



# Contribution of semaphorins to the formation of the peripheral nervous system in higher vertebrates

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## ABSTRACT

Semaphorins are a large family of proteins characterized by sema domains and play a key role not only in the formation of neural circuits, but in the immune system, angiogenesis, tumor progression, and bone metabolism. To date, 15 semaphorins have been reported to be involved in the formation of the peripheral nervous system (PNS) in higher vertebrates. A number of experiments have revealed their functions in the PNS, where they act mainly as axonal guidance cues (as repellents or attractants). Semaphorins also play an important role in the migration of neurons and formation of sensory-motor connections in the PNS. This review summarizes recent knowledge regarding the functions of higher vertebrate semaphorins in the formation of the PNS.

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## Introduction

The ligands that contain sema domains are generically called semaphorins. Semaphorins are secreted or membrane-bound proteins, and their effects are mostly mediated by transmembrane proteins named plexins. Via plexins or alternative receptors, semaphorins are involved in a wide range of growth processes such as neural circuit formation, angiogenesis, and bone metabolism. To date, 20 semaphorins have been identified in higher vertebrates. By focusing on higher vertebrates (eg., mammals, birds, and reptiles), especially on the mouse and the chick, we will review the function of semaphorins in the formation of the peripheral nervous system (PNS) in higher vertebrates.

## Semaphorins in higher vertebrates

Higher vertebrate semaphorins are divided into classes 3–7 subfamilies.<sup>1</sup> Class 3 semaphorins are secreted proteins, and they are subdivided into 7 members (Sema3A–Sema3G). Class 4 semaphorins, also comprising 6 members (Sema4A–Sema4D, Sema4F, Sema4G), are transmembrane proteins known to be regulators of a variety of immune responses. Two class 5 semaphorins (Sema5A and Sema5B) contains 7 thrombospondin repeats. Class 6 semaphorins are transmembrane

proteins. This class contains 4 members with alternatively spliced cytoplasmic regions (Sema6A–Sema6D; Sema6C has not been identified in the chick). The lone class 7 semaphorin (Sema7A) is a glycosylphosphatidylinositol (GPI)-anchored protein.

## Semaphorin receptors in higher vertebrates

### Plexins

Semaphorin signals in higher vertebrates are primarily transduced by 9 plexins which are subdivided into 4 type A plexins (PlexinA1–PlexinA4; PlexinA3 is not identified in the chick), 3 type B plexins (PlexinB1–PlexinB3), PlexinC1 and PlexinD1. Plexins are single-passed transmembrane receptors that are known to interact directly with small GTPases in the cytoplasm.<sup>2</sup> Plexins bind directly to class 4–7 semaphorins and Sema3E to transduce signals intracellularly. Class 3 semaphorins except for Sema3E do not bind directly to plexins but to neuropilins. PlexinAs or PlexinD1 form a complex with neuropilins in which neuropilins act as a binding receptor and plexins as a signal transducer.

### Neuropilins

Neuropilins are cell surface molecules that have a short intracellular domain. In higher vertebrates, 2 homologues–neuropilin-1 (Nrp1) and neuropilin-2 (Nrp2)–have been

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identified. Several class 3 semaphorins utilize neuropilins as a receptor for transducing their signals. *Sema3A* is known to bind to *Nrp1* but not to *Nrp2*.<sup>3</sup> *Sema3A/Nrp1* signals are mediated by *PlexinAs* or *PlexinD1*. In contrast, *Sema3F* binds to *Nrp2* with high affinity but to *Nrp1* with low affinity.<sup>3,4</sup> *Sema3F* signals are transduced by a complex of *Nrp2* and *PlexinAs*. Other class 3 semaphorins, *Sema3B* and *Sema3C* bind to both neuropilins and can transduce signals through them. As mentioned above, unlike other class 3 semaphorins, *Sema3E* binds directly to *PlexinD1*, not to neuropilins. Interestingly, in addition to *PlexinD1*, *Sema3E* requires *Nrp1* to induce axon attraction of subiculo-mammillary neurons in the central nervous system.<sup>5</sup>

