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Review

Eph and ephrin signaling: Lessons learned from spinal motor neurons

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ABSTRACT

In nervous system assembly, Eph/ephrin signaling mediates many axon guidance events that shape the formation of precise neuronal connections. However, due to the complexity of interactions between Ephs and ephrins, the molecular logic of their action is still being unraveled. Considerable advances have been made by studying the innervation of the limb by spinal motor neurons, a series of events governed by Eph/ephrin signaling. Here, we discuss the contributions of different Eph/ephrin modes of interaction, downstream signaling and electrical activity, and how these systems may interact both with each other and with other guidance molecules in limb muscle innervation. This simple model system has emerged as a very powerful tool to study this set of molecules, and will continue to be so by virtue of its simplicity, accessibility and the wealth of pioneering cellular studies.

Contents

1. Introduction

Since its discovery in 1987 [1], the Eph tyrosine kinase receptor has been intensively studied revealing its role in the relay of a multitude of extracellular signals. Eph and ephrin interactions in the developing nervous system influence many processes, including rhombomere formation [2,3], axon guidance [4–6] and synaptogenesis [7,8], highlighting their versatile ability to activate wide-ranging cellular responses. Arguably the best-studied of these functions is the contact-dependent Eph/ephrin-mediated guidance of axons that capitalizes on the complex distribution of Ephs and ephrins to provide the specificity necessary for the formation of precise connections. Eph receptors are divided into A and B classes, that differ mainly in their affinities for specific ephrin-A or B ligands, molecules tethered to the cell membrane by either a glycosylphosphatidylinositol tail or a transmembrane domain and cytoplasmic tail, respectively (Fig. 1, [9,10]). In addition to "forward" signaling from ephrins to Ephs (ephrin:Eph), another layer of complexity of Eph/ephrin function is added by "reverse" signaling or the ability of ephrin ligands to transduce signals from the Eph receptors (Eph:ephrin) [3,11]. Given that these events can also occur between apposing cells (i.e. in *trans*) [12], and in the plane of the same membrane (in *cis*) [13,14], what is the logic of Eph and ephrin signaling

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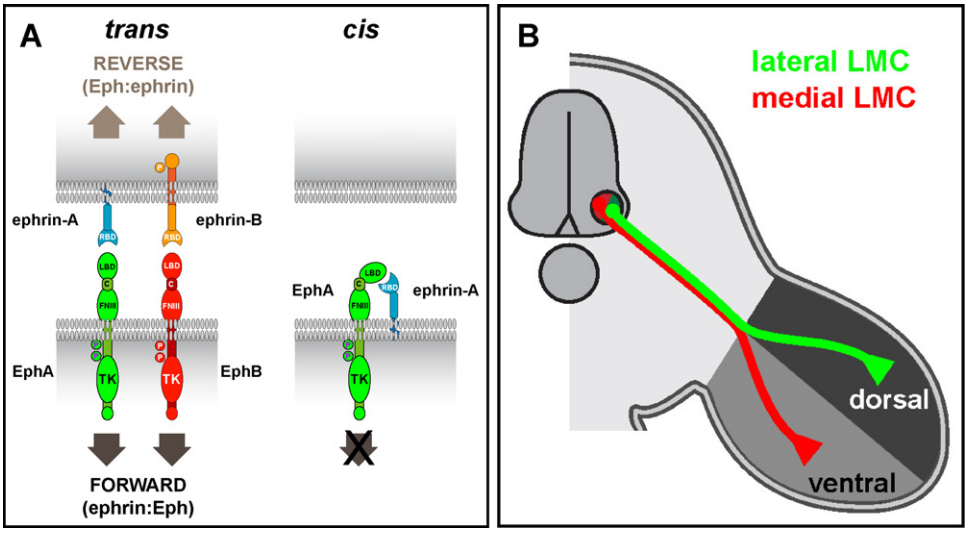

Fig. 1. Eph/ephrin signaling and the innervation of LMC spinal motor neurons to the limb.
(A) Binding of Ephs and ephrins in *trans* can induce bi-directional signaling in Eph (forward signaling) [12] and ephrin (reverse signaling) expressing cells [3,11]. Co-expression of Ephs and ephrins in the same cell can lead to binding in *cis*, attenuating Eph function [13,54].
(B) LMC spinal motor neurons innervate muscle targets in the limb and are subdivided into lateral and medial divisions. The lateral and medial LMC axons arrive at the limb mesenchyme and make an invariant and binary decision into dorsal and ventral limb nerves, respectively [16,17].
Figure modified from [54].

in axon guidance? How can these molecules accurately specify the wiring of so many neuronal circuits?

