repressive complexes in mammalian development and cancer. Nat Rev Mol Cell Biol. 2022 Jun;23(6):444. doi: 10.1038/s41580-022-00495-6. Erratum for: Nat Rev Mol Cell Biol. 2021 May;22(5):326-345.

Portoso M, Ragazzini R, Brencic Z, Moiani A, Michaud A, Vassilev I, Wassef M, Servant N, Sargueil B, and Margueron R (2017). PRC2 is dispensable for HOTAIR-mediated transcriptional repression. EMBO J 36, 981–994. [PubMed: 28167697]

Pownall M, Tucker A, Slack J, Isaacs H. eFGF, *Xcad3* and Hox genes form a molecular pathway that establishes the anteroposterior axis in *Xenopus*. Development. (1996).

Qian P, De Kumar B, He XC, Nolte C, Gogol M, Ahn Y, Chen S, Li Z, Xu H, Perry JM, Hu D, Tao F, Zhao M, Han Y, Hall K, Peak A, Paulson A, Zhao C, Venkatraman A, Box A, Perera A, Haug JS, Parmely T, Li H, Krumlauf R, Li L. Retinoid-Sensitive Epigenetic Regulation of the Hoxb Cluster Maintains Normal Hematopoiesis and Inhibits Leukemogenesis. Cell Stem Cell. 2018 May 3;22(5):740–754.e7. doi: 10.1016/j.stem.2018.04.012. [PubMed: 29727682]

Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E, Chang HY. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell. 2007 Jun 29;129(7):1311–23. doi: 10.1016/j.cell.2007.05.022. [PubMed: 17604720]

Robinson AK, Leal BZ, Chadwell LV, Wang R, Ilangovan U, Kaur Y, Junco SE, Schirf V, Osmulski PA, Gaczynska M, Hinck AP, Demeler B, McEwen DG, Kim CA. The growth-suppressive function of the polycomb group protein polyhomeotic is mediated by polymerization of its sterile alpha motif (SAM) domain. J Biol Chem. 2012 Mar 16;287(12):8702–13. doi: 10.1074/jbc.M111.336115. Epub 2012 Jan 24. [PubMed: 22275371]

Rodríguez-Carballo E, Lopez-Delisle L, Willemin A, Beccari L, Gitto S, Mascrez B, Duboule D. Chromatin topology and the timing of enhancer function at the *HoxD* locus. Proc Natl Acad Sci U S A. 2020 Dec 8;117(49):31231–31241. doi: 10.1073/pnas.2015083117. Epub 2020 Nov 23. [PubMed: 33229569]

Ronshaugen M, Biemar F, Piel J, Levine M, Lai EC. 2005. The Drosophilamicro RNA iab-4 causes a dominant homeotic transformation of halteresto wings. Genes Dev 19:2947 – 2952 [PubMed: 16357215]

Rousso DL, Gaber ZB, Wellik D, Morrisey EE, Novitch BG. Coordinated actions of the forkhead protein Foxp1 and Hox proteins in the columnar organization of spinal motor neurons. Neuron. 2008 Jul 31;59(2):226–40. doi: 10.1016/j.neuron.2008.06.025. [PubMed: 18667151]

Sawai A, Pfennig S, Bulajić M, Miller A, Khodadadi-Jamayran A, Mazzoni EO, Dasen JS. PRC1 sustains the integrity of neural fate in the absence of PRC2 function. Elife. 2022 Jan 7;11:e72769. doi: 10.7554/eLife.72769. [PubMed: 34994686]

Scott MP, Weiner AJ. Structural relationships among genes that control development: sequence homology between the Antennapedia, Ultrabithorax, and fushi tarazu loci of Drosophila. Proc Natl Acad Sci U S A. 1984 Jul;81(13):4115–9. doi: 10.1073/pnas.81.13.4115. [PubMed: 6330741]

Shi Y, Liu JP. Gdf11 facilitates temporal progression of neurogenesis in the developing spinal cord. J Neurosci. 2011 Jan 19;31(3):883–93. doi: 10.1523/JNEUROSCI.2394-10.2011. [PubMed: 21248112]