### Other receptors

Further studies have revealed that a member of immunoglobulin superfamily cell adhesion molecules *L1CAM* and neuron-glial related cell adhesion molecule (*NrCAM*) act as other co-receptors for class 3 semaphorins under certain circumstances.<sup>6,7</sup> In addition, *in vitro* analyses have shown that heparan sulfate proteoglycans mediate *Sema5A*-induced attraction for midbrain axons as components of *Sema5A* receptors.<sup>8</sup> In contrast, the attractive effect by *Sema5A* on growing axons can be converted into repulsion by chondroitin sulfate proteoglycans (CSPGs), suggesting that CSPGs are other components of *Sema5A* receptors.<sup>8</sup> Interestingly, *Sema7A*-induced axon growth of olfactory bulb neurons is mediated by  $\beta 1$  integrins independent of the *PlexinC1* receptor.<sup>9</sup>

### The peripheral nervous system in higher vertebrates

In higher vertebrates, the peripheral nervous system (PNS) is divided into the somatic nervous system, which is composed of cranial and spinal nerves, and the autonomic nervous system. With the exception of the olfactory (n I) and optic (n II) nerves, which are considered to be parts of the central nervous system, the cranial nerves are parts of the PNS.<sup>10</sup> Accordingly, the 10 remaining cranial nerves (n III–n XII) are considered to be included in the PNS in this review. The autonomic nervous system is composed of sympathetic and parasympathetic nervous systems.

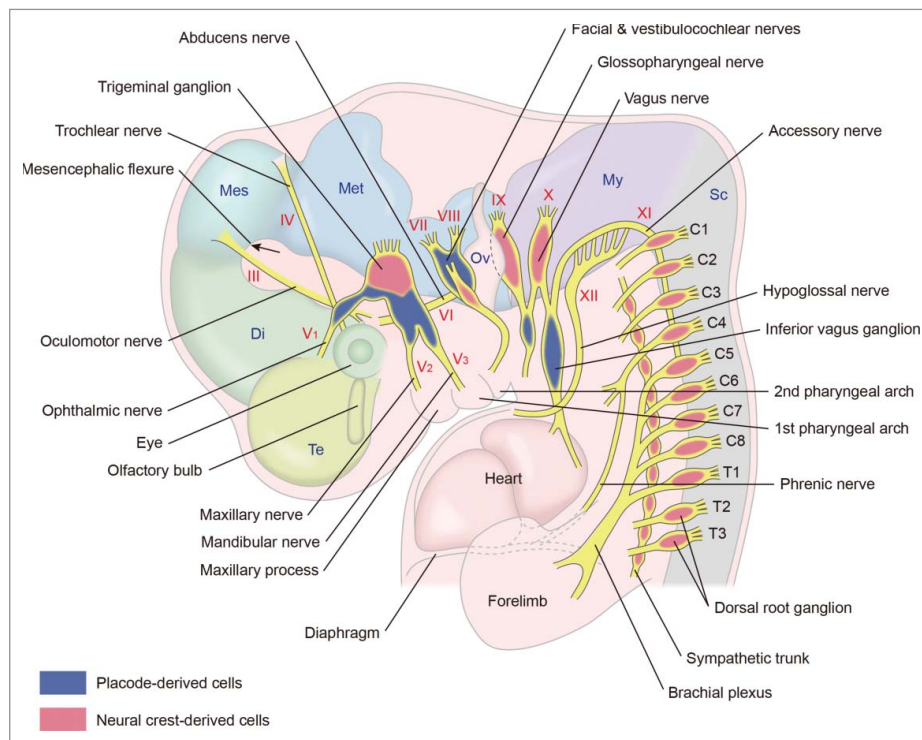
An illustration of the PNS in higher vertebrate embryos briefly shows the pathways of cranial nerves (n III–n XII) and the location of their ganglia (Fig. 1). The oculomotor nerve (n III) projects as a tight motor fiber bundle from the mesencephalic flexure ventrally toward the ciliary ganglion and extraocular muscles. The trochlear nerve (n IV) exits the central nervous system at the dorsal hindbrain-midbrain (mesencephalon-metencephalon) junction and projects as motor fibers to the superior oblique muscle of the eye. The trigeminal nerve

