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Review

Evolution of Patterning Systems and Circuit Elements for Locomotion

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Evolutionary modifications in nervous systems enabled organisms to adapt to their specific environments and underlie the remarkable diversity of behaviors expressed by animals. Resolving the pathways that shaped and modified neural circuits during evolution remains a significant challenge. Comparative studies have revealed a surprising conservation in the intrinsic signaling systems involved in early patterning of bilaterian nervous systems but also raise the question of how neural circuit compositions and architectures evolved within specific animal lineages. In this review, we discuss the mechanisms that contributed to the emergence and diversity of animal nervous systems, focusing on the circuits governing vertebrate locomotion.

The earliest nervous systems are thought to have consisted of distributed populations of sensory neurons and motor neurons (MNs) that enabled animals to detect environmental changes and translate this information into specific motor actions (Holland, 2003). Execution of appropriate motor responses to stimuli is essential to the survival of an organism and one of the most fundamental aspects of nervous system function. Even the most complex regions of vertebrate nervous systems, such as the human cortex, can be considered as processing centers whose primary role is to interpret sensory information and transform it into specific motor commands.

In vertebrates, much of the activity of the CNS is channeled into the brain stem and spinal cord with the sole purpose of coordinating the activation of muscles. The most well-studied motor circuits in vertebrates are those that control walking and breathing, yet we know very little about the genetic modifications that facilitated the emergence of even these relatively simple animal behaviors. In the vertebrate lineage, fundamental changes in the nervous system coincided with the transition from aquatic to terrestrial terrains, and necessitated the modulation and rewiring of existing locomotor and respiratory neuronal networks. A major goal has been to resolve how these essential motor circuits are constructed during development, and to determine how they evolved and diversified.

Comparisons of transcription factor profiles among diverse bilaterian species suggest deep conservation in the intrinsic signaling pathways controlling early nervous system patterning. Perhaps the most dramatic example is seen in the development of the visual system. Studies in mice and flies have demonstrated that key aspects of early eye development are controlled by a relatively small number of conserved fate determinants (Gehring, 2014). For example, the transcription factor Pax6/eyeless has a central role in the development of photodetection systems in both vertebrates and insects, and misexpression of mouse Pax6 can generate ectopic eyes in imaginal discs of *Drosophila* embryos (Halder et al., 1995). More recent studies indicated that a large number of transcription factors involved in early patterning along the dorsoventral (DV) and rostrocaudal axes are conserved in both vertebrates and invertebrates (Denes et al., 2007; Lowe et al., 2003), implying that the nervous system of the common ancestor to all bilaterians was already quite sophisticated (De Robertis and Sasai, 1996).

Given the remarkable conservation in the expression of key patterning genes, how did nervous systems evolve to generate new motor behaviors within various animal lineages? In this review, we discuss how alterations in developmental pathways enabled nervous systems to construct, and in some cases deconstruct, motor circuits that govern genetically predetermined locomotor behaviors. Because the link between neuronal identity and circuit connectivity has been closely examined in the spinal cord, we focus on the circuits governing the development of vertebrate motor systems, and describe how early intrinsic patterning systems impact circuit assembly and function. We discuss evidence that small changes in transcription factor activity can act as a major driving force for evolutionary modification of circuit architectures. We argue that within the spinal cord a flexible system involving modulation of rostrocaudal positional information, acting in the context of a relatively uniform DV patterning system, can act to modify neuronal organization and connectivity within circuits governing a specific locomotor output.

Ancestral Origins of Neural Induction and Early Patterning

During the earliest phases of neural development, regions of ectoderm are allocated to acquire neuronal characteristics. Naive neural ectoderm subsequently acquires regional identities that prefigure the organization of motor circuits in the adult. On the surface, there appears to be fundamental differences in how nervous systems develop in distantly related species. Subsequent to neural induction, the majority of neurons in *Drosophila* are specified in lineages that are governed through temporal specification codes, and a single progenitor can give rise to multiple neuronal classes (Kohwi and Doe, 2013). In contrast, patterning in the vertebrate neural tube is driven by extrinsic morphogen-based signaling, and progenitors typically give rise

to only a few classes of neurons (Jessell, 2000). Despite these significant differences, many species appear to use a common set of intrinsic determinants during early neural patterning. In this section, we compare and contrast the mechanisms of neural induction and global patterning within the two major superphyla of bilaterians, protostomes (which includes arthropods and annelids) and deuterostomes (which includes chordates, hemichordates, and echinoderms) (Figure 1A).

Neural Induction and DV Patterning in Bilaterians

The formation of bilaterian nervous systems is initiated through neural induction, a process where the neural plate is specified within a restricted region of ectoderm. In most species, neural induction involves bone morphogenetic protein (Bmp) signaling along the DV axis (De Robertis, 2008). Bmp signaling suppresses neural differentiation, the default fate of ectodermal cells, and promotes epidermal differentiation. In vertebrates, Bmp antagonists (noggin, chordin, and follistatin) are secreted from the dorsal organizer, thereby differentiating ectodermal cells into neural tissue. Subsequently, gradients of dorsal Bmps, in conjunction with ventral sonic hedgehog (Shh) signaling, establish subdivisions of progenitor domains along the DV axis of the neural tube in chordates (Jessell, 2000).

Although there are significant morphological differences among bilaterian nervous systems, Bmp signaling plays a conserved role in both protostomes and deuterostomes (Figures 1A and 1B). For example, the vertebrate Bmp antagonist Chordin acts similarly to its *Drosophila* homolog sog (short gastrulation) in promoting neuronal fate (Holley et al., 1995). The *Drosophila* Bmp homolog dpp can phenocopy Bmp4 activity when expressed in *Xenopus*. Early Bmp expression is inversely correlated with the position where the CNS develops in both protostomes and deuterostomes, although the relative position of where the nervous system forms is distinct in both phyla. In protostomes the nerve cord forms ventrally, while in deuterostomes the nerve cord forms dorsally (Figure 1A). This relationship suggested a DV inversion hypothesis, where the CNSs of all bilaterians have a common origin, and an inversion of the DV axis occurred during deuterostome evolution (Arendt and Nübler-Jung, 1994; De Robertis and Sasai, 1996).

Further support for a common origin of bilaterian nervous systems has emerged from studies of neural development in protostome annelids. These studies revealed that the transcriptional regulatory networks required for early DV patterning in the vertebrate nerve cord are present in protostomes (Denes et al., 2007). Like in other bilaterians Bmp signaling has a key role in annelid neural induction. Annelids also show a higher degree of similarity with vertebrates than *Drosophila* in the expression of neural patterning genes (Figure 1B). For example, the ventral determinants Nkx2.2 and Pax6 are expressed in mutually exclusive domains in both vertebrates and annelids, but this pattern is not conserved in fly (Kammermeier et al., 2001). In addition, like vertebrates, annelid MNs are generated from a ventral domain characterized by expression of the transcription factor Hb9, and these neurons are cholinergic. This contrasts with the embryonic CNS of *Drosophila,* where MNs are generated in multiple lineages and are typically glutamatergic.

Repression of neural induction by Bmps appears to have been lost in hemichordates, although Bmp-Chordin signaling and orthologs of DV target genes are expressed (Lowe et al., 2006). This phenomenon may be due to its unique nervous system organization that consists of two nerve cords, one dorsal and one ventral, and a diffuse basiepidermal nerve net (Holland, 2003; Nomaksteinsky et al., 2009). A possible explanation provided by Arendt and colleagues is that hemichordates, such as acorn worms, might have modified their trunk neuroarchitecture due to the evolutionary changes in locomotor behaviors (Denes et al., 2007). Furthermore, a recent study provided additional evidence for conserved DV patterning cues in hemichordates. The hedgehog receptor *patched* is expressed ventrally in the collar nerve cord, while *hedgehog* is expressed in the endoderm of the buccal tube and the stomochord, similar to the relationship between *ptc* in the neural tube and *Shh* in the floor plate and notochord of vertebrates (Miyamoto and Wada, 2013).