Shin MM, Catela C, Dasen J. Intrinsic control of neuronal diversity and synaptic specificity in a proprioceptive circuit. Elife. 2020 Aug 18;9:e56374. doi: 10.7554/eLife.56374. [PubMed: 32808924]

Simon JA, Kingston RE. Mechanisms of polycomb gene silencing: knowns and unknowns. Nat Rev Mol Cell Biol. 2009 oct;10(10):697–708. doi: 10.1038/nrm2763. Epub 2009 Sep 9. [PubMed: 19738629]

Singh NP, Mishra RK. A double-edged sword to force posterior dominance of Hox genes. Bioessays. 2008 Nov;30(11-12): 1058–61. doi: 10.1002/bies.20847. [PubMed: 18937351]

Soshnikova N, Duboule D. Epigenetic temporal control of mouse Hox genes in vivo. Science. 2009 Jun 5;324(5932):1320–3. doi: 10.1126/science.1171468. [PubMed: 19498168]

Soshnikova N, Montavon T, Leleu M, Galjart N, Duboule D. Functional analysis of CTCF during mammalian limb development. Dev Cell. 2010 Dec 14;19(6):819–30. doi: 10.1016/j.devcel.2010.11.009. [PubMed: 21145498]

Stern CD. Initial patterning of the central nervous system: how many organizers? Nat Rev Neurosci. 2001 Feb;2(2):92–8. doi: 10.1038/35053563. [PubMed: 11252999]

Stifani N. Motor neurons and the generation of spinal motor neuron diversity. Front Cell Neurosci. 2014 Oct 9;8:293. doi: 10.3389/fncel.2014.00293. [PubMed: 25346659]

Stock JK, Giadrossi S, Casanova M, Brookes E, Vidal M, Koseki H, Brockdorff N, Fisher AG, Pombo A. Ring1-mediated ubiquitination of H2A restrains poised RNA polymerase II at bivalent genes in mouse ES cells. Nat Cell Biol. 2007 Dec;9(12):1428–35. doi: 10.1038/ncb1663. Epub 2007 Nov 25. [PubMed: 18037880]

Sweeney LB, Bikoff JB, Gabitto MI, Brenner-Morton S, Baek M, Yang JH, Tabak EG, Dasen JS, Kintner CR, Jessell TM. Origin and Segmental Diversity of Spinal Inhibitory Interneurons. Neuron. 2018 Jan 17;97(2):341–355.e3. doi: 10.1016/j.neuron.2017.12.029. Epub 2018 Jan 4. [PubMed: 29307712]

Tanzer A, Amemiya CT, Kim CB, Stadler PF. 2005. Evolution of microRNAs located within Hox gene clusters. J Exp Zoolog B Mol DevEvol 304:75–85.

Tavares L, Dimitrova E, Oxley D, Webster J, Poot R, Demmers J, Bezstarosti K, Taylor S, Ura H, Koide H, Wutz A, Vidal M, Elderkin S, Brockdorff N. RYBP-PRC1 complexes mediate H2A ubiquitylation at polycomb target sites independently of PRC2 and H3K27me3. Cell. 2012 Feb 17;148(4):664–78. doi: 10.1016/j.cell.2011.12.029. Epub 2012 Feb 9. Erratum in: Cell. 2012 Jun 22;149(7):1647-8. [PubMed: 22325148]

Tolhuis B, Blom M, Kerkhoven RM, Pagie L, Teunissen H, Nieuwland M, Simonis M, de Laat W, van Lohuizen M, van Steensel B. Interactions among Polycomb domains are guided by chromosome architecture. PLoS Genet. 2011 Mar;7(3):e1001343. doi: 10.1371/journal.pgen.1001343. Epub 2011 Mar 24. [PubMed: 21455484]

Tsuboi M, Kishi Y, Yokozeki W, Koseki H, Hirabayashi Y, Gotoh Y. Ubiquitination-Independent Repression of PRC1 Targets during Neuronal Fate Restriction in the Developing Mouse Neocortex. Dev Cell. 2018 Dec 17;47(6):758–772.e5. doi: 10.1016/j.devcel.2018.11.018. [PubMed: 30562514]