(n V) is the largest and most complex nerve among cranial nerves. It contains motor and sensory fibers. The ophthalmic branch ( $V_1$ ) of the trigeminal nerve projects to the frontal part of the head. The maxillary branch ( $V_2$ ) projects to the maxillary process. The mandibular branch ( $V_3$ ) projects to the first pharyngeal arch (mandibular arch). The abducens nerve (n IV) is derived from the basal plate of the hindbrain (metencephalon) and projects as motor fibers to the lateral rectus muscle. The facial nerve (n VII) projects to the second pharyngeal arch. It contains motor and sensory fibers. The pathways of this nerve are variable. The vestibulocochlear nerve (n VIII) is derived from the otic placode, which forms the otic vesicle. The glossopharyngeal nerve (n IX) projects to the third pharyngeal arch (the third pharyngeal arch is not shown in Figure 1 because it is behind the second pharyngeal arch). The vagus nerve (n X) is the longest cranial nerve. Its motor and sensory fiber elements are derived from the basal plate of the hindbrain (myelencephalon) and cranial neural crest cells, respectively. The vagus nerve has a large ganglion called the inferior (nodose) ganglion. Most of the accessory nerve fibers (n XI) grow out of neurons located in the 1st–5th cervical spinal cord. They are motor fibers and pass rostrally between the dorsal and ventral roots. The hypoglossal nerve (n XII) is also comprised of motor fibers and is derived from the basal plate of the hindbrain (myelencephalon).

Figure 1 also shows that fibers of the 5th–8th cervical spinal nerves and of the 1st thoracic spinal nerve converge to form the brachial plexus at the proximal region of the forelimb. In contrast, the sympathetic branches from the sympathetic ganglia in the thoracic 2nd–4th segments of the spinal cord project to the cardiac plexus (not shown in Fig. 1).

### Discovery of semaphorins

Semaphorins have been implicated in the formation of the PNS from the time they were first identified. *Sema3A* is the first semaphorin whose role in the PNS has been closely examined. *Sema3A* was initially discovered to have an ability to collapse growth cones of dorsal root ganglion (DRG) neurons and to repel their axons *in vitro*.<sup>11</sup> After this discovery, several studies using *in vitro* assays have revealed that other PNS axons, such as sympathetic and spinal motor axons, are also repelled by *Sema3A*. Subsequently, other higher vertebrate semaphorins have been examined by the same *in vitro* assays and/or genetic experiments using knock-out mice or RNAi. In this review, we will focus on several semaphorins (*Sema3A*, *Sema3F*, *Sema6A*, *Sema6C* and *Sema6D*), as mutant mice with these semaphorins have varied



**Figure 1.** A schematic lateral view of the peripheral nervous system in a higher vertebrate embryo at the early stages. The 3rd–12th cranial nerves with their ganglia, spinal nerves with dorsal root ganglia and sympathetic trunk with its ganglia are shown. C1–C8 and T1–T3 indicate 1st–8th cervical and 1st–3rd thoracic spinal nerves, respectively. Di: diencephalon, Mes: mesencephalon, Met: metencephalon, My: myelencephalon, Ov: otic vesicle, Sc: spinal cord, Te: telencephalon.

phenotypes with respect to the formation of the PNS. Furthermore, we will show additional data of both the *in vitro* and *in vivo* functions of other semaphorins and their receptors in Tables 1 and 2, respectively.

