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Guidance of motor axons: where do we stand?

Frédéric Clotman and Fadel Tissir

Motor neurons are among the longest projection neurons and their axons extend into the periphery following highly stereotypical pathways. Motor neurons share similar guidance molecules with other projection neurons. Hence, one challenge in the field has been to understand how the vast complexity of connections can be regulated by a relatively small number of factors. In this review, we describe the transcriptional programs and signaling pathways that guide motor axons as they navigate toward their muscle targets. In particular, we underscore how signals triggered by these pathways are integrated in time and space to increase the diversity of the steering mechanisms and improve the accuracy of axon path finding.

Address
Université Catholique de Louvain, Institute of Neuroscience, Division of Cellular and Molecular, Brussels, Belgium

Corresponding author: Tissir, Fadel (fadel.tissir@uclouvain.be)

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Introduction

Cell bodies of spinal motor neurons coalesce into longitudinal columns in the ventral horn and their axons extend along highly stereotypic pathways to control effector muscles in the periphery. Spinal motor neurons comprise somatic and visceral neurons. Somatic motor neurons directly innervate skeletal muscles of the body, whereas visceral motor neurons innervate the sympathetic ventral chain wherein they synapse onto noradrenergic neurons of the paravertebral ganglia, which regulate cardiac or smooth muscle contractions, and gland activity [1,2]. Further diversification into specialized motor neuron subsets defines and coordinates neuronal identity and axon projections.

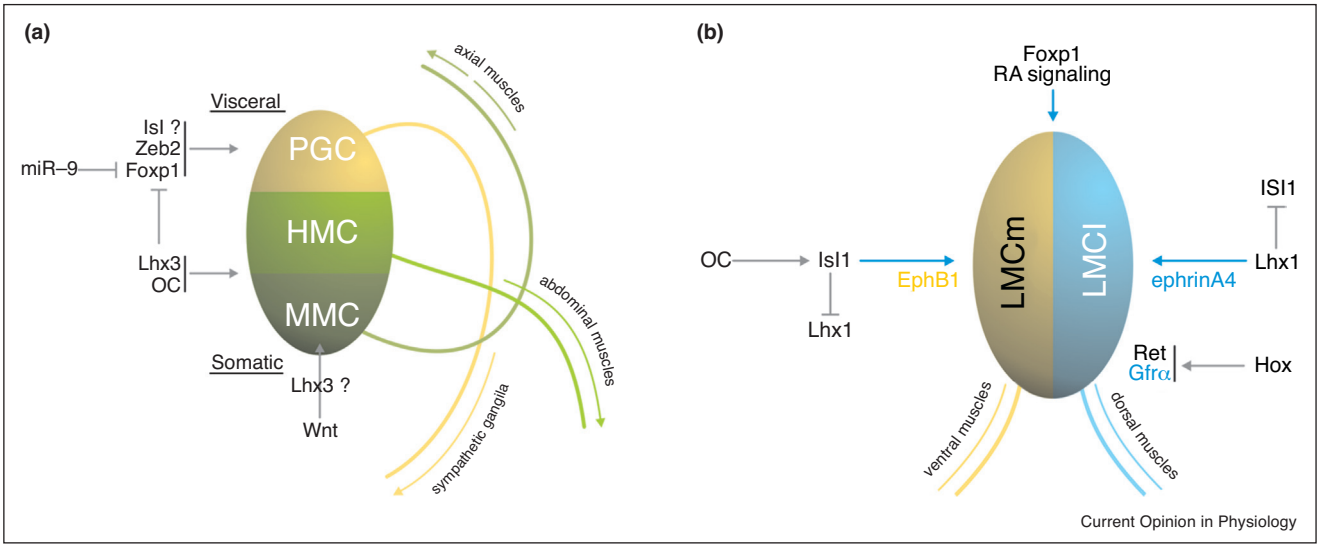
Motor neuron identity determines the repertoire of guiding cues

The transcriptional regulation of motor column formation and patterning is beyond the scope of the present article and has been extensively reviewed [1,2]. Here, only factors involved in fate decisions that directly and critically impact on motor axon guidance are summarized.