Conservation of Rostrocaudal Patterning Cues in Bilaterians

Soon after neural induction in vertebrates, cells from the neural plate acquire rostrocaudal positional identities and segregate into four major regions: the forebrain, midbrain, hindbrain, and spinal cord. The anterior neural plate has three primary signaling centers that produce morphogens involved in rostrocaudal patterning: (1) the anterior neural ridge (ANR), (2) zona limitans intrathalamica (ZLI), and (3) isthmic organizer (IsO). These neuroectodermal signaling centers were thought to have originated in the vertebrate CNS since they are either absent or divergent in other chordates (Bertrand et al., 2011; Holland et al., 2000; Imai et al., 2009; Irimia et al., 2010; Shimeld, 1999; Takatori et al., 2002). Recently, Lowe and colleagues provided evidence that inductive centers homologous to the ANR, ZLI, and IsO are present in hemichordates, suggesting that they are ancient patterning systems that were present in early deuterostomes (Pani et al., 2012). Additionally, extensive analysis from Kirschner and colleagues revealed that the hemichordate nervous system shows remarkable conservation in rostrocaudal patterning (Lowe et al., 2003). While there are some differences in the rostrocaudal expression domains within the 22 orthologs of chordate neural patterning genes that were tested, the relative expression domains are very similar to vertebrates (Figure 1C).

Although the corresponding extrinsic signaling centers are absent from protostomes, early anteroposterior patterning has been reported in several species indicating that compartmental-like boundaries existed in the common bilaterian ancestor (Figure 1C). For example, recent studies revealed that the *Drosophila* brain has a tripartite ground plan similar to vertebrates and displays conserved expression of transcription factors that are key to the development of vertebrate nervous systems (*otx2*, *gbx2*, *fezf*, *irx*, *pax2/5/8*, and *Hox*) (Hirth et al., 2003; Irimia et al., 2010). Similarly, the segmental expression pattern of *otx*, *gbx*, and *Hox* genes in the protostome annelids parallels the pattern in *Drosophila* (Steinmetz et al., 2011). These results support the hypothesis that the nervous system of the common urbilaterian ancestor of all bilaterians had an organized CNS that was patterned by shared intrinsic signaling programs (De Robertis and Sasai, 1996).

Neuronal Class Specification, Guidance Systems, and Neuronal Organization

In vertebrates, early patterning systems act on neuronal progenitors to prefigure cells to express a set of cell identity

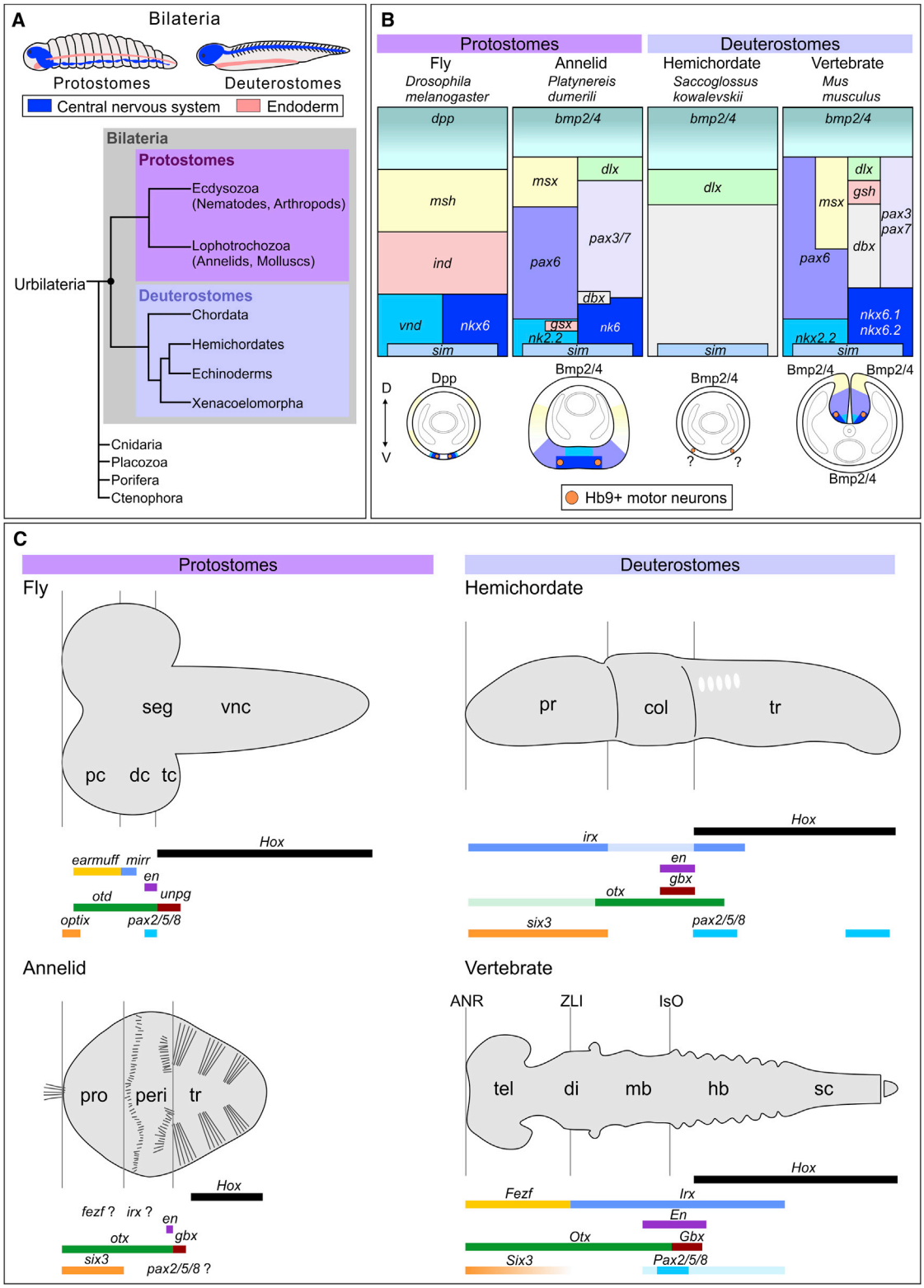

Figure 1. Neural Induction and Early Patterning in Bilateria

(A) Traditional classification of bilateria. Bilaterians are a subgroup of eumetazoan animals characterized by a bilaterally symmetrical body plan and triploblastic development. Bilaterians are subdivided into protostomes (mouth first) and deuterostomes (mouth second). (Top) The CNS (in blue) forms ventrally in protostomes and dorsally in deuterostomes. (Bottom) A simplified phylogenetic tree showing the evolutionary relationships among bilaterians and other metazoan phyla.

(legend continued on next page)

determinants at the time of cell-cycle exit. The pattern of transcription factor expression in newly born neurons generates a remarkable diversity in cell types, a defining feature of most animal nervous systems. How neuronal cell types are specified is a first step toward elucidating how neurons are interconnected to establish a specific circuit. Here we outline the mechanisms through which large classes of neurons are specified, and the strategies through which neuronal subtypes essential within motor systems emerged in the vertebrate lineage. Recent evidence indicates that, in some cases, a transcription factor class present in multiple species can target the same genes that define the core physiological properties of a neuronal type.

Cell Fate Specification and Neurotransmitter Identity

Near the time of terminal differentiation, transcription factors act to define the core physiological properties of neurons as well as features that allow them to establish their initial connectivity patterns. The nervous systems of many species contain thousands of molecularly and anatomically distinct cell types, and it has historically been challenging to establish a unifying classification scheme (Masland, 2004). For simplicity, we define the steps through which neurons acquire their identities as class and subtype specification programs. In vertebrates, neurons within a class typically derive from a single molecularly defined progenitor domain, use a common neurotransmission system, and form connections with similar types of neurons. Subtypes of neurons within a class are more loosely defined, but often express different sets of transcription factors, establish connections that are distinct from other subtypes, and can be morphologically distinct. In terms of evolutionary changes, neuronal classes are often present throughout animal species, whereas subtypes show the greatest evolutionary diversification.