### **Sema3A in the formation of the PNS**

Receptors for Sema3A are PlexinA1–PlexinA4 and PlexinD1 via co-receptor Nrp1 and L1CAM.<sup>6,12</sup>

#### **DRG fibers**

At the early stages before DRG fibers arrive at the dorsal spinal cord, *Sema3A* is expressed in the notochord, the myotome and a subset of spinal motor neurons (medial motor column [MMC] neurons; Fig. 2A).<sup>13</sup> *In vitro* assays have shown that both myotome- and notochord-derived Sema3A strongly repel early DRG axons to block the aberrant innervation toward the myotome and the notochord (Fig. 2A).<sup>14</sup>

After DRG fibers have reached the dorsal spinal cord, *Sema3A* expression spreads over the ventral spinal cord (Fig. 2B). *In vitro* assays have also shown that tropomyosin receptor kinase A (TrkA)-positive DRG fibers (cutaneous afferents) are inhibited by Sema3A derived from the ventral spinal cord, suggesting that Sema3A may serve as a barrier to stop further projection of TrkA-

positive DRG fibers into the ventral spinal cord (Fig. 2B).<sup>15,16</sup> In contrast, TrkC-positive DRG fibers (Ia afferents) do not respond to Sema3A-induced repulsion, and they can project into the ventral spinal cord without blocking by Sema3A (Fig. 2B). Consistent with these results, a subset of TrkA-positive DRG fibers in *Sema3A* mutants extend into the ventral region of the spinal cord.<sup>17</sup> Similar defects in the projection of TrkA-positive DRG fibers are observed in *Nrp1<sup>sema-</sup>* mutants (In *Nrp1<sup>sema-</sup>* mutants, the expression of the Nrp1 protein is normal but Sema/Nrp1 signaling is abolished).<sup>18</sup>

#### **Spinal motor fibers**

*In vitro* studies have revealed that spinal motor axons at embryonic stages are repelled by Sema3A.<sup>13,19</sup> At early stages at the brachial or lumbar level, *Sema3A* is expressed in the notochord, the perinotochordal mesenchyme, the presumptive epaxial muscle (myotome), MMC neurons and the limb (Fig. 3A).<sup>20,21</sup> In wild-type embryos, fibers of lateral motor column (LMC) neurons reach the base of the limb in a fasciculated manner, but do not enter the limb (Fig. 3A).<sup>20</sup> In *Sema3A*-deficient mice, fibers of LMC neurons show defasciculation when they extend to the limb, indicating that the Sema3A signal derived from the myotome and notochord regulate fasciculation of fibers of LMC neurons.<sup>20</sup> Furthermore,

**Table 1.** *In vitro* function of semaphorins and their receptors in the PNS of higher vertebrates.

Semaphorins	Receptors*	<i>In vitro</i> function in the PNS of higher vertebrates	Refs.
Sema3A	PlexinA1–A4, PlexinD1, Nrp1, L1CAM	collapses DRG growth cones / repels DRG axons collapses spinal motor growth cones / repels spinal motor axons collapses sympathetic growth cones / repels sympathetic axons collapses growth cones of oculomotor axons (III) collapses trigeminal (sensory) growth cones / repels trigeminal (sensory) axons (V) repels trigeminal (motor) axons (V) repels abducens motor axons (VI) collapses geniculate (sensory) growth cones / repels geniculate (sensory) axons (VII) repels facial (motor) axons (VII) repels glossopharyngeal (motor) axons (IX) repels vagus (sensory) axons (X) avoids the neural crest cell migration	11,15 13,19,52 22,23 25 27,29,53 19 19 27,28,29 19 19 29 31
Sema3B	Nrp1/2, NrCAM	repels sympathetic axons	16,23
Sema3C	PlexinA1, A2, PlexinD1, Nrp1/2	repels sympathetic axons collapses growth cones of oculomotor axons (III)	16,23 25
Sema3E	PlexinD1, Nrp1	collapses DRG growth cones / repels DRG axons	54,55
Sema3F	PlexinA1–A4, Nrp1/2, NrCAM	repels sympathetic axons	4,36
Sema3G	Nrp2	repels sympathetic axons	56
Sema4D	PlexinB1, B2, PlexinC1	promotes DRG axonal growth	57
Sema4F	nd	mediates the interaction between Schwann cells and DRG axons	58
Sema5A	PlexinA1, A3**, PlexinB3, CSPG, HSPG	attracts DRG axons	59
Sema5B	PlexinA1, A3**	collapses DRG growth cones / inhibits DRG axonal growth	60,61
Sema6A	PlexinA1, A2, A4	collapses sympathetic growth cones / repels sympathetic axons regulates dendritic growth of LMC motor neurons	45,46 42
Sema6B	PlexinA2, A4	inhibits sympathetic axonal growth	45
Sema6C**	PlexinA1	collapses growth cones of DRG axons	49,62
Sema6D	PlexinA1	collapses growth cones of DRG axons	49
Sema7A	PlexinC1, $\beta$ 1 integrins	promotes DRG axonal growth	9