At the thoracic level of the spinal cord (Figure 1a), segregation of somatic vs visceral motor neurons directs subsequent motor axon growth toward different targets (i.e. skeletal muscles or sympathetic ganglia, respectively). The Hox cofactor Foxp1 [3,4] regulated by the miR-9 micro-RNA [5] and the 2-handed zinc finger/homeodomain protein Zeb2 [6] promote visceral motor neuron differentiation. By contrast, the Lim protein Lhx3 [7,8], a repressor of Foxp1 [3,4], and Onecut (OC) factors 1–3 [6] favor their somatic counterpart (Figure 1b). Among somatic motor neurons, Wnt4/5a/5b stimulate the production of medial motor column (MMC) neurons that innervate axial muscles, at the expense of hypaxial motor column (HMC) cells, which target abdominal muscles [9], possibly by stimulating Lhx3 expression [10]. In each motor column, change in cell fate concomitantly changes axon targeting, indicating that the identity of motor neurons determines the trajectory of their axons [4,6,7,9].

This relationship has been more extensively emphasized at limb level. Motor neurons that innervate limb muscles settle in the lateral motor columns (LMC). Production of LMC neurons requires Foxp1 [[24•]] and retinoic acid signaling [11]. A first set of LMC cells forms the medial division of the LMC (LMC_m). In the LMC_m, OC factors promote Isl1 expression [[13•]], which prevents Lhx1 transcription and targets the LMC_m axon toward the ventral limb [12]. Retinoic acid synthesis by the LMC_m neurons then promotes the production of lateral LMC (LMC_l) cells [11], which innervate the dorsal limb, and wherein Lhx1 inhibits Isl1 expression [12]. However, among LMC neurons, escaping retinoic acid signaling is necessary to generate motor neurons innervating digit muscles [13•]. ephrins/Ephs partners are the main effectors connecting LMC neuron identity to axon guidance. OC factors and Isl1 are required in LMC_m neurons for expression of EphB1 [14], which prevents axonal growth toward the dorsal limb. In mirror, Lhx1 in LMC_l cells drives ephrinA4 production [15], which inhibits progression of the growth cone into the ventral limb (see below). Furthermore, Hox proteins and their cofactors stimulate the expression of the GDNF receptors Ret and Gfrα1 [16], which cooperate with EphA/ephrinA reverse signaling to strengthen the dorsal trajectory of LMC_l axons. Subsequently, in collaboration with extrinsic signals arising from the periphery including BDNF [17] and HGF [18], unique combinations of Hox factors subdivide LMC neurons into distinct pools that each innervate a single

Figure 1



Transcriptional networks and signaling pathways determine motor neuron fate and motor axon guidance. **(a)** At thoracic levels, Foxp1, Zeb2 and possibly Isl proteins stimulate visceral motor neuron differentiation, whereas OC factors and Lhx3 favor their somatic counterpart. Among somatic motor neurons, the Wnt pathway promotes MMC fate, possibly by regulating Lhx3. **(b)** At the limb level, retinoic acid (RA) signaling and Foxp1 stimulate LMC motor neuron differentiation. Isl1 promotes the production of LMC_m neurons, which express EphB1, whereas Lhx1 favors the differentiation of LMC_l cells, which produce ephrinA4. In LMC_l neurons, Hox proteins additionally stimulate Ret and GFRα expression (see text for additional details).

appendicular muscle [19]. Hox proteins can achieve this variety of functions using specific motifs that are required, sometimes through interactions with specific cofactors, for different aspects of motor neuron differentiation (e.g. LMC induction or pool specification) [20]. Besides regulating fate decision and axon guidance, Hox cofactors of Pbx family, along with the cadherin-catenin signaling, are necessary for proper motor neuron clustering [21•]. Finally, Neuropilin (Nrp)1 and Eph/ephrin signalings contribute to the fasciculation of motor and sensory axons via surround repulsion mechanisms [22,23] in a hierarchical fashion that recapitulates the successive phylogenetic emergence of peripheral axon types in the vertebrate lineage [24•].

Exiting the central nervous system

A unique and distinctive feature of motor neurons is their capacity to send axons out of the central nervous system (CNS) and innervate muscles in the periphery. Branchiomotor and viscero-motor neurons extend axons from dorsal exit points in the hindbrain. Spinal and cranial somatic motor neurons extend axons via ventral exit points (except for the trochlear and spinal accessory nerves [25]). In mice lacking the chemokine Cxcl12 (formerly known as stromal-derived factor-1) or its receptor Cxcr4, a subset of ventral motor axons is rerouted dorsally within or after exiting the spinal cord [26]. Cxcl12 is expressed by mesenchymal cells flanking the spinal cord and brainstem, whereas Cxcr4 is produced by ventral motor neurons. Whether the Cxcl12/Cxcr4 system behaves as chemoattractant or modulates the activity of other cues remains unclear [27]. Zebrafish with mutation in the Plexin A3 exhibit errors in axons' exit point [28], indicating that other mechanisms are also at play. Recent data additionally suggest that signals produced by meninges of the spinal cord may attract motor axons toward the periphery and allow their exit from the neural tube, whereas interneuron axons would be repelled and confined inside the CNS [29].

