Livin' On The Edge: Glia Shape Nervous System Transition Zones

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Abstract

The vertebrate nervous system is divided into two functional halves; the central nervous system (CNS), which includes the brain and spinal cord, and the peripheral nervous system (PNS), which consists of nerves and ganglia. Incoming peripheral stimuli transmitted from the periphery to the CNS and subsequent motor responses created because of this information, require efficient communication between the two halves that make up this organ system. Neurons and glial cells of each half of the nervous system, which are the main actors in this communication, segregate across nervous system transition zones and never mix, allowing for efficient neurotransmission. Studies aimed at understanding the cellular and molecular mechanisms governing the development and maintenance of these transition zones have predominantly focused on mammalian models. However, zebrafish has emerged as a powerful model organism to study these structures and has allowed researchers to identify novel glial cells and mechanisms essential for nervous system assembly. This review will highlight recent advances into the important role that glial cells play in building and maintaining the nervous system and its boundaries.

Introduction

The vertebrate nervous system is a remarkably complex organ that consists of an intricate network of distinct populations of neurons and supporting glial cells that form neural circuits that allow for organismal survival. This elaborated system allows for peripheral sensory input and its ultimate central integration as well as the generation and fine-tuning of motor output. Communication between the central and peripheral nervous systems (CNS and PNS, respectively) occurs via peripheral nerves, which are composed of motor and/or sensory axons and their associated glia that cross between the two halves of the nervous system via specialized structures known as transition zones (TZ).

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In the early nineteenth century, the Scottish surgeon Charles Bell and the French physiologist François Magendie reported from their independent observations the separation of sensory and motor nerves into and out of the spinal cord, disproving the previous belief that spinal ventral nerve roots were a mix of sensory and motor axons capable of signaling in both directions [1]. We now know that in the vertebrate spinal cord, developing motor neurons migrate to reach their final destination and extend their axons into the periphery through permissive gaps in the spinal cord margins known as motor exit point (MEP) TZs, while their cell bodies remain in the CNS. Conversely, sensory afferent axons derived from sensory neurons in the PNS enter the spinal cord in distinct locations known as dorsal root entry zones (DREZ), which are also characterized by gaps in the basal lamina of the spinal cord [2,3]. These transition zones reflect a selectively gated boundary between the two halves of the nervous system, segregating not only neurons and axons, but also central and peripheral glia.

In the CNS, oligodendrocytes myelinate axons, while Schwann cells in the PNS perform the same task along peripheral axons [4,5]. Oligodendrocytes were first described in 1921 by the neuroanatomist Pío del Río Hortega and Schwann cells were named after the anatomist and physiologist Theodor Schwann in 1871 [6,7]. Both were identified with the development of pioneering histological staining methods still used today. While these myelinating glial cells have been extensively studied by neuroscientists for over a century, the first studies dedicated to understanding the boundary between the CNS and PNS only emerged in the late 1960s, with the invention of transmission electron microscopy (TEM), a nanometer-scale resolution technique developed after the end of World War II.

How a selective barrier allows pathfinding axons to freely cross, while neuronal cell bodies and select populations of highly migratory glial cells segregate to their respective half of the nervous system, is intriguing, but unknown. Elucidating the mechanisms that mediate how axons and glia travel across TZs is a fascinating developmental question that also has implications when considering how circuits reconnect and rewire after injury. In the injured adult mammalian nervous system, damaged neurons attempt to regrow their axons to rebuild functional circuits. Peripherally, this regrowth is mostly successful and motor and sensory axons can regrow and reinnervate target organs. However, regrowth of sensory axons into the CNS fails [8]. The disparity between the CNS and PNS in terms of self-repair ability is particularly interesting when you consider that regrowth fails when axons needs to cross through TZs. Both developmentally and after injury and disease, these specialized transition zones have recently spurred many studies which shed light on the cellular mechanisms responsible for TZ development, maintenance and involvement in disease [9–16]. However, the molecular mechanisms that mediate these cellular behaviors are still poorly understood.

In this article, we review new insights into the cellular, molecular and functional aspects of spinal cord TZs, including mammalian boundary cap (BC) cells as well as recent findings that raise the hypothesis that BC cell function may be covered by a combination of different subtypes of glia in zebrafish that include MEP and perineurial glia [11,12,15].