A defining characteristic of neurons within a single class is the expression of genes encoding elements of neurotransmitter systems, including proteins involved in neurotransmitter synthesis and release. Expression of neurotransmitter genes appears to rely on the actions of transcription factors expressed in postmitotic cells, the identities of which have been resolved only in recent years. This question has been worked out in greatest clarity in *C. elegans*, where cohorts of genes involved in neurotransmission are controlled by a relatively small number of transcription factors acting on common *cis*-regulatory elements (Hobert, 2011). As these factors are capable of controlling a large number of genes that act in the same synthetic pathway, they have been called terminal selectors. Terminal selectors are typically expressed throughout the life of an organism, and their expression can be maintained through positive transcriptional autoregulation (Deneris and Hobert, 2014). Many of the regulatory proteins defined in *C. elegans* are functionally conserved in vertebrates. For example the *C. elegans* ETS family transcription factor ast-1 plays a critical role in regulating the battery of genes involved in dopamine synthesis (Flames and Hobert, 2009). In vertebrate olfactory neurons, the ast-1 homolog Etv-1 directly controls the terminal synthetic enzyme required for dopamine synthesis, tyrosine hydroxylase. Similar conservation is observed in the regulation of glutamatergic fates by Lim homeodomain (HD) factors (Serrano-Saiz et al., 2013). The regulatory factors that control neurotransmitter synthesis in *C. elegans* also are tied to programs that regulate other features of a neuronal class, such as expression of ion channels, cell adhesion molecules, and determinants of axonal and dendritic morphology (Kratsios et al., 2012; Serrano-Saiz et al., 2013). These observations indicate that terminal selectors act on common *cis* elements to establish and maintain the identity of a neuron throughout an animal's lifespan.

Similar to *C. elegans*, regulation of neurotransmitter identity in vertebrates is linked to gene networks governing multiple aspects of neuronal identity and connectivity (Figure 2A). The MNs of vertebrates use acetylcholine (Ach) as the primary neurotransmitter to activate muscle and other neurons. Cholinergic gene batteries are directly regulated through complexes formed between the Lim HD proteins Isl1 and Lhx3 and their cofactor Lbd1 (Cho et al., 2014; Lee et al., 2012). This complex also is required to regulate the gene encoding the transcription factor *Hb9* (Lee et al., 2008), a key determinant of multiple facets of MN subtype differentiation (Arber et al., 1999; Thaler et al., 1999). While vertebrates use Lim HD proteins to orchestrate Ach synthesis in MNs, *C. elegans* uses a distinct class of transcription factor, the COE family member unc-3 (Kratsios et al., 2012). Nematodes do, however, use Lim HD factors to regulate Ach synthesis in interneuron subtypes (Zhang et al., 2014). Another layer of complexity is apparent when one considers how MNs activate muscles in different model organisms. While vertebrates and *C. elegans* MNs use the cholinergic system, embryonic MNs of *Drosophila* activate muscles using glutamate, although both flies and mice require the same set of transcription factors (Hb9, Isl1, and Lhx3) for diversifying MNs into subtypes.

(B) Conservation of gene expression patterns along the DV axis in protostomes (flies and annelids) and deuterostomes (hemichordates and vertebrates). In both protostomes and deuterostomes, expression of neural identity genes is patterned by Bmps along the DV axis of the nerve cord (Esteves et al., 2014). Ventral patterning cues are not portrayed here as they are not homologous in different species (e.g., Dorsal in flies, Shh in vertebrates). As in vertebrates, cholinergic Hb9$^+$ MNs derive from pax6$^+$nk6$^+$ progenitors and directly innervate muscles in annelids (Denes et al., 2007). In flies, there are MN populations (not depicted here) in addition to Hb9$^+$ MNs. Although Bmp-Chordin signaling is present in hemichordates, many DV-patterning genes are not expressed by the neuroectoderm (e.g., nk2.2 in endoderm). The *Mnx* gene, which shares high homology with Hb9 homeodomain, is expressed in the hemichordate ventral ectoderm, implicating possible conservation in MN specification (Lowe et al., 2006). Homologous genes are color coded. Schematics on the bottom represent cross-sections of the embryos.

(C) Conservation of anteroposterior-patterning systems in bilaterians. Although protostomes do not have analogous neuroectodermal-signaling centers present in developing vertebrate brains, key genes determining their boundaries are conserved along the anteroposterior axis. The *en* gene is also expressed at parasegmental boundaries in the epidermis of flies and annelids. In hemichordates, the expression of *fezf* (not shown here) is not adjacent to that of *irx*. Homologous genes are color coded for comparison. Pc, protocerebrum; dc, deutocerebrum; tc, tritocerebrum; seg, subesophageal ganglion; vnc, ventral nerve cord; pro, prostomium; peri, peristomium; tr, trunk (both in annelid and hemichordate); pr, proboscis; col, collar; tel, telencephalon; di, diencephalon; mb, midbrain; hb, hindbrain; sc, spinal cord. Comparisons between species represented in (A and B) do not take into account gene expression differences and, therefore, do not represent a true cladistics analysis. Furthermore, this model does not fully take into account the development of animals with unsegmented nervous systems, such as in molluscs. (A) is modified from De Robertis (2008) and Philippe et al. (2011; (B) is modified from Denes et al. (2007) and Mizutani and Bier (2008).

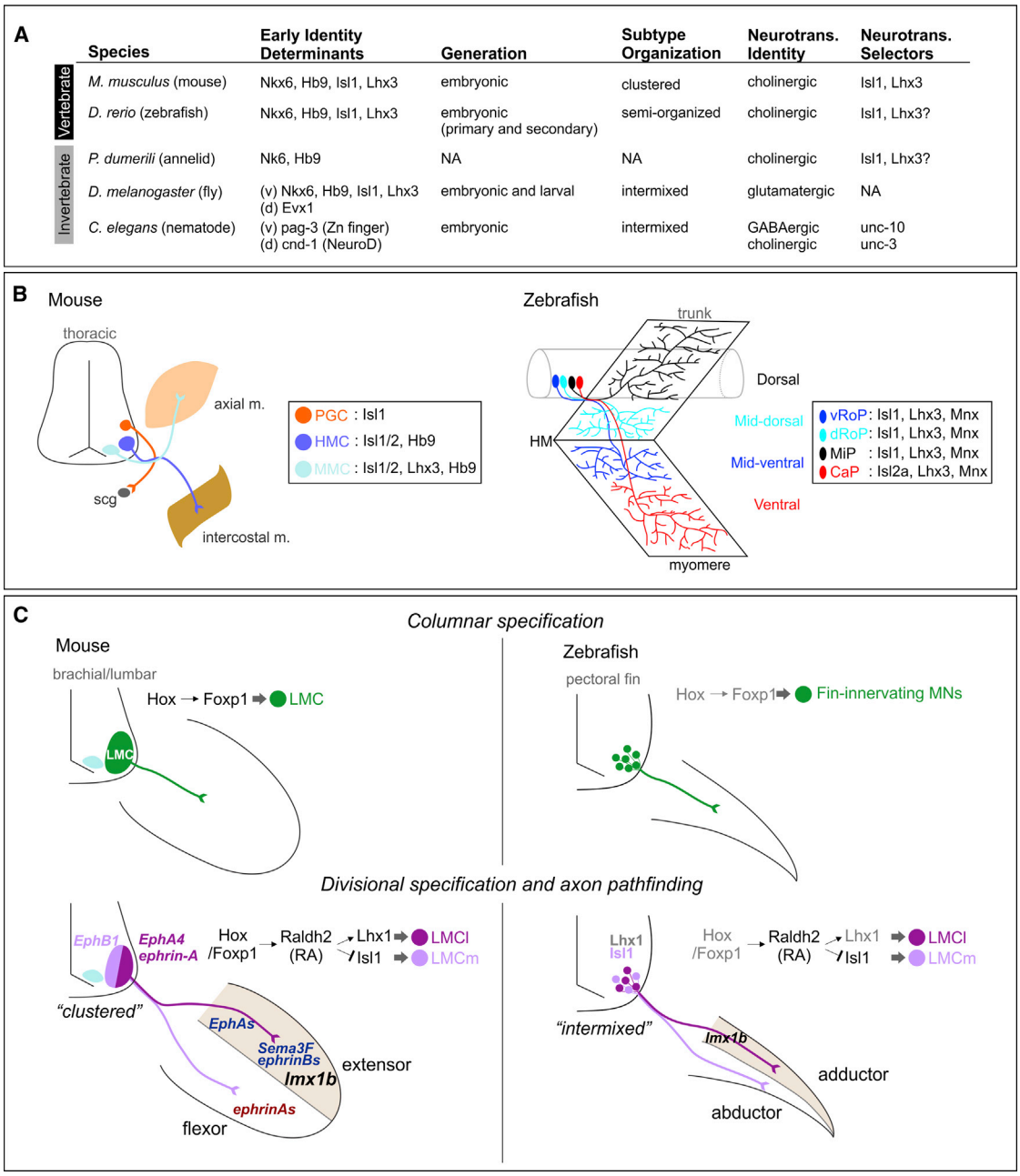

Figure 2. Motor Innervation Programs in Bilaterians

(A) Conservation and divergence of MN cell fate specification programs in invertebrates and vertebrates, emphasizing known conserved transcription factors. Several key transcription factors involved in MN specification are not indicated. NA, not assessed.