Vagnozzi AN, Garg K, Dewitz C, Moore MT, Cregg JM, Jeannotte L, Zampieri N, Landmesser LT, Philippidou P. Phrenic-specific transcriptional programs shape respiratory motor output. Elife. 2020 Jan 16;9:e52859. doi: 10.7554/eLife.52859. [PubMed: 31944180]

Venkatasubramanian L, Mann RS. The development and assembly of the Drosophila adult ventral nerve cord. Curr Opin Neurobiol. 2019 Jun;56:135–143. doi: 10.1016/j.conb.2019.01.013. Epub 2019 Feb 28. [PubMed: 30826502]

Voncken JW, Roelen BA, Roefs M, de Vries S, Verhoeven E, Marino S, Deschamps J, van Lohuizen M. Rnf2 (Ring1b) deficiency causes gastrulation arrest and cell cycle inhibition. Proc Natl Acad Sci U S A. 2003 Mar 4;100(5):2468–73. doi: 10.1073/pnas.0434312100. Epub 2003 Feb 14. [PubMed: 12589020]

Wang H, Wang L, Erdjument-Bromage H, Vidal M, Tempst P, Jones RS, Zhang Y. Role of histone H2A ubiquitination in Polycomb silencing. Nature. 2004 Oct 14;431(7010):873–8. doi: 10.1038/nature02985. Epub 2004 Sep 22. [PubMed: 15386022]

Wang R, Taylor AB, Leal BZ, Chadwell LV, Ilangovan U, Robinson AK, Schirf V, Hart PJ, Lafer EM, Demeler B, Hinck AP, McEwen DG, Kim CA. Polycomb group targeting through different binding partners of RING1B C-terminal domain. Structure. 2010 Aug 11;18(8):966–75. doi: 10.1016/j.str.2010.04.013. [PubMed: 20696397]

Wang W, Cho H, Kim D, Park Y, Moon JH, Lim SJ, Yoon SM, McCane M, Aicher SA, Kim S, Emery B, Lee JW, Lee S, Park Y, Lee SK. PRC2 Acts as a Critical Timer That Drives Oligodendrocyte Fate over Astrocyte Identity by Repressing the Notch Pathway. Cell Rep. 2020 Sep 15;32(11):108147. doi: 10.1016/j.celrep.2020.108147. [PubMed: 32937136]

Wang X, Gu J, Zhang MQ, Li Y. 2008. Identification of phylogenetically conserved microRNA cis-regulatory elements across 12 Drosophila species. Bioinformatics 24:165 –171 [PubMed: 18037683] Sprecher Simon G., Müller Martin, Kammermeier Lars, Miller David F.B., Kaufman Thomas C., Reichert Heinrich, Hirth Frank. Hox gene cross-regulatory interactions in the embryonic brain of Drosophila. Mechanisms of Development. Volume 121, Issue 6. 2004

Wani A, Boettiger A, Schorderet P et al. Chromatin topology is coupled to Polycomb group protein subnuclear organization. Nat Commun 7, 10291 (2016). 10.1038/ncomms10291 [PubMed: 26759081]

Woltering JM, Durston AJ. The zebrafish hoxDb cluster has been reduced to a single microRNA. Nat Genet. 2006 Jun;38(6):601–2. doi: 10.1038/ng0606-601. [PubMed: 16736008]

Wu L, Murat P, Matak-Vinkovic D, Murrell A, Balasubramanian S. Binding interactions between long noncoding RNA HOTAIR and PRC2 proteins. Biochemistry. 2013 Dec 31;52(52):9519–27. doi: 10.1021/bi401085h. Epub 2013 Dec 17. [PubMed: 24320048]

Yaghmaeian Salmani B, Monedero Cobeta I, Rakar J, Bauer S, Curt JR, Starkenberg A, Thor S. Evolutionarily conserved anterior expansion of the central nervous system promoted by a common PcG-Hox program. Development. 2018 Apr 5;145(7):dev160747. doi: 10.1242/dev.160747. [PubMed: 29530878]