Glia orchestrate the development of the nervous system at TZs

In mammals, an astrocytic barrier is generally found at the TZs between the CNS and the PNS (Figure 1). This barrier, consisting of a thick layer of astrocytic endfeet, is continuous with the glia limitans that covers the surface of the spinal cord but has perforations, allowing axons to cross [2]. Following irradiation of the spinal cord, Schwann cells (SC) are able to pass through the damaged glia limitans (Figure 2c). Additionally, remyelination by SCs in the damaged CNS only occurs in restricted areas where astrocytes are absent, strongly suggesting that astrocytes and SCs repel each other [17,18]. However, oligodendrocyte (OL) and SC intermixing is never seen in normal animals [19]. In line with this, the first electron microscopy ultrastructural study of a rat cranial nerve TZ described the transitional region along myelinated axons as an abrupt change between central OLs and peripheral SCs [8]. While this study did not demonstrate the existence of a cell type other than OLs or SCs, ultrastructural and molecular studies have recently shed light on novel glial populations that reside at TZs and mediate the selective segregation of cells observed under normal conditions [20,21].

Boundary cap (BC) cells are neural crest derivatives that migrate along the outer edge of the neural tube and are thought to transiently reside in clusters at the TZs between the CNS and PNS, precisely at the sites where axons exit and enter the CNS (Figure 2a) [22]. Electron microscopy studies revealed that BC cells prefigure the DREZ, but were never detected at the presumptive MEP TZ prior to motor axons exiting the ventral spinal cord. They were only observed after axons left the spinal cord and were at a significant distance away from the MEP TZ [21]. Analysis of the temporal and spatial pattern of expression of the zinc finger transcription factor *Krox-20* (also known as *Egr2*) during murine development revealed the presence of an early group of *Krox20*-expressing neural crest cells that stopped at both the DREZ and MEP TZ [20,23]. *Krox-20* is first expressed in rhombomeres 3 and 5 and in early neural crest cells and its expression persists in neural crest-derived SCs and BC cells [20]. This molecular study concluded that BC cells arise from the neural crest at embryonic day 10.5 (E10.5) and migrate along the neural tube to reach the MEP where *Krox-20* expression is detected in BC cells [24,25].

In fish and mammals, *Krox-20* plays a master role in SC development and function, and in its absence, SCs do not progress past the pro-myelinating stage and do not myelinate axons after radial sorting [26,27]. In spite of being the most widely accepted marker of BC cells, *Krox-20* might have a different outcome in these cells, as BC cells do not initiate myelination but generate SCs, which in turn, are capable of myelinating axons. Functionally, BC cells play an essential role in spinal motor nerve development (Figure 2b). Before they become Schwann cells at the MEP, DREZ and in the dorsal root ganglia (DRG), BC cells in mammals also give rise to nociceptive neurons, satellite glial cells and terminal glia, and above all, they act as a gate-keeper [9,10,13,24,28]. Surgical and genetic ablation of BC cells results in ectopic motor neuron cell bodies and CNS glia mispositioned along ventral roots (Figure 2b) [9,10,13,29]. Vermeren *et al.* showed that ablation of neural crest cells, including both BC cells and SCs, performed by surgically removing the dorsal half of the neural tube of E2 chick embryos, resulted in the abnormal presence of motor neurons along ventral nerve roots [9]. They also stated that the segregating function lost after neural crest cell

ablation was due to the loss of BC cells, as conditional genetic ablation of *Krox-20*-expressing BC cells in mouse embryos, obtained by targeting diphtheria toxin to the *Krox-20* locus, also resulted in the emigration of motor neurons from the spinal cord to the root [9]. However, the potential presence of ectopic CNS glia along the motor nerve was not examined in this study and this genetic manipulation also affected neural crest-derived Schwann cells. In a more directed BC ablation study, Coulpier *et al.* reported that the inactivation of *Krox-20* in mouse resulted in oligodendrocytes and astrocytes invading both dorsal and ventral nerve roots, and in oligodendrocytes ultimately myelinating peripheral axons [13]. In the same study, the authors also showed a transient presence of ectopic motor neurons along the nerve root of the Schwann cell depleted mice at E12.5 but not at E17.5. Regrettably, the authors did not look at the motor neuron phenotype in more depth and their work did not lead to a hypothesis that explained the role of *Krox-20* in motor neuron segregation at TZs.