(B) Comparisons of MN organization and innervation patterns between mouse and zebrafish at trunk levels. Core MN determinants, Isl1/2, Hb9, and Lhx3, are expressed in different combinations in three distinct thoracic columns in mouse. Scg, sympathetic chain ganglia. Zebrafish embryos contain four classes of primary MNs as follows: vRoP (ventrally projecting rostral primary), dRoP (dorsally projecting RoP), MiP (medial primary), and CaP (caudal primary); they do not organize into tightly clustered columns (Menelaou and McLean, 2012). They are classified by their specific innervation of axial muscles from dorsal to ventral. The stereotypic innervation patterns of each primary MN are depicted here. Although three Mnx proteins are detected within each primary MN subtype in zebrafish, Mnx proteins are only required in MiP MNs (Seredick et al., 2012).

(C) MN organization and specification programs at limb/fin levels in mouse and zebrafish. In zebrafish, pectoral fin innervating MNs are considered to be secondary due to their late development and ventrolateral position relative to primary MNs (Myers, 1985). A GFP reporter under control of an *Isl1* enhancer indicates that Isl1^{+} pectoral fin MNs selectively innervate abductor muscles (Uemura et al., 2005). Untested aspects of these models are shown in gray.

Protostome annelids also express class determinants similar to vertebrates, and their MNs are cholinergic (Denes et al., 2007). This observation supports the idea that the urbilaterian ancestor contained MNs that were similar to those of modern vertebrates. Flies and nematodes therefore may have evolved distinct mechanisms for controlling neurotransmitter systems in MNs.

Convergence of cell fate determinants and neurotransmitter systems is also apparent when comparing different neuronal classes that share the same neurotransmitter identity. In addition to spinal MNs, cholinergic neurons are present in specific neurons of the vertebrate forebrain. Interestingly, the logic of the transcription factor network regulating cholinergic gene batteries is very similar in both regions. In MNs, Lhx3 and Isl1 have key roles in regulation of cholinergic genes, while Lhx8 and Isl1 serve similar roles in the forebrain (Cho et al., 2014; Lopes et al., 2012). Thus, in the context of neurotransmitter gene batteries, key targets can be regulated through highly conserved *cis*-regulatory elements. Evolutionary diversification of neurons using the same neurotransmitter system in principle could be achieved by utilization of multiple members of the same transcription factor family.

Ancestry and Evolution of Genetic Programs for Muscle Innervation

In addition to neurotransmitter systems, a defining feature of neurons within a specific class is the types of cells with which they establish connections. Because of their central role in motor circuits, we emphasize the connectivity programs of MN subtypes. The MNs of most species are characterized by the extension of axons outside the CNS, local connectivity with certain classes of interneurons and sensory neurons, as well as descending inputs from supraspinal areas. The basic program of peripheral connectivity with muscle is likely to be conserved across many bilaterian species, since determinants necessary for the selectivity of their peripheral projections are conserved in protostomes and deuterostomes. In mice and flies, ventrally projecting MNs can be defined by the expression of Hb9, Nkx6, and Lim HD proteins, with each class member also acting at later stages to define the peripheral connectivity of MN subtypes.

A common feature of motor systems in many protostome and deuterostome species is the innervation of segmentally organized axial muscles by MNs. In tetrapods the selection of axial muscles is largely determined by the actions of Lim HD proteins and Hb9 (Figure 2B). Dorsal epaxial and ventral hypaxial muscles are innervated by motor columns that are defined by the expression of these factors. Hypaxial muscles, which include intercostal and abdominal muscles, are innervated by ventrally projecting MNs that express Isl1 and Hb9, while dorsal epaxial muscles are innervated by MNs expressing Lhx3 and Hb9 (Figure 2B). Lhx3 has a central role in differentiating dorsally from ventrally projecting MN subtypes, as misexpression of Lhx3 can suppress all other MN subtype specification programs and force motor axons to select a dorsal trajectory (Dasen et al., 2008; Sharma et al., 2000). In other species, the logic of the Lim code with respect to the peripheral trajectories of motor axons is distinct. In zebrafish, there is no clear correlation between the selection of DV trajectories of primary MNs and the expression of specific Lim HD proteins (Figure 2B), although MN subtypes can be distinguished based on differential expression of these factors (Appel et al., 1995). Similarly in *Drosophila*, the basic decision to project dorsally or ventrally involves a different class of transcription factors, where the Evx1 homolog even-skipped is required in dorsally projecting MNs, with Lim HD factors and Hb9 acting to define subtypes of ventrally projecting populations both in the embryonic and adult nervous system (Lacin et al., 2014; Landgraf and Thor, 2006).

A significant evolutionary advancement in the vertebrate lineage was the generation of MN subtypes that enabled the articulation of muscles in the limb. However, it is largely unknown at what stage in vertebrate evolution the program for limb innervation emerged. In vertebrates, limb innervating MNs are organized into the lateral motor column (LMC), and are defined by the expression of the transcription factor Foxp1 and the retinoic acid synthetic enzyme Raldh2 (Figure 2C; Dasen and Jessell, 2009). Among Foxp1^{+} limb MNs, those projecting to the dorsal limb compartment express Lhx1, while those projecting ventrally express Isl1 (Dasen et al., 2008; Tsuchida et al., 1994). The establishment of this Lim HD code is essential for the peripheral connectivity of LMC axons. In the case of limb-innervating MNs, the effectors of these cell fate determinants have been well characterized and include members of the Eph/ephrin-signaling system, which are regulated by Lim HD proteins and determine the response of motor axons to ephrin signaling in the limb mesenchyme (Kao et al., 2012).

Analysis of limb-level MNs in other species suggests that some, but not all, aspects of appendage innervation programs are conserved among vertebrates (Figure 2C). Representatives of each of the four main classes of tetrapods (birds, reptiles, amphibians, and mammals) express similar profiles of transcription factors in LMC neurons (Jung et al., 2014). In zebrafish, the Lim HD code that defines the DV selection of motor axons appears to be conserved at the level of the pectoral fin (Uemura et al., 2005), and expression of Raldh2 has been reported in pectoral fin-level MNs (Begemann et al., 2001). However, selective expression of *Foxp1* by fin-level MNs has not been reported, nor is there any direct evidence that rostrocaudal positional identity determinants (e.g., *Hox* genes) have any role in MN subtype specification.

Many arthropod species also bear appendages involved in walking, although it appears that their leg innervation program arose independently. The common ancestor to protostomes and deuterostomes is thought to have lacked appendages, and this limbless state was preserved in early chordates, suggesting that the Foxp1/Lim HD code emerged in the vertebrate lineage. As a consequence of the independent origins of limb innervation programs, many basic features of MN organization and connectivity have diverged between vertebrates and invertebrates.

Evolution of MN Somatotopic Organization

A highly varied feature of bilaterian motor systems is reflected in how MNs are organized. In tetrapods, MNs projecting to a common target zone or specific muscle are clustered in longitudinally arrayed columnar and pool groups. This organization creates a somatotopic map within the spinal cord that links cell body position to the peripheral trajectory of motor axons. The clustering of MNs is present in all tetrapods that have been examined, as well as some species of fish (Fetcho, 1987; Jung et al., 2014). In *Drosophila* and *C. elegans*, as well as aquatic vertebrates such as zebrafish, MNs targeting specific muscles do not cluster into coherent columnar groups (Thor and Thomas, 2002), although there is evidence that zebrafish MNs are dorsoventrally organized based on their activation at different locomotor speeds (Ampatzis et al., 2013). These observations raise the

question of what is the significance of the clustering of MNs in tetrapods, and what evolutionary advantages it provides in terms of motor circuit connectivity and function. One possibility is that the complexity of vertebrate limb musculature necessitated a strategy to ensure that MNs receive selective inputs from other neuronal classes (e.g., interneurons and sensory neurons) on the basis of their position, rather than through specific molecular determinants.