Yen YP, Hsieh WF, Tsai YY, Lu YL, Liau ES, Hsu HC, Chen YC, Liu TC, Chang M, Li J, Lin SP, Hung JH, Chen JA. *Dlk1-Dio3* locus-derived lncRNAs perpetuate postmitotic motor neuron cell fate and subtype identity. Elife. 2018 Oct 12;7:e38080. doi: 10.7554/eLife.38080. Erratum in: Elife. 2020 Feb 05;9: [PubMed: 30311912]

Youmans DT, Gooding AR, Dowell RD, Cech TR. Competition between PRC2.1 and 2.2 subcomplexes regulates PRC2 chromatin occupancy in human stem cells. Mol Cell. 2021 Feb 4;81(3):488–501.e9. doi: 10.1016/j.molcel.2020.11.044. Epub 2020 Dec 17. [PubMed: 33338397]

Young T, Deschamps J. Hox, Cdx, and anteroposterior patterning in the mouse embryo. Curr Top Dev Biol. 2009;88:235–55. doi: 10.1016/S0070-2153(09)88008-3. [PubMed: 19651307]

Young T, Rowland JE, van de Ven C, Bialecka M, Novoa A, Carapuco M, van Nes J, de Graaff W, Duluc I, Freund JN, Beck F, Mallo M, Deschamps J. Cdx and Hox genes differentially regulate posterior axial growth in mammalian embryos. Dev Cell. 2009 Oct;17(4):516–26. doi: 10.1016/j.devcel.2009.08.010. [PubMed: 19853565]

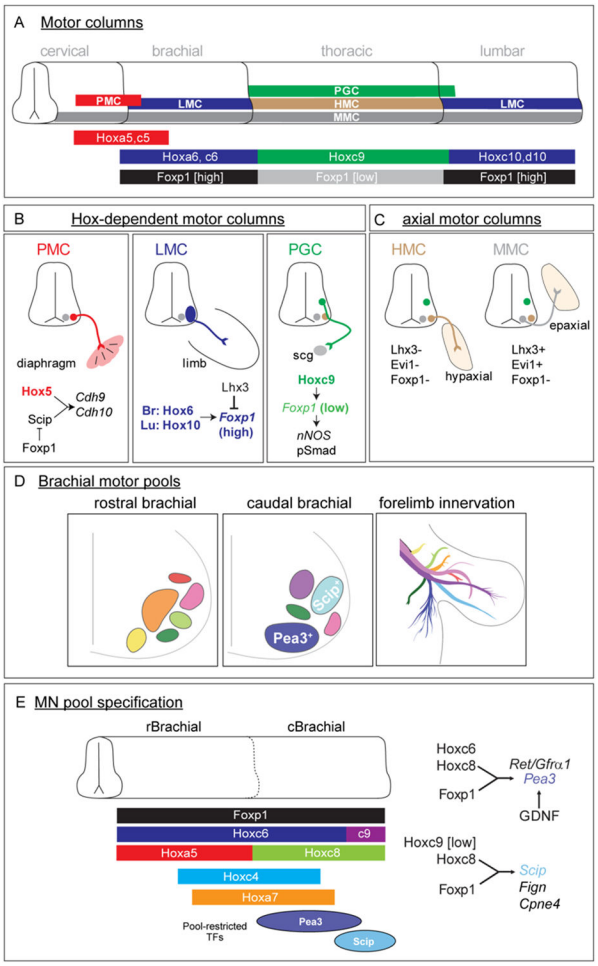

Fig. 1. *Hox* gene profiles and function during spinal MN development.

(A) Organization of spinal motor columns at cervical, brachial, thoracic, and lumbar levels in mice. Hox genes expressed by segmentally restricted motor columns are shown. Phrenic motor column (PMC) neurons express *Hox5* genes (*Hoxa5* and *Hoxc5*), brachial and lumbar lateral motor column (LMC) neuron express Hox6 and Hox10 genes, respectively. *Hoxc9* is expressed by thoracic preganglionic column (PGC) and hypaxial motor column (HMC) neurons. Medial motor column (MMC) neurons are present at all segmental levels. Foxp1 is expressed at high levels in LMC neurons and reduced levels in PGC neurons. **(B)** *Hox* regulatory interactions involved in motor column specification. Downstream targets of Hox proteins in motor columns are shown. Peripheral target muscles of motor columns are also indicated. **(C)** Axial motor column markers and innervation pattern. MMC neurons express Lhx3 and Evi1, while no definitive embryonic TF markers for HMC neuron are currently known. **(D)** Organization of motor pools and innervation pattern of mouse forelimb at e12.5 (based on Catela et al., 2016). Motor pools expressing the TFs Pea3 (also known as Etv4) and Scip (Pou3f1) are shown, and each color-coded pool corresponds to a specific axonal trajectory in the limb. **(E)** Hox expression in brachial LMC neurons and regulatory interactions involved in motor pool specification. Hoxc6 and Hoxc8 function in conjunction with Foxp1 to promote expression of *Ret* and *GFRα1*, which are expressed in Pea3^{+} motor