Although neural crest-derived BC cells exist and have the same gating role in birds and mammals, they have not been observed, *per se*, in fish. However, motor neuron cell bodies are perfectly segregated to the spinal cord in fish as well, and various subpopulations of glial cells beautifully orchestrate spinal nerve development, architecture and maintenance (Figure 3a). Several lines of evidence suggest that distinct CNS-derived glial populations work jointly to ensure TZ segregation in zebrafish [11,14–16].

Perineurial glia, which also exist in mice, are *nkx2.2a*-expressing cells that originate from the lateral floor plate (p3 domain) of the spinal cord and migrate into the PNS via MEP TZs (Figure 3a). Ultimately, these cells migrate along motor axons, ensheath the motor nerve by tightly associating with one another via tight-junctions to ultimately form the perineurium, a protective layer that ensheaths both axons and Schwann cells into fascicles [11,14]. Experiments in zebrafish show that perineurial glia prefigure motor axon exit points and form a selective barrier that confines motor neurons to the CNS. *Nkx2.2a* loss of function leads to ectopic motor axon exit from the spinal cord and motor neuron cell bodies escaping the CNS (Figure 3c) [11]. Consistent with these findings, in mice lacking *Nkx2.2*, ectopic motor neuron cell bodies were found outside of the spinal cord along peripheral axons and the latter were defasciculated [14]. Together, these data highlight the crucial function that CNS-derived perineurial glia have in motor nerve development and maturation.

Not long ago, another fundamental CNS-derived glial cell type was described in the PNS, regularly spaced at each MEP TZ outside of the spinal cord, where they ensheath and myelinate spinal motor nerve root axons (Figure 3a) [12,15]. These cells, known as MEP glia, originate from the pMN domain of the ventral neural tube, where they acquire *olig2* expression, and exit the spinal cord to reach the motor roots at about 50 hours post fertilization (hpf) in zebrafish, after motor axon outgrowth is completed but before oligodendrocyte progenitor cells (OPC) start migrating and filling the CNS [12,15]. MEP glia sit along the ventral motor root like BC cells, and although they share a common marker, *wnt-inhibitory factor 1* (*wif1*), they do not express the hallmark BC cell marker *krox-20* [15,30]. Using *in vivo* imaging coupled with single cell laser ablation of MEP glia, we demonstrated that OPCs exit the spinal cord by pinching through the MEP TZ to colonize motor axons when these cells are absent (Figure 3b) [15]. These findings

demonstrate that MEP glia are responsible for restricting central myelinating glia to the CNS. However, there is no evidence that they are involved in motor neuron positioning.

Taken together, these studies investigating perineurial glia and MEP glia demonstrate that oligodendrocytes and motor neurons are restricted to the spinal cord by distinct mechanisms, mediated by specific CNS-derived peripheral glial populations. Overall, these CNS-derived glia share an important gating function with the mammalian neural crest-derived BC cells during nervous system assembly in spite of the noteworthy differences in their origin and gene expression. However, because *in vivo* live imaging is impossible during murine embryonic development and *in situ* hybridization does not allow for accurate fate-mapping, we cannot rule out the possibility that a subset of mammalian BC cells are also CNS-derived. Moreover, because *in situ* hybridization does not offer precise cellular resolution, it is also possible that the *wif1* expression observed in mouse BC cells may be restricted to a subset of this population [30].

Molecular mechanisms mediating cell segregation at TZs

BC cells restrict motor neuron cell bodies to the CNS, most likely by releasing repulsive signals, but allow axons to cross (Figure 2a). To identify the molecular mechanisms that mediate this selective permeability at TZs, studies have targeted essential axon pathfinding pathways, including Netrin, Semaphorins and Plexins (Table 1) [29,31–35].

The Netrin family is an evolutionarily conserved family of bifunctional secreted guidance molecules that can function as chemoattractants or chemorepellents, depending on the class of receptor expressed by the responding neuron. Recently, Laumonnerie *et al.* demonstrated that Netrin 1 plays a role in the confinement of spinal interneuron axons to the CNS. Netrin 1 attracts spinal interneurons to the ventral spinal cord, through Deleted in colorectal cancer (DCC), and prevents them from exiting the CNS via the DREZ [31]. A more recent study demonstrated that another member, Netrin 5 (Ntn5), which is expressed by BC cells, prevented motor neuron migration out of the CNS by repulsion, likely mediated by DCC receptors expressed by motor neurons [32].