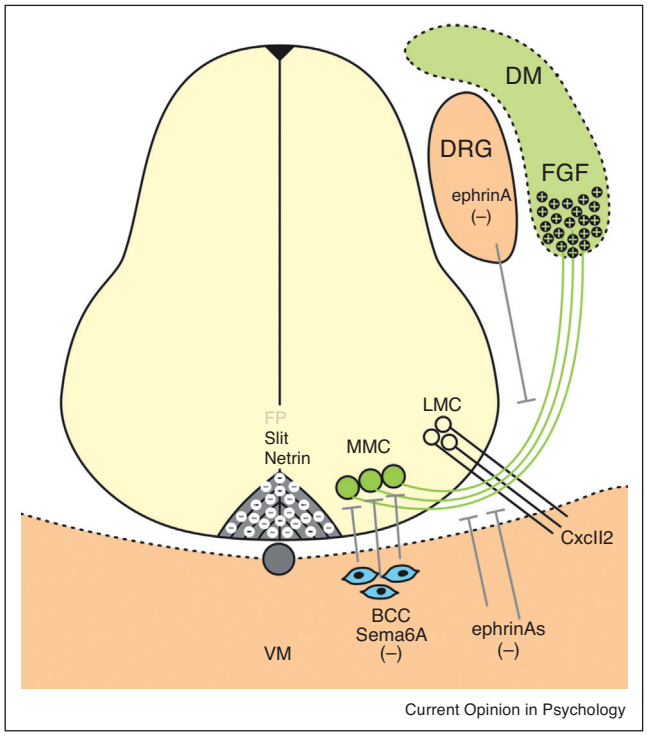
A corollary issue of motor axons guidance to the periphery is how to prevent cell bodies from leaving CNS? Even though the molecular mechanisms are not fully understood, a role for the boundary cap cells has been clearly demonstrated, as their ablation results in translocation of cell bodies along axons and ectopic localization of motor neuron nuclei in peripheral nerves [30]. Boundary cells secrete repulsive cues as semaphorins (Sema6A and Sema3s) that might bind to Nrp2 and PlexinA1/A2 [31] receptor complexes on motor neurons. Yet, intricate mechanisms must discriminate the axons from cell bodies and ensures that Sema(s) repel only the latter. One possibility is that boundary cells associate with and act on motor neurons of the ventral root only when axons have left the spinal cord and extended distally beyond the exit point [32]. Another possibility is that the repulsive action of Sema is specifically 'silenced' in motor axons by interactions with other signaling systems such as Cxcl12/Cxcr4. In support of interactions between guidance systems is the fact that axons lacking Cxcl12 or Cxcr4 invade

the floor plate and DRG despite the presence of several repellents in these territories (e.g., Netrins, Sema(s), Slits, ephrinBs in floor plate, ephrinAs in DRG) [26]. Recent observations suggest that the position of motor neurons along the dorso-ventral axis is regulated by a balanced activity of attractive Netrin/DCC and repulsive Slit/Robo signalings regulated by Isl proteins [33•] (Figure 2).

Guidance of MMC axons

As soon as MMC axons leave the spinal cord, they come across a first decision point where they turn dorsally toward axial muscles. MMC axons seem to be attracted by their targets as ablation of the target muscles reorients their trajectory [34]. This chemoattractive activity is mediated by FGF Receptor 1 (FgfR1) expressed by MMC neurons and FGFs produced by the dermomyotome [7]. MMC axons are also repelled from inappropriate areas, such as the limb and DRG [35]. Whether FgfR1 and EphAs signalings work cooperatively or independently to elicit attraction and repulsion awaits further investigations. Nevertheless, FgfR1 and EphA4 are component of a hetero-signaling complex and share downstream effectors such as ephexin1, a guanine nucleotide exchange factor for Rho-mediated axon repulsion [36]. Furthermore, these receptors were shown to potentiate each other's signaling [37]. Sema3A is another repulsive cue released from the DRGs and may contribute to guidance of MMC axons, even though MMC and their target muscles also express Sema3A. The precise spatio-temporal regulation of Sema3A/Nrp1 (e.g. the coexpression of Sema3A and Nrp1 by MMC, which restricts the availability of receptor on the growth cone) might fine-tune the response of MMC axons to Sema(s) at each choice point [38] (Figure 3).