In tetrapods, the organization of MN cell bodies is controlled by signaling pathways that determine the migratory and adhesive properties of columnar and pool subtypes. A MN pool that targets a single muscle in the limb is clustered into groups of ~50–200 MNs that occupy a stereotypic intrasegmental and rostrocaudal position within the spinal cord. Members of the type II cadherin family have been implicated in pool clustering, as they display columnar- and pool-specific patterns of gene expression, and genetic manipulations that perturb cadherin expression or signaling randomizes MN position (Demireva et al., 2011; Price et al., 2002). Expression of type II cadherins is regulated by intrinsic signaling systems, including columnar-specific transcription factors such as Foxp1 and pool-restricted factors such as the Ets protein Pea3 (Dasen et al., 2008; Livet et al., 2002). In *Foxp1* mutants, cadherin expression is lost in LMC neurons and the position of MNs targeting a muscle is randomized within the spinal cord (Dasen et al., 2008; Sürmeli et al., 2011).

The phenotype of *Foxp1* mutants provides a means to test the hypothesis that settling position is a determining factor in the specificity of connections that MNs establish centrally. Consistent with this idea, mutation in *Foxp1*, which scrambles MN cell body position but otherwise preserves core features of MN identity, leads to formation of inappropriate connections between MNs and proprioceptive sensory neurons (Sürmeli et al., 2011). This observation is consistent with the hypothesis that the organization of MNs into clustered groups may have evolved to facilitate synaptic specificity within the context of an increased diversity of limb muscles in tetrapods (Fetcho, 1987).

Evolutionary Diversification of Effector Neurons in Motor Systems

The evolution of motor networks can be easily appreciated when one considers the diversity of locomotor behaviors exhibited by animals (e.g., swimming, walking, flying, and hopping). During vertebrate evolution, fundamental changes in motor circuits accompanied the acquisition of paired appendages and the transition of tetrapods from the sea to land (Figure 3A). The most primitive vertebrates are thought to have lacked paired appendages and are represented in modern species by agnathan (jawless) fish including lamprey and hagfish. Locomotion in agnathans is achieved through propagation of sinusoidal waves of muscle contraction along the body axis, and this locomotor strategy is observed in a range of species including nematodes, insect larvae, and snakes. While some modern fish can utilize the fins to generate a walking-like form of locomotion on the sea floor (Macesic and Kajiura, 2010), the predominant role of paired appendages in aquatic species is for steering, not propulsion. Therefore, the basic locomotor strategy in most fish is axial-based undulation, which has led to the proposal that motor circuits for walking evolved in species similar to modern amphibians (Murakami and Tanaka, 2011). Amphibians and reptiles appear to represent an intermediate step in the emergence of walking circuits, as some species display a combination of both undulatory and ambulatory locomotor behaviors (Figure 3A).

Limb-based locomotion requires the precise coordination of individual muscles in the limb and, hence, a more complex peripheral innervation program than is needed for undulatory locomotion. Insights into how motor circuits for walking emerged can be gleaned from understanding the mechanisms that fostered evolutionary changes in MN organization and connectivity.

Hox Networks in Spinal MN Diversity and Organization

While all MNs share certain core properties, they are a highly diverse population that has evolved unique functions in different animal lineages. The spinal MNs of tetrapods are topographically organized into columns and pools, and express subtype identity determinants that allow them to make selective connections with their peripheral targets (Dasen and Jessell, 2009). In addition to the motor columns targeting epaxial (MMC), hypaxial (HMC), and limb muscles (LMC), several additional columnar subtypes appeared at different stages of vertebrate evolution. In tetrapods, the preganglionic column (PGC) is generated at thoracic levels and innervates the sympathetic chain ganglia (Figures 3B and 3C). Because sympathetic neurons are derived from neural crest cells, a migratory population that evolved in early chordates (Bronner and LeDouarin, 2012), PGC neurons likely emerged during neural crest diversification. A group of specialized MNs involved in respiratory function appeared later in vertebrate evolution. Phrenic motor column (PMC) neurons are generated at cervical levels of the spinal cord and innervate diaphragm muscle. The PMC is unique to mammals, and is absent from birds, reptiles, and amphibians (Figure 3C). At limb levels, neurons within the LMC fractionate into ~50 motor pools, and this diversity likely emerged concomitantly with the increased complexity of tetrapod limb musculature.

How are MN columnar and pool subtypes specified during development? MN diversification relies on the large family of *Hox* genes, an evolutionarily conserved family of transcription factors essential in governing animal body plans along the rostrocaudal axis (McGinnis and Krumlauf, 1992). In tetrapods, *Hox* genes are arrayed in four chromosomal clusters and their expression is governed through opposing FGF and rostral RA signaling gradients acting on neural progenitors (Dasen et al., 2003; Liu et al., 2001). Although *Hox* genes are restricted to specific rostrocaudal levels, they are widely expressed by most neuronal classes within the hindbrain and the spinal cord (Dasen et al., 2005). Efforts to elucidate their functions during neural patterning have focused largely on their roles in specifying MN subtype identities.

The role of *Hox* genes in MN specification has been investigated by genetic manipulation of their activities in mice and chick. The generation of segmentally restricted MN subtypes (PMC, PGC, HMC, LMC neurons, and LMC pools) is governed by *Hox* genes expressed at specific levels, although the strategies involved vary significantly depending on the MN population. The columnar identity of limb-innervating MNs is controlled by multiple redundant Hox inputs, and only through combined deletion of the *HoxA* and *HoxC* clusters is LMC identity erased at forelimb levels (Jung et al., 2014; Lacombe et al., 2013). In contrast, at thoracic levels, MN subtypes rely on the single