Many insights into these questions have been gleaned from the visual system (reviewed in [15]), where the topographic innervation of the tectum/superior colliculus is largely dependent upon Eph and ephrin signaling. However, recently, the axon pathways formed by developing motor nerves in the vertebrate limb [16] have begun emerging as a model system that is considerably expanding our understanding of Eph/ephrin signaling and its contribution to nervous system development. Lateral motor column (LMC) motor neurons that innervate the limb exist in two divisions (medial and lateral) that can be further subdivided into motor pools that innervate individual muscles [17]. LMC axons exit the spinal cord, traverse the anterior somite and other axon guidance decision points, then diverge at the base of the limb: lateral LMC axons select the dorsal limb nerve while medial axons select the ventral limb nerve [16]. These nerves continue branching, with bundles of axons from specific motor pools innervating individual limb muscles [16].

In this review, we will focus on Eph/ephrin signaling in LMC motor axon guidance. Studies of this system have revealed roles for forward ephrin: Eph signaling at multiple decision points, as well as in the topographic innervation of musculature (Fig. 2, [4,18–22]). Additionally, this model circuit has been used to characterize both *cis* and reverse modes of Eph/ephrin interactions, how these proteins may be regulated by electrical activity, and how this activity might alter innervation of muscles via regulation of Eph/ephrin signal transduction.

2. The initial trajectory of spinal motor axons

Shortly after their exit from the ventral root of the spinal cord, motor axons are channeled into the anterior portion of somites, giving rise to the segmented appearance of peripheral nerves [23,24]. *In vitro* observations of motor axons and sclerotomal cell interactions have suggested that the contact-dependent repulsion by the posterior sclerotome might be responsible for this restriction [25,26]. Among several contact repellents enriched in the posterior sclerotome are ephrin-B1 and ephrin-B2 while their EphB2 receptor is expressed by motor axons. Pre-clustered ephrin-Bs repel motor axons *in vitro*, providing the first evidence for ephrin-B:EphB signaling as a contact repulsive cue for spinal motor axons [22]. Surprisingly, *EphB2*/*EphB3* double knockout or *ephrin-B2* null mice show no defect in spinal nerve segmentation [22,27,28] hinting at a functional redundancy in this system; an idea supported by expression of Eph receptors in spinal motor axons, and ephrin-As in somites [5,20,29,30]. Additionally, other contact repellents, including semaphorins [31], peanut agglutinin-binding glycoproteins [32,33], GPI-linked T-cadherin [34], and F-spondin [35] are expressed in the posterior somites and repel motor axons, thus possibly contributing to motor axon guidance.

In addition to this early evidence of chemorepellent activities, some have proposed that chemoattractants, such as hepatocyte growth factor and scatter factor, as well as attractive Eph:ephrin reverse signaling, may also play a role in spinal nerve segmentation [22,36–38]. The G-protein couple receptor CXCR4, highly expressed in motor neurons, and its cytokine ligand CXCL12, expressed in the mesenchyme surrounding the spinal cord, regulate the outgrowth of spinal motor axons exiting the ventral root [39]. Shirasaki et al. [40] also demonstrated that fibroblast growth factors (FGFs) are selective chemoattractants for motor neuron subsets in this context [40]. Since Eph receptors can be coupled with both CXCR4 [41] and FGF receptors (FGFRs) [42], CXCL12:CXCR4 and/or FGF:FGFR signaling interaction with ephrin:Eph signaling in motor axons is an interesting possibility. If this interaction is additive then the motor axon trajectories of mice lacking both Eph receptors and CXCR4 (or FGFRs) might be more severely disrupted than those of CXCR4 null mice. Additional attractive cues such as netrin found in the peripheral targets of motor neurons could also mediate the proper trajectory of spinal motor axons [43–45]. Interestingly, ephrin-A5 in the anterior sclerotome has been demonstrated to attract subsets of EphA4-expressing motor axons, implying an alternative way of forward ephrin:Eph signaling to mediate spinal nerve segmentation in attractive fashion [46].

3. LMC axon guidance in the limb

3.1. Concurrent trans-forward and trans-reverse signaling

LMC axons execute a ternary trajectory choice at the base of the limb: lateral LMC axons grow into the dorsal limb, most medial