Figure 2

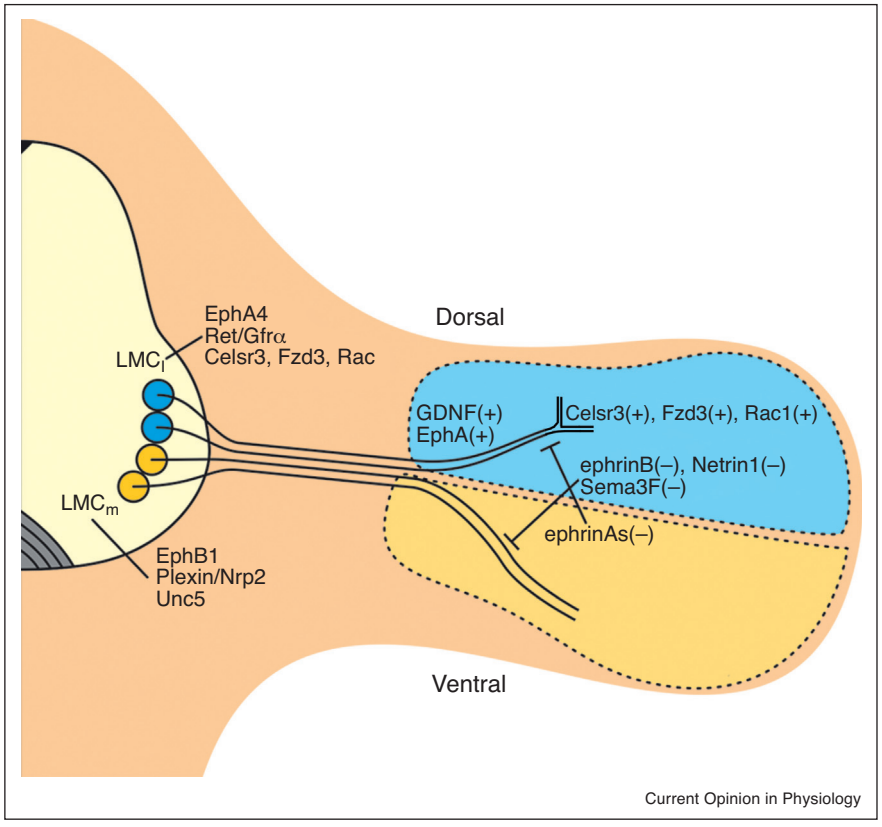


Outgrowth of motor axons from the spinal cord and guidance of MMC axons. Motor neurons are initially repelled from the midline by Slit/Robo and attracted ventrally toward the ventral exit point by Netrin/DCC. Axons are also attracted to the periphery by the chemokine Cxcl12 expressed in territories flanking the neural tube which binds to its cognate receptor Cxcr4 on motor axons. Cell bodies are confined to the CNs by Sema6A expressed by boundary cap cells (BCC). Upon leaving the spinal cord, axons of MMC neurons are channeled dorsally by repulsive ephrinAs from dorsal root ganglia (DRG) and ventral mesenchyme (VM). They are also attracted by fibroblast growth factors (FGF) 4/8 which bind to FgfR1 on MMC axons. Axons of LMC_l are insensitive to ephrinAs and extend ventro-laterally to the limb. *Abbreviations*: DM, dermomyotome; FP, floorplate; VM, ventral mesenchyme.