BC cells also mediate motor neuron confinement to the spinal cord through repulsive semaphorin-plexin interactions [10,29]. Plexins are receptor components for semaphorins and their chemotropic activities are well described in axonal growth and pathfinding. Loss of Neuropilin-2 (Npn-2), a high affinity receptor for class 3 semaphorins in chick and mouse, leads to ectopic motor neuron cell bodies in the PNS. Additionally, BC cells express the transmembrane protein Semaphorin6A (Sema6A), whose loss of function phenocopies *Npn-2* mutants, resulting in motor neurons mispositioned along motor axons in the PNS [29]. Studies conducted in zebrafish also reveal the conserved role of semaphorin-plexin signaling in cellular restriction at MEP TZs, as *neuropilin 1* and *Sema3b* knockdown lead to the mispositioning of a subset of primary motor neurons and ectopic axonal exit from the CNS [36].

Interestingly, Bron *et al.* [10] reported that the ectopic motor neuron cell bodies observed in *Sema6A* and *Npn-2* null mouse embryos were confined to caudal regions of the trunk and

that, on the contrary, no significant defects were observed at the forelimb and thoracic levels. Intriguingly, in *Ntn5* mutant mice, Garrett *et al.* observed that the defects were mainly located rostrally. This heterogeneous distribution of the defects along the antero-posterior axis led the authors to suggest that BC cell-derived Netrin 5 might signal towards a specific subtype of motor neuron [32]. An alternative explanation for this spatial pattern is that the proteins cited above and involved in restricting motor neurons within the CNS might be expressed at different levels in the neural tube, following opposite gradients along the antero-posterior axis. Moreover, we cannot rule out the possibility that there might be redundancy with the pathways involved, as it is well known that the semaphorin, plexin and netrin families all consist of numerous members and various combinations of ligands and receptors.

More recently, a study linked defects in radial glial scaffolding to defective CXCL12 signaling and the presence of mispositioned BC cells inside the spinal cord (Figure 2c). The meninges-derived chemokine CXCL12 binds to the CXCR4 receptor expressed by BC cells. In *Cxcl12* and *Cxcr4* mouse mutants, the pial layer that surrounds the CNS is dotted with gaps and BC cells were observed inside the developing spinal cord at these perforated sites [37]. Further analysis revealed that CXCL12/CXCR4 signaling activated integrin β1, which in turn increased radial glial endfeet adhesiveness to pial basement membrane components. This study demonstrated that CXCL12 signaling was an extrinsic factor regulating radial glial scaffold integrity during spinal cord development. In keeping with previous observations in mouse mutants, Zhu *et al.* stated that in the *Cxcl12* and *Cxcr4* null mice, ectopic BC cells were almost exclusively found in the lumbosacral region of the spinal cord and were rare in the rostral region, in spite of the homogeneous presence of radial glia defects [37].

In the mature mammalian nervous system, the presence of peripheral glial cells in the CNS is generally attributed to a disruption of the astrocytic layer that forms the glia limitans on the surface of the spinal cord [19,38]. Peripheral glia, such as SCs, are found in the CNS following spinal cord injury or experimental irradiation and the prevalence of SC invasion in intact spinal cords becomes more prevalent with age [17,19,39,40]. Recently, this was also described in the developing nervous system of zebrafish. Smith *et al.* demonstrated that before radial glial maturation, peripheral glial precursors can freely transgress the MEP TZ (Figure 3d). However, this migration stops once radial glial endfeet mature [16]. This study, along with the MEP and perineurial glial analyses, demonstrate the synchronization of several glial populations that allow for unidirectional migration across the MEP TZ [11,15]. These studies also highlight an intriguing phenomenon where glia freely pass the boundary between the CNS and PNS during development but have to ultimately differentiate on one half of the TZ. Once they definitively sit in the PNS, they function as a gate-keeper to maintain selective and unidirectional migration across the CNS/PNS boundary, in addition to their wrapping function. Altogether, these studies illustrate a developmental mechanism conserved among vertebrates including mice, chick and fish in motor neuron and glial segregation to the CNS.