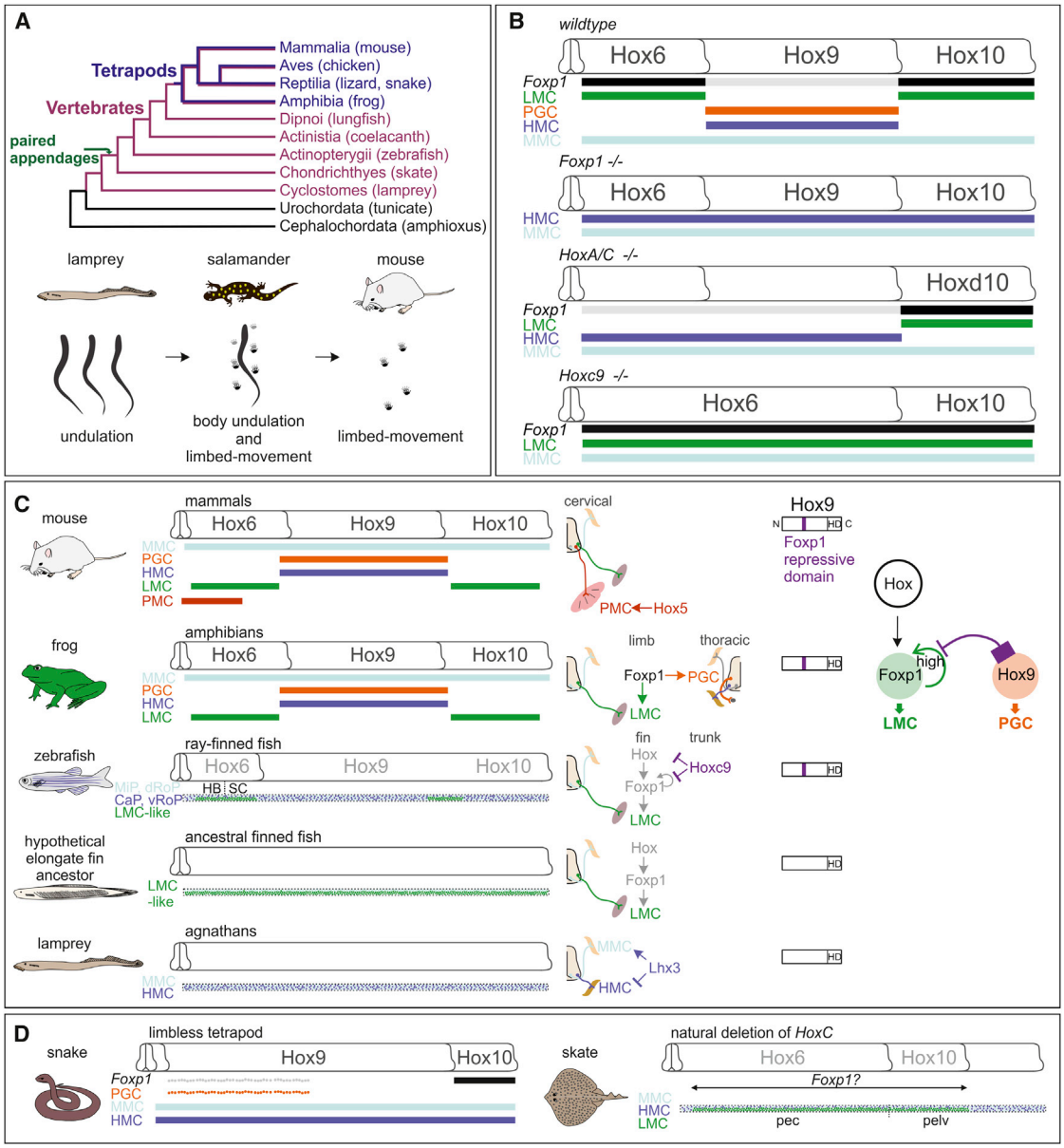

Figure 3. Evolutionary Diversity of Spinal MNs

(A) Evolution of locomotor strategies. (Top) A chordate phylogeny showing representative species of tetrapods (dark purple) and vertebrates (light purple). Chondrichthyans represent the most primitive species bearing paired appendages. (Bottom) Comparisons of locomotor behaviors in lamprey, salamander, and mouse.

(B) Altered MN columnar organization in *Foxp1* and *Hox* mutants. In *Foxp1* mutants, Hox-dependent spinal MN columns (LMC and PGC) are transformed into an HMC-like ground state, which may represent a primitive condition. PMC neurons are present in *Foxp1* mutants, but not depicted. Loss of LMC neurons at brachial levels is achieved only when *HoxA* and *HoxC* gene clusters are mutated. Lumbar LMC neurons are preserved in *HoxA/C* cluster mutant mice due to Hoxd10 activity. Deletion of the *Hoxc9* gene causes global derepression of brachial *Hox* genes, resulting in an extension of the brachial LMC throughout thoracic levels. MMC neurons are considered Hox independent as their molecular profiles are preserved in each of these mutants.

(C) A model showing how MN organization has evolved with changes in body plans. A subset of MNs in agnathan vertebrates (represented by modern lampreys) may have lost Lhx3 activity, permitting the generation of HMC-like neurons. The acquisition of paired appendages promoted the generation of LMC-like populations, which may have been present initially at most spinal levels. A repressive domain within Hox9 proteins necessary to suppress LMC specification appears to have emerged when the elongate fin split into pectoral and pelvic fins. Studies in zebrafish suggest the pectoral fin MNs were initially positioned in both the hindbrain and spinal cord (Ma et al., 2010). Pelvic fin-innervating MNs do not align with *Hox10* gene expression (Murata et al., 2010). In mammals, PMC neurons are specified by Hox5 proteins and are Foxp1 independent (Philippidou et al., 2012).

(D) In snake embryos, expansion of *Hoxc9* expression blocks LMC generation. The enlarged-finned fish skate, which naturally has lost the *HoxC* cluster, may have extended LMC population along the anteroposterior axis of the spinal cord.

Hoxc9 gene, and in the absence of *Hoxc9* function, HMC and PGC neurons acquire an LMC fate (Jung et al., 2010). This phenotype is due, in part, to the derepression of *Hox* genes expressed at forelimb levels (Figure 3B). At limb levels, a network of ~20 *Hox* genes establishes the identity and connectivity of motor pools targeting limb muscles (Philippidou and Dasen, 2013). Given their central roles in MN subtype specification, modulation in Hox protein activities likely had a key role in the evolutionary diversification of motor circuits.

Origins of MN Diversity

If *Hox* genes are involved in the diversification of MNs, at what stage in vertebrate evolution did this program first appear, and what are the ancestral MN populations that *Hox* genes acted upon? Insight into this question has emerged through analysis of a primary target of Hox proteins in MNs, the transcription factor Foxp1. In quadrupeds, a critical function of Hox proteins expressed by MNs is to regulate expression of *Foxp1*. The majority of limb-level Hox proteins can induce high levels of *Foxp1* when ectopically expressed in thoracic MNs, while the thoracic Hoxc9 protein represses *Foxp1* levels when expressed at limb levels (Jung et al., 2014; Lacombe et al., 2013). In mice lacking the *Foxp1* gene, MNs fail to express essential molecular determinants of Hox-dependent subtypes, and MNs that have lost *Foxp1* retain expression of markers for thoracic HMC neurons (Dasen et al., 2008; Rousso et al., 2008). In contrast, dorsally projecting MMC neurons are unaffected by loss of *Foxp1.* As a consequence, mice with *Foxp1* deletion consist largely of HMC and MMC subtypes extending throughout the spinal cord (Figure 3B).

The organization of MNs in *Foxp1* mutants appears to resemble an ancestral state of the motor system present in primitive aquatic vertebrates lacking limbs, similar to modern agnathan species. Lamprey locomotion is driven by MMC-like neurons innervating segmentally iterated axial muscles that drive undulatory locomotion (Fetcho, 1992). While patterned *Hox* expression is present in lampreys (Takio et al., 2007), in this context Hox proteins presumably have no influence on spinal MN subtype diversification. The tetrapod motor system, therefore, likely co-opted a preexisting Hox network to allow Foxp1 to be induced in HMC-like precursors. The emergence of LMC neurons in appendage-bearing vertebrates likely required evolutionary changes in functions of Hox proteins and/or modification of *cis*-regulatory elements within the *Foxp1* gene.

Further evidence that HMC neurons serve as the evolutionary substrate for Hox-dependent MN diversification programs comes from analyses of the development of respiratory neurons in mammals. In mice, PMC neurons require the actions of two *Hox5* genes, *Hoxa5* and *Hoxc5*. In *Hox5* mutants, molecular determinants for PMC neurons are lost and the diaphragm fails to be properly innervated, leading to respiratory failure (Philippidou et al., 2012). In other tetrapod classes, Hox5 proteins are expressed by cervical LMC neurons and were likely co-opted in mammals for regulating PMC-specific genes. This process may have been facilitated by partial duplication of cervical segments of the spinal cord (Hirasawa and Kuratani, 2013), which may have served to allow a new MN population to utilize *Hox5* function in PMC specification, while preserving their function in LMC subtype specification. A similar strategy of co-option appears to have occurred during the development of insect nervous systems, as *Hox* genes recently have been shown to be instrumental in the development of peptidergic interneurons and leg-innervating MNs in *Drosophila* (Baek et al., 2013; Karlsson et al., 2010).

Hox Genes and the Evolutionary Diversification of Motor Effector Systems

Did changes in the profiles of *Hox* gene activity contribute to the evolutionary diversification of motor circuits? Comparisons of Hox expression patterns among limb-bearing and limbless tetrapods have provided insights into this question. Snakes evolved from limb-bearing reptiles but presumably no longer require the MN subtypes necessary for limb motility. Analysis of *Hox* gene expression in snake embryos revealed that expression of the thoracic *Hoxc9* gene is broadly extended along the rostrocaudal axis, and may account for the absence of LMC neurons (Figure 3D; Jung et al., 2014). Furthermore, the ability of the Hoxc9 protein to repress limb-innervation programs relies on an N-terminal peptide motif present only in Hox9 proteins of vertebrates bearing paired appendages. This motif acts by blocking an autoregulatory circuit activated by limb-level Hox proteins that promote high levels of Foxp1 expression in LMC neurons (Figure 3C). The repressive motif in Hoxc9 is present in both aquatic and terrestrial vertebrate species, including modern representatives of the most primitive fin-bearing vertebrates. These observations indicate that the repressive functions of Hoxc9 emerged at the time vertebrates acquired paired appendages.

Analysis of *Hoxc9* activities suggests that Hox signaling contributed to the evolution of motor systems in early vertebrates. This interpretation is surprising, given that many Hox-dependent programs, such as clustering of MNs into columnar groups and the alignment of Hox expression to specific MN subtypes, are apparently not present in bony fish such as zebrafish (Appel et al., 1995; Murata et al., 2010). One possible explanation is that the utilization of Hox signaling in MNs may be more relevant in marine species that use fins as the primary mode of locomotion, such as in batoid chondrichthyans (rays and skates). In skates, for example, the pectoral and pelvic fins develop adjacent to each other with no intervening thoracic level (Maxwell et al., 2008). Moreover, stingrays have a population of fin-innervating MNs extending over ~80 segments (Droge and Leonard, 1983). Interestingly, whole genome analysis of chondrichthyans revealed that elasmobranchs, which include skates and rays, lack the entire *HoxC* cluster (King et al., 2011). It is possible that removal of *Hoxc9* gene in batoids allowed for the extension of fin-innervating MNs along the rostrocaudal axis of the spinal cord (Figure 3D).