pools. A subset of Scip^{+} MNs express *Fign* and *Cpne4*, and target forelimb digit muscles (Mendelsohn et al., 2017).

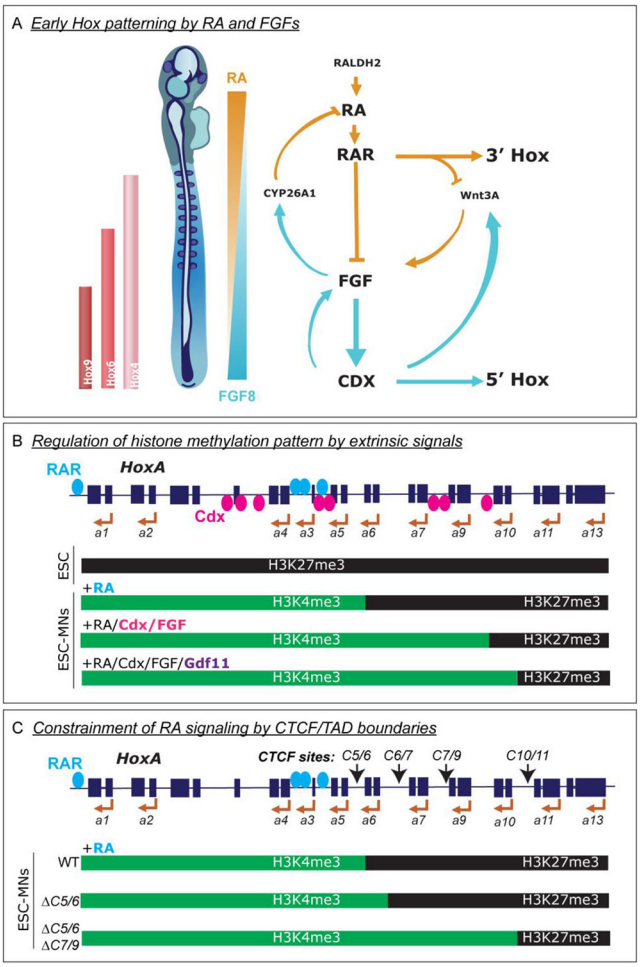

Fig. 2, Regulation of *Hox* expression and chromatin structure by extrinsic cues.

(A) During vertebrate axial elongation, *Hox* genes are sequentially induced by RA and FGF. In neural progenitors RA functions to regulate *Hox1-Hox5* genes in rostral segments while FGF regulates *Hox6-Hox9* genes in caudal regions. Stage 12 chick embryo shown. Regulatory interactions between RA and FGF signaling pathways are shown on the right. **(B)** Model for the effects of morphogens on distribution of histone marks associated with active (H3K4me3) and repressed (H3K27me3) *Hox* genes in ESC-MNs. In general, the presence of H4K4me3 is associated with *Hox* expression, while H3K27me3 with *Hox* repression. Signaling through RA and FGF depletes H3K27me3 from *Hox* genes and establishes presumptive rostrocaudal expression boundaries. Genes in *HoxA* cluster is shown. RA clears H3K27me3 marks from *Hox1-Hox5* genes through RAR-RXR recruitment. Binding of CDX following FGF treatment results in the clearance of H3K27me3 from *Hox6-Hox9* genes. Gdf11 is likely required to clear H3K27me3 from more caudal *Hox10* genes. Approximate binding sites for RAR and CDX are shown. **(C)** Role of CTCF in insulating the effects of RA in H3K27me3 clearance. CTCF sites in the *HoxA* cluster are shown. Mutation in CTCF binding sites results in an extension of H3K4me3 signal at more 5’ ends of *HoxA* cluster after RA treatment in ESC-MNs, and a concomitant

spreading of activating H3K4me3 marks. Changes in chromatin marks after CTCF site mutation results in ectopic expression of progressively more 5' *HoxA* genes.

