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Dispatches

Axon Guidance: Ephrins at WRK on the Midline

Recent findings indicate that the embryonic motor neurons act as gatekeepers to regulate midline crossing during development of the nematode *Caenorhabditis elegans*. The newly identified protein WRK-1 and ephrins cooperate to prevent longitudinal axons from crossing the midline.

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At least 600 million years ago a great evolutionary innovation occurred that established bilateral symmetry, a type of body plan that is organized around a midline with left–right symmetry. This fundamental shift in organization allowed for anterior–posterior and dorsal–ventral specializations, permitting the development of the central nervous system. The bilaterally symmetric body plan required a communication system to coordinate the two sides of the body, meaning that axons had to cross the midline of the central nervous system. Thus, it is no wonder that the nervous system midline has long fascinated developmental neuroscientists.

As developing axons reach the midline they must decide whether to turn and migrate longitudinally along the same side or to cross the midline and migrate along the opposite side. This decision is crucial because it underlies the ability of the nervous system to coordinate events that occur on each side of the body. Studies of *Drosophila* and mice have revealed that axon crossing is regulated by specialized midline cells that act as gatekeepers (Figure 1A,B). These midline cells produce a variety of cues that can prevent midline crossing. For instance, in *Drosophila*, the midline glia cells secrete Slit, a guidance cue that can prevent axons from crossing the midline [1]. Likewise, in mice, the floorplate cells secrete Slit to prevent axons from crossing the midline [2]. In both organisms, longitudinal axons are repelled from Slit through the action of Robo, a receptor for Slit that is expressed on the surface of the axons [3]. Robo can be temporarily suppressed to allow for midline crossing [4–6].

The simple anatomy and powerful genetic techniques available in the nematode *Caenorhabditis elegans* are ideal for studying midline guidance. Our understanding of the *C. elegans* midline has been limited, however, by the inability to clearly establish which cells act as gatekeepers to regulate midline crossing. A paper published recently in *Current Biology* [7] reports that the embryonic moterneurons (eMNs) act as gatekeepers to regulate midline crossing in *C. elegans* (Figure 1C). In addition to identifying gatekeeper cells, the authors also identify a novel midline cue, an immunoglobin superfamily protein known as WRK-1. They report that WRK-1 is expressed by the gatekeeper cells and acts with the ephrins to inhibit axon crossing. The Eph tyrosine kinase VAB-1 functions in the longitudinal axons as a receptor for ephrins and WRK-1.

A key implication of this study is that neither WRK-1 nor the ephrins can independently activate VAB-1, rather it is a joint effort. There are several ways in which WRK-1 could cooperate with the ephrins to activate VAB-1. It is possible that VAB-1 activation requires simultaneous binding to both

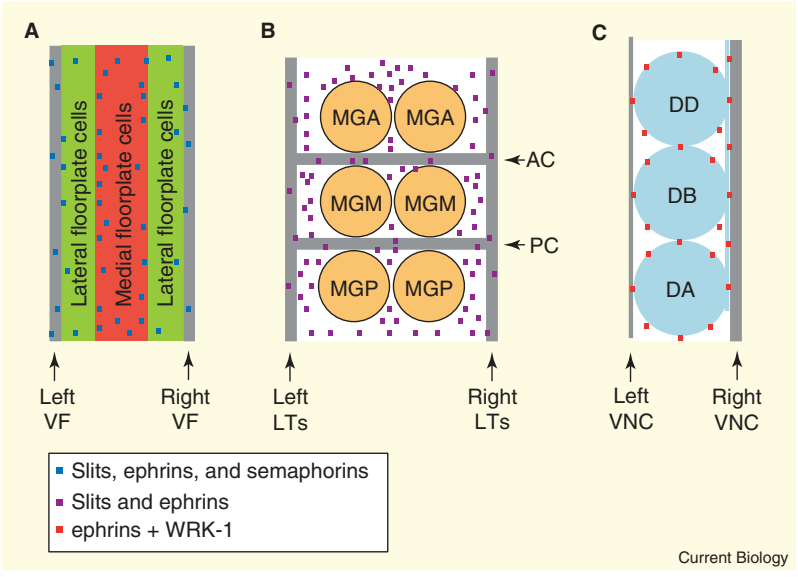

Figure 1. Specialized midline cells act as gatekeepers to regulate midline crossing in vertebrates and invertebrates.