Evolution of the Neural Circuits Governing Locomotion

While analyses of MN specification programs have revealed important insights into how peripheral innervation patterns have evolved, locomotor behaviors in vertebrates are driven largely through assemblies of rhythmically active neural circuits residing in the brain stem and spinal cord. These networks, termed central pattern generators (CPGs), are composed of several classes of locally connected interneuron subtypes that provide the primary drive to MNs during basic motor actions. Both axial-based undulatory and limb-based ambulatory locomotion rely on CPG activities (Grillner and Jessell,

2009), and there is emerging evidence that limb CPGs evolved from co-option of preexisting undulatory motor circuits (Bagnall and McLean, 2014; Sillar et al., 2008).

The most thoroughly investigated CPG circuits in tetrapods are the locomotor networks residing within the spinal cord and the respiratory rhythm generator in the brain stem. Recent studies indicate that locomotor CPG circuits are constructed in a modular fashion, and alteration in their neuronal components can have a dramatic effect on gait characteristics. Changes in the composition and connectivity of neurons within CPGs likely contributed to evolutionary adaptations in motor behaviors.

Commissural Interneurons and Locomotor CPG Output

In tetrapods, spinal CPGs can facilitate two types of locomotor output, one that ensures coordination between left and right halves of the spinal cord in animals that walk (L-R CPGs), and a second that facilitates reciprocal activation of extensor and flexor muscles within an appendage (E-F CPGs) (Goulding and Pfaff, 2005). Among vertebrate species there is considerable diversity in how CPG circuits are organized. While bipedal and quadrupedal animals typically alternate left and right limbs during walking, most avian species and several terrestrial species (e.g., rabbits, kangaroos, bats, and desert jerboas) utilize CPGs that activate muscles in both limbs synchronously. The mode of L-R CPG operation appears to be a consequence of the activities of excitatory and inhibitory commissural interneurons (CINs) that project their axons across the midline of the spinal cord (Vallstedt and Kullander, 2013). CINs control L-R CPGs via the connections that they establish with MNs and interneurons. Hemisection of the spinal cord leads to discoordination in L-R CPGs, while preserving E-F CPG function, indicating the circuits governing limb alternation rely on CINs (Kjaerulff and Kiehn, 1996; Lanuza et al., 2004).

The activities of L-R CPGs are coordinated through two distinct CIN-driven microcircuits (Bernhardt et al., 2013). One circuit ensures L-R alternation through reciprocal inhibition of the contralateral half of the spinal cord when the ipsilateral side is active. A parallel CPG circuit facilitates synchronous activity and predominates in the absence of inhibitory CIN connections. Evidence supporting this model comes from genetic manipulations that shift the balance in excitation-inhibition ratios across the midline (Figure 4A). Eph/ephrin signaling plays an essential role in CIN guidance, where expression of *ephrinB3* in midline spinal populations prevents the crossing of excitatory EphA4-positive ipsilaterally projecting interneurons (IINs). Mutation in *EphA4* or its ligand *ephrinB3* in mice leads to inappropriate excitatory projections to the contralateral side of the spinal cord (Kullander et al., 2003). Mice lacking *EphA4* or *ephrinB3* display a hopping-like motor behavior, likely due to a shift in the balance from inhibitory toward excitatory connections with MNs. The spinal autonomy of this L-R CPG defect was confirmed using fictive locomotor assays that measured the activities of MN through ventral root recordings in isolated spinal cords (Figure 4B). Complete L-R synchrony is also observed in mice mutant for the midline attractant *Netrin1*, which disables the ability of inhibitory CINs to cross the midline (Rabe et al., 2009). Netrin signaling also controls commissural axon guidance in *Drosophila*, suggesting deep conservation in the signaling pathways ensuring communication between both halves of the nerve cord (Zarin et al., 2014).

The role of specific classes of spinal interneurons in locomotor CPGs has been examined closely in mice, and many of the cell types required in L-R CPG circuits have been characterized genetically (Arber, 2012). Interneurons originating from the p0 progenitor domain generate two types of postmitotic CIN subtypes, V0v and V0d, characterized by specific transcription factor profiles and neurotransmitter systems (Figure 4C). All V0 neurons derive from progenitors expressing the transcription factor Dbx1. This population further segregates into an excitatory population expressing Evx1 (V0v) and an inhibitory population expressing Pax7 (V0d). Mutation in *Dbx1* leads to discoordination in left-right alternation characterized by episodes of synchronous activity (Lanuza et al., 2004). Genetic silencing of both V0 populations leads to synchronous activation of both sides of the spinal cord (Talpalar et al., 2013). These genetic manipulations indicate that V0 neurons have a key role in establishing the L-R CPG spinal circuitry. Given the variety of vertebrate species capable of synchronous muscle activation, it seems likely that modification in V0 interneuron subtype distributions, and/or changes in the levels of Eph/ephrin or netrin signaling, could account for evolutionary adaptations of L-R CPG output.

Diversification of Locomotor Gaits

While genetic manipulation in L-R CPGs can transform locomotor output from walking to hopping, most animals are capable of displaying a variety of gaits that are intermediates of these extremes. Within a species, gait changes are often associated with the speed of locomotion, as well as how movements are coordinated between the forelimbs and hindlimbs. In quadrupeds, the relative phase of locomotor gaits between left and right limbs tends to shift from purely L-R alternation at slow speeds, such as walking, to more synchronized at higher speeds. Switch in gaits at different speeds appears to involve several populations of spinal interneurons. Consistent with this idea, mutations that affect the relative distribution of interneuron subtypes display phenotypes at specific locomotor velocities (Talpalar et al., 2013). For example, ablation of the inhibitory V0d populations leads to locomotor discoordination at low speeds but normal alternation at high speeds, while ablation of excitatory V0v interneurons leads to hopping only at medium and high locomotor speeds. Additional interneuron populations, including excitatory V3 domain-derived CINs and V2a-ipsilateral excitatory interneurons, play critical roles in securing left-right alternation (Crone et al., 2009; Zhang et al., 2008). These studies indicate multiple interneuron classes are involved in the maintenance and fine-tuning of CPG function (Figure 4D).

A more direct test of the role of cell fate determinants in the evolution of locomotor behaviors has emerged from analyses of the genetic determinants controlling gait patterns in horses. Certain horse breeds are capable of a special gait called pacing, where two legs on one side of the body are moved in a synchronized manner. Genome-wide association analysis identified a single transcription factor, Dmrt3, that when mutated allows for the ability to perform pacing (Andersson et al., 2012). Heterozygote mutant horses also display an alternate gait, suggesting this mutation acts as a dominant negative. In mice, Dmrt3 is expressed by a single class of dorsal (di6) spinal interneurons, and mutation of this gene causes locomotor discoordination at high locomotor speeds.