Although the question of how neurons become segregated to either the CNS or PNS is old, many recent studies have begun to shed light on the important role of several cell

populations in vertebrate models, including BC cells in preventing neurons from crossing the borders of the spinal cord while selectively allowing axonal pass through [9,10,29]. However, how myelinating glia are precisely segregated to the two halves of the vertebrate nervous system is still not thoroughly understood. Fascinatingly, we currently know of two peripheral glial populations that are strictly restricted to the PNS, MEP glia and perineurial glia, but which are generated in the neural tube and must migrate across MEP TZs or spinal motor nerve development and maturation is perturbed. The absence of these glial cells along the nerves causes the ectopic migration of CNS populations, including motor neurons and OPCs, into the periphery [11,14,15]. Understanding how TZs balance cell-specific restriction and selective permeability is an intriguing question that not only sheds light on the mechanisms required for nervous system development, but also those mechanisms that could be perturbed in disease.

Conclusion

The assembly of the nervous system is orchestrated during development by multiple glial cells that migrate freely across spinal cord transition zones. A better understanding of the mechanisms that define these specialized points, as well as the molecular pathways involved in the selective cellular restrictions observed at these boundaries in development and disease will provide a deeper comprehension of the mechanisms underlying neuropathies. Studies in vertebrates such as zebrafish will not only continue to increase our knowledge on TZ structure, but will also allow for the establishment of new therapeutic strategies for neuropathies and demyelinating diseases.

Acknowledgments

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Highlights

- Glial cells segregate neurons and glia at nervous system transition zones.

- Glia are essential to build and maintain the nervous system boundaries.

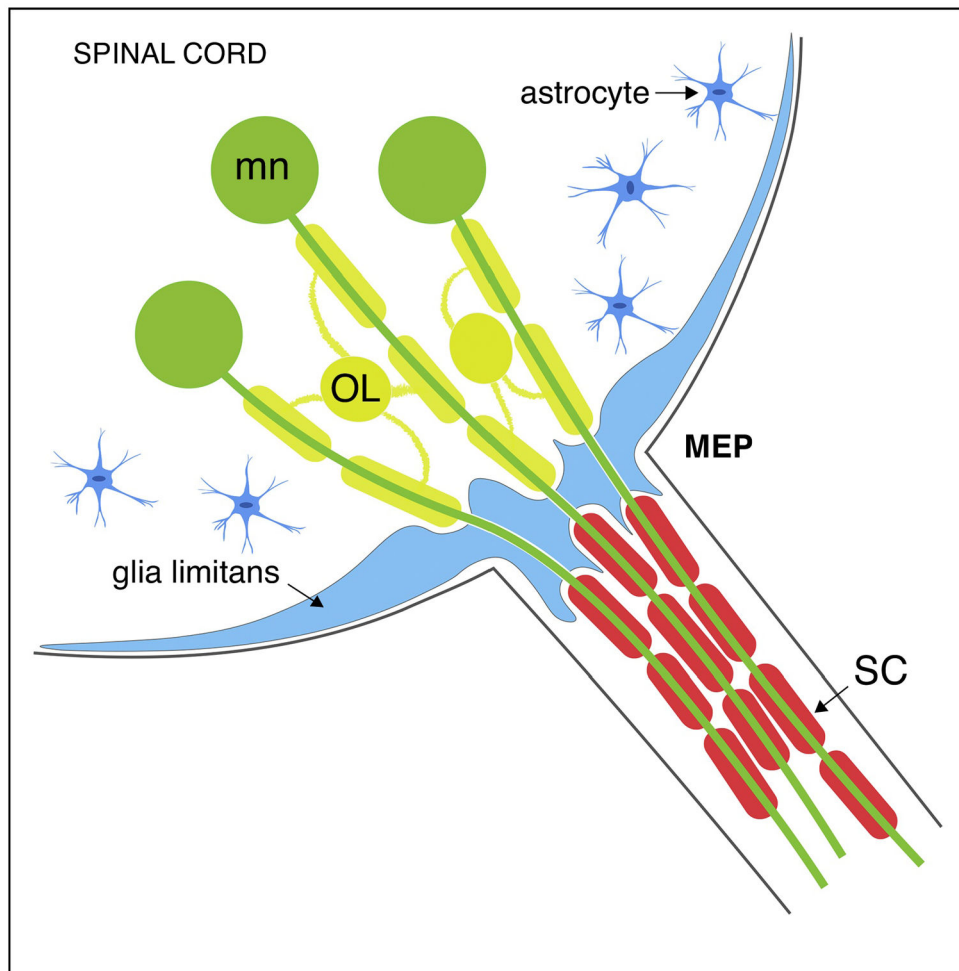- Novel glia described in zebrafish show conserved mechanisms among vertebrates.