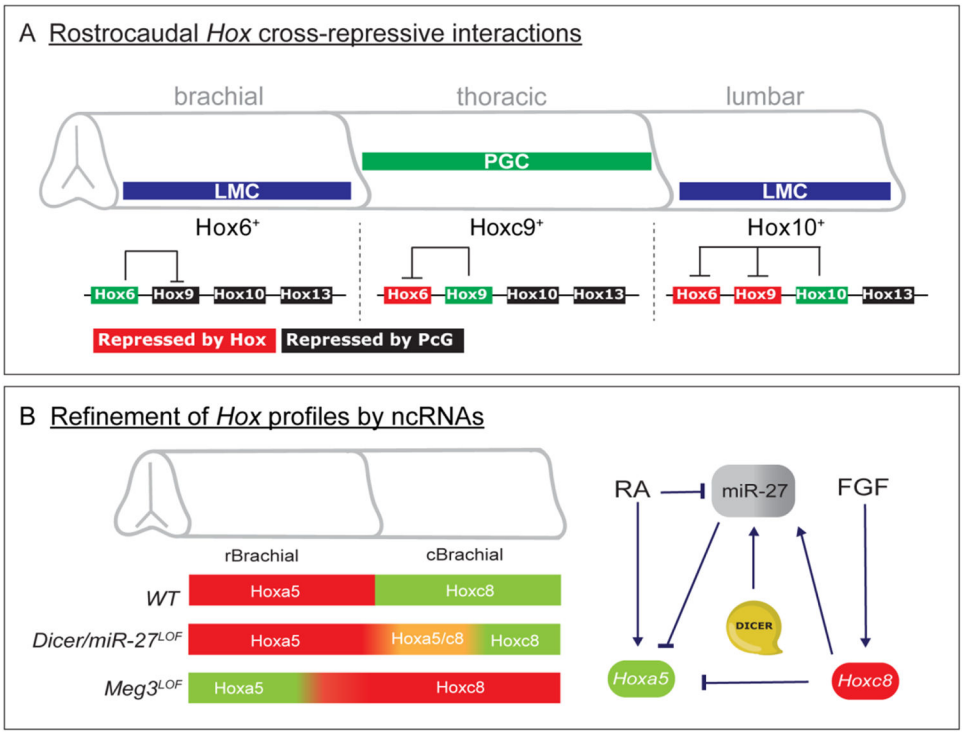

Fig. 3. Establishment and refinement of *Hox* boundaries in postmitotic MNs.

(A) Hox boundaries along the rostrocaudal axis are established through cross-repression and PcG-mediated silencing. For simplicity only *Hox6, Hox9, Hox10*, and *Hox13* paralogs are shown. Limb-innervating LMC neurons and thoracic PGC neurons are shown. Cross-repressive interactions appear to be conserved among paralogs (e.g. Hoxa9, Hoxb6, Hoxc9, and Hoxd9 all repress *Hoxc6*). At thoracic levels, Hox9 proteins repress brachial *Hox6* genes, while more caudal *Hox10* and *Hox13* genes are silenced by PcG proteins. **(B)** The activity of miRNAs fine-tunes expression of *Hoxa5* and *Hoxc8* at the boundary between rostral (r) and caudal (c) brachial segments. Depletion of miR-27 or Dicer function leads to erroneous expression of *Hoxa5* in caudal Hoxc8^{+} brachial segments. Loss of the lncRNA *meg3* in MNs results in the expansion of *Hoxc8* into rostral brachial segments and reduced Hoxa5 expression.