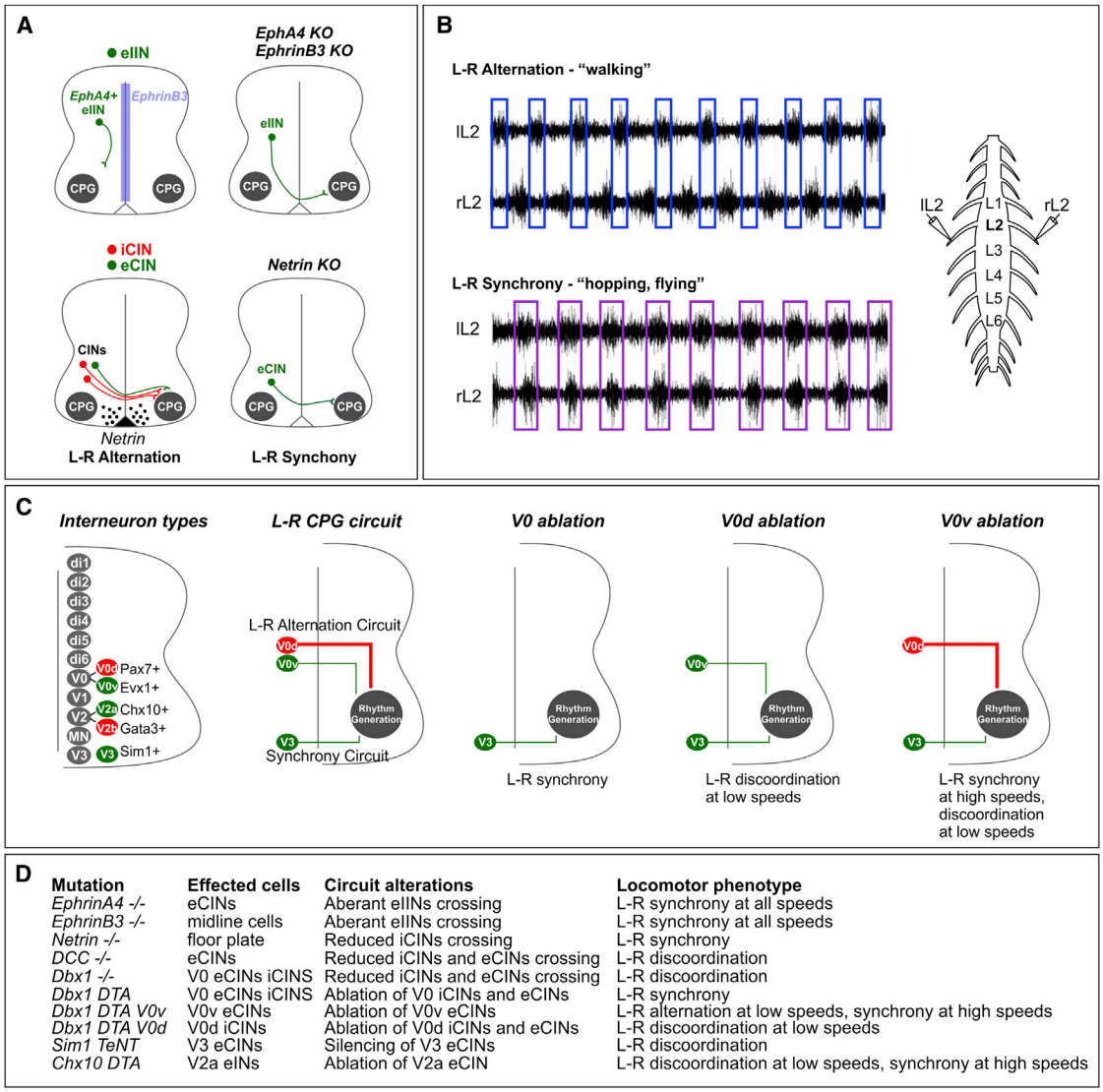

Figure 4. CPGs and Locomotor Behaviors

(A) Genetic mutations in guidance systems that lead to synchronous bilateral activation of limb-level MNs (hopping) in mice. Mutations in *EphA4* or *ephrinB3* cause multiple classes of excitatory ipsilaterally projecting interneurons (eIINs) to aberrantly cross the ventral midline. Mutation in *netrin* causes fewer inhibitory commissural interneurons (iCINs) to cross, but preserves some eCINs projections.

(B) Examples of fictive locomotor assays in mice. Ventral root recordings from lumbar level L2 showing bursts of MN activation at regular intervals. In control mice bursts recorded from left L2 (lL2) and right L2 (rL2) alternate. In *netrin* mutants both sides of the spinal cord burst in phase. Images are modified from Rabe et al. (2009).

(C) Intrinsic factors involved in CIN specification. Excitatory and inhibitory CINs are derived from multiple progenitor domains that are defined by transcription factor expression. Factors expressed by postmitotic neurons are indicated. Both V0d and V0v interneurons are derived from progenitors expressing Dbx1. Genetic silencing of V0 populations causes changes in the connections between CINs and target cells on the contralateral side of the spinal cord.

(D) Partial list of genetic manipulations that affect left-right alternation. Locomotor phenotypes described represent analysis using either fictive locomotor or behavior assays or combinations of both.

Rostrocaudal Positional Information and Locomotor Circuits

The organization of CPG circuits within the vertebrate nervous system also appears to rely on rostrocaudal positional information. Lesion studies in rats indicate that locomotor CPG circuits reside at specific rostrocaudal levels of the spinal cord. For example, the CPGs controlling hindlimb muscles extend from lower thoracic to upper lumbar levels (Kjaerulff and Kiehn, 1996). A classic set of experiments underscoring the role of rostrocaudal positional identities involved neural tube transposition experiments in chick embryos (Narayanan and Hamburger, 1971; Straznicky, 1963). When brachial (wing) levels of chick neural tube is grafted to lumbar levels, hatched chicks lose left-right alternation and instead exhibit synchronous activation of leg muscles, resulting in hopping motor behaviors. Conversely, when brachial neural tube is replaced by lumbar tissue, chicks alternate wing movements. These studies suggest that positional specification along the rostrocaudal axis is

essential for the development of CPG circuits associated with level-specific locomotor output. It is tempting to speculate that the same Hox-dependent pathways that confer MN subtype identities along the rostrocaudal axis are similarly used in local CPG circuits to establish species-specific locomotor behaviors.

Further evidence suggesting a role for *Hox* function in locomotor circuits has emerged from studies of the embryonic nervous system in *Drosophila*. Fly larvae display segmental-level-specific patterns of peristaltic locomotion that are used in exploratory behaviors. Recent genetic experiments have shown that these motor patterns can be displayed in the absence of brain function, indicating they reflect the activities of CPG circuits residing within the nerve cord (Berni et al., 2012). Larvae with combined mutations in the *Hox* genes *Ubx* and *abdA* show no peristaltic movement of abdominal segments, while ubiquitous misexpression of either of these genes extends the number of segments displaying abdominal-specific muscle contractions (Dixit et al., 2008). Although the neurons responsible for the altered behaviors have not been fully resolved, these observations implicate key roles for *Hox* genes in larval locomotion. Collectively, work in vertebrates and flies suggests that changes in rostrocaudal positional information provided by *Hox* genes can impact locomotor behaviors through modifying CPG organization.

Perspectives

Studies on the evolution and development of locomotor circuits have provided key insights into the mechanisms through which nervous systems have diversified to establish new motor behaviors. Although comparative studies in vertebrates and invertebrates provide evidence for conservation in transcription factor profiles during early neural patterning, in the future it will be informative to determine whether these ancestral relationships extend to the target genes they regulate. A recent study on the gene networks involved in segmentation of the hindbrain indicates that many of the *cis*-regulatory elements controlling the expression of *Hox* genes and their targets in mice are functionally conserved in lamprey (Parker et al., 2014). Because aspects of class features are often shared between vertebrate and invertebrate neurons, such as the neurotransmitter systems of mammalian and annelid MNs, it is plausible that, in many cases, conservation will extend to target gene regulation.

Modulation in the rostrocaudal expression profiles of *Hox* genes appears to be capable of eliciting global changes in the organization of effector neurons within locomotor circuits. These observations suggest that modulation in the expression of a small number of key regulatory factors can reorganize the structure of preexisting circuits, independent of changes in the downstream genes they regulate, or creation of new neuronal classes. However, in many cases, the development of new circuits relies on the generation of completely novel cell types. The appearance of a new *cis*-regulatory element in the *Fezf2* gene fostered the generation of corticospinal MNs in mammals (Shim et al., 2012), a population of projection neurons essential for communication between the brain and spinal cord. Changes in *cis* elements also were likely essential in the establishment of the gene regulatory network controlling neural crest lineages in vertebrates and the developmental of the peripheral nervous systems (Bronner and LeDouarin, 2012).

While this review has focused on spinal circuits controlling relatively simple locomotor behaviors, in the future it will be important to resolve how the circuits governing more refined motor tasks evolved in vertebrate lineages. Perhaps the most relevant for understanding mammalian evolution is the development of circuits controlling articulation of muscles in the hand. Recent studies have defined many of the anatomical features and functional properties of the neurons responsible for skilled forelimb movement in mice (Azim et al., 2014; Esposito et al., 2014). Given the vast number of novel motor behaviors that were enabled through the development of circuits for hand control, it will be revealing to determine how spinal and supraspinal networks evolved to establish these sophisticated motor functions.

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