(A) In mice, Slits, ephrins, and Semaphorins are secreted by the floorplate cells. These cues prevent axons that are migrating in the ventral funiculus from recrossing the midline [2]. (B) In *Drosophila*, Slits and ephrins are secreted by the midline glia cells: MGA, MGM and MGP. These cues prevent axons that are migrating in the lateral tracts from crossing or recrossing the midline [1]. (C) In *C. elegans,* the ephrins and WRK-1 are tethered to the surface of the embryonic motor neurons: DD, DB, and DA [7]. These cues prevent axons that are migrating in the ventral nerve cords from crossing the midline. VF, ventral funiculus; LTs, lateral tracts; AC, anterior commissure; PC, posterior commissure; VNC, ventral nerve cord. (*Drosophila* midline diagram was adapted from [16].)

WRK-1 and ephrins. Alternatively, WRK-1 might modulate the presentation of the ephrins. Although the precise mechanism of WRK-1 requires further investigation, its role in ephrin signaling should stimulate further research into the role of its vertebrate homolog, a protein known as MAM domain-containing glycosylphosphatidylinositol anchor 1 (MDGA1). In addition to being expressed in floor plate cells, MDGA1 is expressed in subsets of neurons throughout the nervous system. In the cortex, MDGA1 is expressed in an area and layer specific manner [8]. Likewise, MDGA1 is expressed within a specific motor column in the spinal cord [9]. While these expression patterns are very provocative and suggestive of a role in axonal patterning, a role for MDGA1 in axon guidance has not yet been established.

One of the advantages of studying the *C. elegans* ventral midline is that its anatomy is simpler than that of *Drosophila* or mouse, allowing the spatial relationships of cells and axons to be easily observed. For example, Boulin *et al.* [7] used electron microscopy to observe the relationship between the longitudinal axons and the eMNs. They found that the longitudinal axons grow in direct contact with the eMNs. If the eMNs repel the longitudinal axons away from the midline, how are the longitudinal axons able to grow in contact with the eMNs?

An attractive hypothesis is that the migratory path of the longitudinal axons is determined by the relative balance of signals that promote adhesion and signals that inhibit adhesion. Adhesion can be modulated by effecting opposite changes in the phosphorylation state of the focal adhesion kinase (FAK). For example, activation of the Eph receptor can cause dephosphorylation of FAK to inhibit adhesion [10], while activation of DCC/UNC-40 or the integrins can cause phosphorylation of FAK to promote adhesion [11–13]. The longitudinal axons might simultaneously receive adhesion-inhibiting signals from the eMNs and adhesion-promoting signals from the other axons, the epidermis, or the flanking muscle cells.

To allow the longitudinal axons to migrate in contact with the eMNs, the adhesion-inhibiting signals from the eMNs might be countered by the adhesion-promoting signals from other cells. The longitudinal axons would be unable to depart from the nerve tract to cross the midline, because this would cause a loss of the adhesion-promoting signal and the inhibitory signals from the eMNs would prevent further migration. This hypothesis would also account for the crossing defects that are seen with the loss of adhesion-promoting proteins such as INA-1/integrin [14] or various extracellular matrix molecules [15], where the resulting imbalance between adhesion-promoting and adhesion-inhibiting signals can result in midline crossing defects.

While midline guidance has been studied for many years, the study of this process in *C. elegans* can lead to new insights. For example, the role of WRK-1 in ephrin signaling was observable in *C. elegans* because of powerful genetic analysis and the ability to follow midline crossing with single axon resolution. These findings might readily be transferable to vertebrate models, as MDGA1 is expressed by floor plate cells [9], the gatekeepers of midline crossing in vertebrates. Meanwhile, future studies of midline crossing in *C. elegans* are likely to reveal further insights into this important process.

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