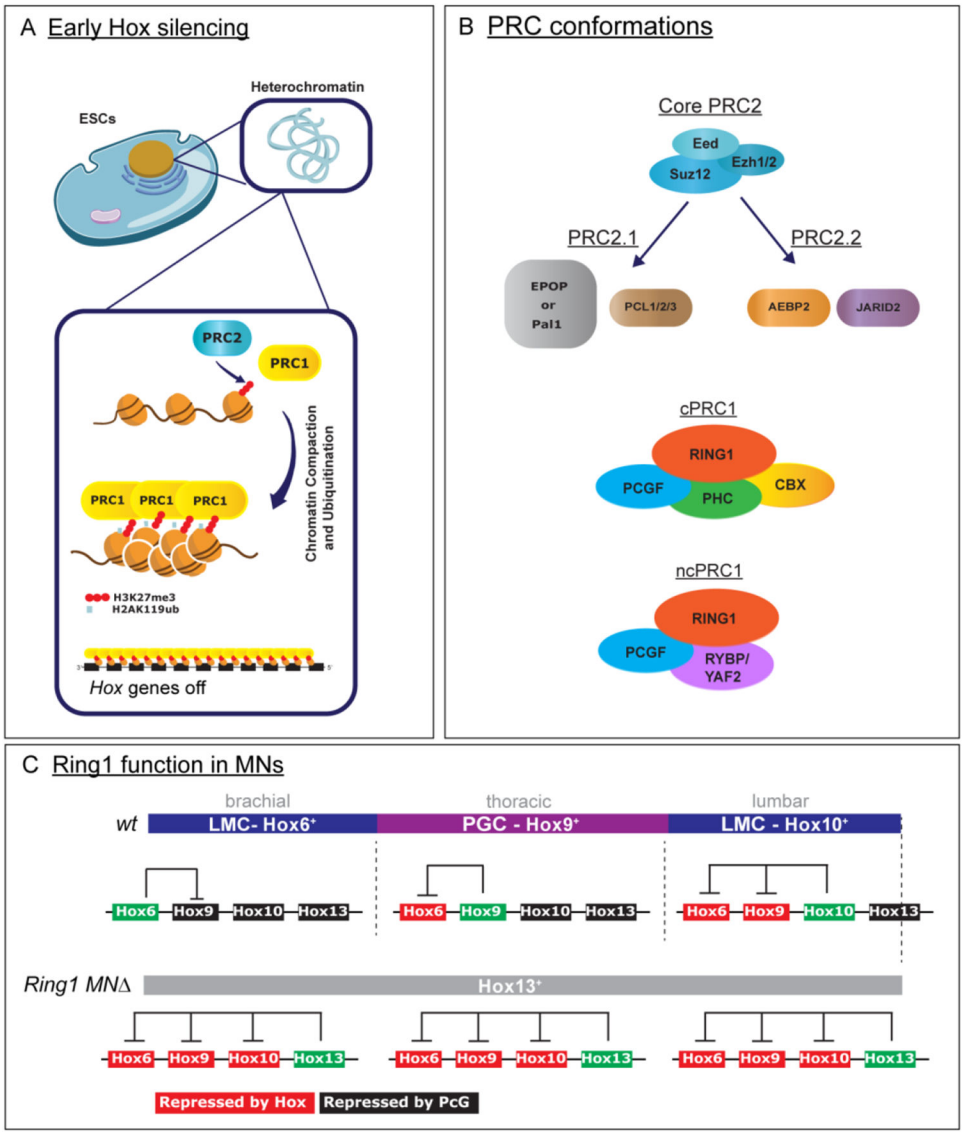

Fig. 4. Diversity and function of PRCs during MN differentiation.

(A) In early embryos and mouse embryonic stem cells, *Hox* clusters are kept transcriptionally silent through PRC1 and PRC2, which modify histones and compact chromatin. **(B)** Alternate PRC2 and PRC1 subunit compositions. PRC2 can be subdivided into PRC2.1 and PRC2.2. Canonical (c) PRC1 contains PHC and CBX proteins while non-canonical (nc) PRC1 contains RYBP or YAF2. **(C)** Function of Ring1 during MN differentiation. In the absence of *Ring1* function (*Ring1A* and *Ring1B*), *Hox13* genes are ectopically expressed in rostral spinal segments, leading the repression of *Hox4-Hox10* gene expression. For simplicity only *Hox6, Hox9, Hox10*, and *Hox13* paralogs are shown.