Figure 1. Diagram of a transverse section of a mammalian MEP TZ
Motor neurons (green) project their axons into the periphery through the MEP through an astrocytic glia limitans (blue). Oligodendrocytes (yellow) myelinate the central portion of motor axons while SCs (red) myelinate their peripheral portion. The glia limitans layer, which is thicker at the TZ, segregate OLs from SCs.
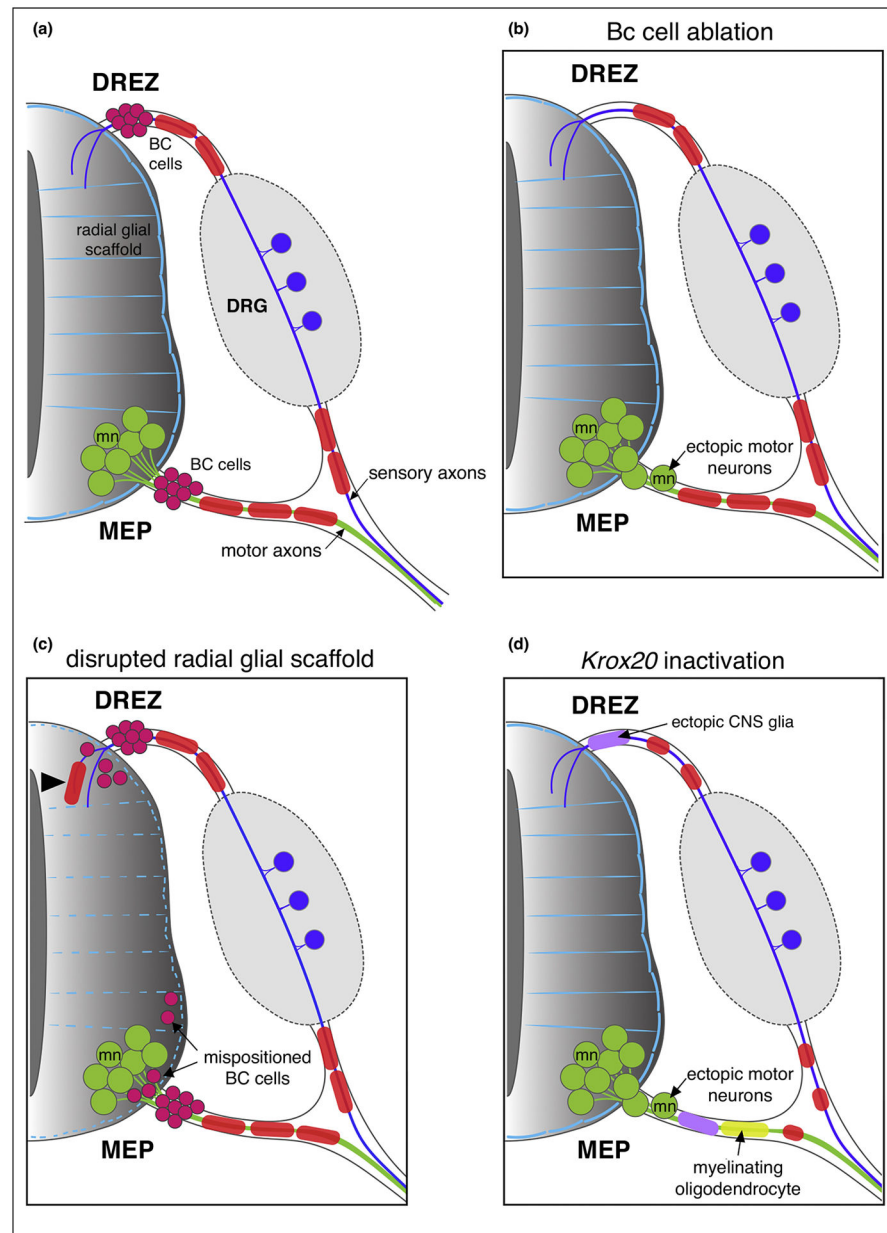
Figure 2. Mammalian TZs