Guidance of LMC axons

After leaving the spinal cord, axons of LMC neurons ignore the dorsal turning point (a typical feature of MMC axons) and grow ventrally to the base of the limb. Axons are redistributed within plexuses so that they emerge as two main fascicles. The first selects a dorsal trajectory where most extensor muscles develop, whereas the second extends ventrally toward the developing flexor muscles. This dorsoventral segregation is exquisitely regulated and mirrors the patterning of LMC. Motor neurons of the LMC_l innervate dorsal limb muscles, whereas those of the LMC_m innervate the ventral limb muscles [39]. Manipulating the dorsoventral patterning of the limb results in rerouting of motor axons so that innervation of extensor muscles by LMC_l and flexors by LMC_m axons is preserved. This strongly suggests that signals located in the proximal limb steer LMC axons [15]. Chief among these (signals) is the ephrin/Eph system. EphrinA ligands produced by ventral limb mesenchyme bind to EphA4 receptors repelling LMC_l axons from the ventral limb [15,40••,41,42]. EphAs are also expressed in the dorsal limb where they serve as 'ligands' for ephrin-As expressed by LMC_l axons in the so called 'reverse signaling', which, contrary to the forward signaling, potentiates growth of motor axons and attracts LMC_l dorsally. Bidirectional interactions occur thanks to (i) the differential distribution of EphAs and ephrinAs in discrete sub-regions of the membrane fostering interactions in *trans* (i.e. with ligands on different cells) [43••], and (ii) *cis*-attenuation mechanisms such as the proteolytic cleavage of the EphA4 ectodomain in the limb mesenchyme, which reduces the effective concentration of ephrinAs and the EphA4-mediated forward signaling in the motor axons [44]. Similarly to the ephrinA/EphA signal, which plays a key role in guiding LMC_l axons, the ephrinB/EphB guides LMC_m axons to the ventral limb. LMC_m neurons express EphB receptors, which facilitate repulsion from ephrinB2 ligands in the dorsal limb [14]. In mouse, mutations in ephrinB2, or EphBs genes disrupt the trajectory of LMC_m axons that select an aberrant dorsal trajectory.

Figure 3



Guidance of the LMC axons. Axons of LMC_m neurons (yellow) express Nrp2 and EphB1 are repelled by Sema3F, Netrin1, and EphrinB in the dorsal limb. They select a ventral trajectory toward muscles in the ventral limb. Axons of LMC_l neurons (blue) are guided to the dorsal limb muscles by a combination of repulsive and attractive signals. At the base of the limb, ephrinAs in the ventral mesenchyme trigger 'forward' repulsive signaling through axonal EphA4. EphAs expressed in the dorsal limb trigger an attractive 'reverse' signaling through axonal ephrinAs in cooperation with GDNF, which binds to Ret and/or Gfrα1 on LMC_l axons, enforcing their dorsal choice. More distally, Celsr3 and Fzd3, which are members of the Wnt/PCP, and the actin cytoskeleton regulator Rac1 are required for extension of LMC_l axons.

Other guidance systems act in parallel to or in collaboration with ephrin/Eph signaling to improve the reliability of dorsoventral choice but also the extension of motor axons in the distal limb. These systems include the Sema3/Nrp signaling which contributes to LMC axon navigation [45]. Nrp2 receptor expressed by LMC_m neurons mediates the repulsive signaling activated by Sema3F in the dorsal limb to direct LMC_m axons ventrally. Netrin/Unc5 acts in synergy with the ephrinB/EphB to guide LMC_m axons [46]. GDNF is expressed by the limb mesenchyme and binds to Gfrα1 on LMC_l axons in a Ret-dependent manner. On the other hand, Ret is also required to the EphA/ephrinA reverse signaling. Hence, it behaves as a shared co-receptor for multiple ligands that altogether secure the dorsal targeting of LMC_l axons [40••,47••]. The ephrinA/EphA, but not ephrinB/EphB, signaling is mediated by the Rho-GTPase-activating protein α2-chimaerin [48].

In the dorsal hindlimb, MMC_l axons, which have segregated from LMC_m and form the peroneal nerve, encounter another choice point and select either the deep (ventral) or superficial (dorsal) component of the peroneal nerve. Further extension of axons from this choice point is regulated by the Wnt/Planar cell polarity (PCP) genes Celsr3 and Fzd3 [49,50•,51], as well as Rac1, a regulator of the actin cytoskeleton [52•]. In mice bearing null mutations in these genes, LMC_l axons fail to grow distally and stall at the branching point [49,52•,53]. Importantly, the Wnt/PCP signaling is also involved in neuromuscular junction formation [54], suggesting that the final extension of motor axon and nerve-muscle recognition might be mechanistically linked.

In summary, studies of motor system development have revealed that the large diversity of axon guidance mechanisms is generated through context-dependent expression and use of a relatively low number of molecules that cooperatively steer axons at different checkpoints.

Conflict of interest statement

Nothing declared.

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