(a) Schematic of a mammalian neural tube showing BC cells (magenta) sitting at the DREZ and MEP TZs. (b) In the absence of BC cells, motor neurons (mn; green) transgress the MEP TZ and invade the motor root. (c) When the radial glial scaffold (light blue) that covers the spinal cord is absent or disrupted, peripheral glial cells (SCs; red, and BC cells) enter the CNS ectopically. (d) Following *Krox20* inactivation, CNS glia (purple) invade the dorsal and ventral roots, oligodendrocytes (yellow) myelinate peripheral axons and SCs (red) fail to develop.
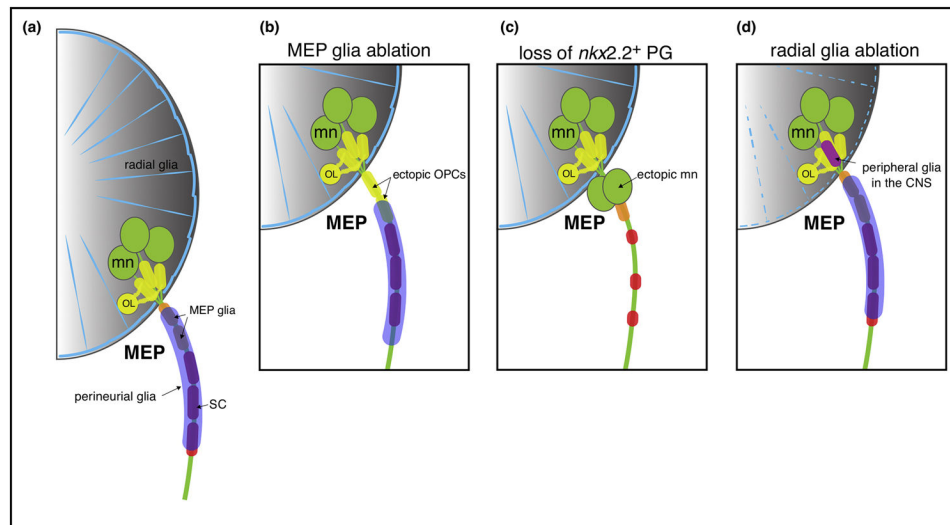
Figure 3. Zebrafish MEP TZs

(a) Schematic of a zebrafish MEP TZ and the diverse populations of glial cells orchestrating this boundary. MEP glia (orange) restrict the oligodendrocyte lineage (OL; yellow) to the spinal cord. Perineurial glia (purple), which make the perineurium around axon bundles, also constrain motor neurons (mn; green) to the ventral spinal cord. Radial glia (blue) cover the surface of the spinal cord and prevent peripheral glia from entering the CNS. (b) In the absence of MEP glia, OPCs ectopically migrate out of the spinal cord and ensheath motor root axons. (c) In the absence of perineurial glia, motor neurons ectopically exit the spinal cord and are found along the motor root and SCs (red) fail to develop. (d) Following radial glial ablation, peripheral glial cells ectopically enter the spinal cord. Note that MEP and perineurial glia originate in the ventral spinal cord and must migrate into the periphery to restrict CNS components.

Table 1

Signaling governing nervous system transition zones

MUTANT/KNOCK-DOWN	EXPRESSION	PHENOTYPE	ORGANISM	REFERENCES
Ntn1/Dcc	Dorsolateral spinal cord	Interneuron exit the DREZ ectopically	Mouse	28
Ntn5/Dcc	BC cells	Ectopic motor neurons	Mouse	29
Krox20	BC cells, SCs	Ectopic motor neurons and CNS glia in the motor nerve root		11
Nkx2.2	Perineurial glia	Ectopic motor neurons in the motor nerve root	Zebrafish/Mouse	9, 12
Npn1	Motor neurons	Ectopic motor neurons in the motor nerve root	Zebrafish	33
Npn2	Motor neurons	Ectopic motor neurons in the motor nerve root	Chick/Mouse	8, 27
Sema6a	BC cells	Ectopic motor neurons in the motor nerve root	Mouse	27
Sema3ab	Posterior half of the somite	Mispositioned motor neurons	Zebrafish	33
PlexinA3 (*sidetracked*)	Motor neurons	Mispositioned MEP	Zebrafish	30–32
Erbb3	PNS glia	Ectopic oligodendrocytes along motor nerves	Zebrafish	41