nd, not determined

\*These data are referred from refs. 2, 12, 35, 41, 63, 64

\*\*No homologue has been identified in the chick.

NrCAM: neuron-glia related cell adhesion molecule, CSPG: chondroitin sulfate proteoglycan, HSPG: heparan sulfate proteoglycan

fibers of LMC neurons aberrantly enter the limb in advance, thus showing that the Sema3A signal also determines the timing of growth of these fibers to the limb.<sup>20</sup>

At later stages, *Sema3A* expression within the limb becomes weaker (Fig. 3B).<sup>20,21</sup> In wild-type embryos, the fibers of both lateral and medial LMC (LMCl and LMCm, respectively) neurons diverge after entering the limb. LMCl fibers project toward the dorsal limb and LMCm fibers toward the ventral limb (Fig. 3B).<sup>20</sup> In *Sema3A*-deficient mice, both fibers grow aberrantly in the dorso-ventral direction of the limb. These findings suggest that Sema3A signaling controls the dorso-ventral choice for the limb innervation by a subpopulation of spinal motor neurons.<sup>20</sup> Furthermore, intrinsic Sema3A in MMC motor neurons is also involved in the pathway formation of their fibers.<sup>21</sup> Loss- and gain-of-function experiments in chick embryos show that intrinsic Sema3A in MMC neurons modulates their axonal responses to environmental Sema3A, allowing MMC fibers to grow and project into the myotome/epaxial muscle regardless of the presence of Sema3A in these tissues (Fig. 3A and 3B).

### Sympathetic fibers

Sema3A has been shown not only to collapse the growth cones of sympathetic axons but to repel sympathetic

axons.<sup>22,23</sup> Sema3A is synthesized by Purkinje fibers in the developing heart and displays an inverse distribution pattern with sympathetic fibers.<sup>24</sup> A detailed examination of *Sema3A* mutants has shown that Sema3A plays a crucial role in the cardiac innervation by sympathetic fibers. *Sema3A* mutants lack this innervation, with this sympathetic dysfunction leading to sinus bradycardia. Overexpression of *Sema3A* in the heart also leads to reduced sympathetic innervation and sudden death.<sup>24</sup> Taken together, these findings indicate that the appropriate expression of *Sema3A* in the heart is required for the normal innervation pattern of sympathetic fibers to control the heart rate.

### Cranial nerves

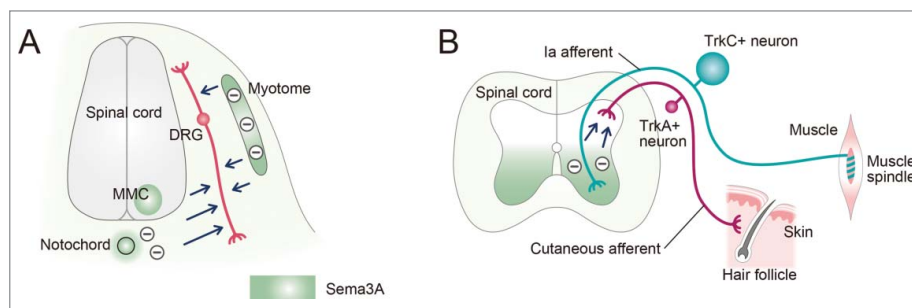
As mentioned above, the oculomotor nerve (n III) projects into the extraocular muscles. *Sema3A* is expressed in developing extraocular muscles and has been shown to collapse growth cones of oculomotor axons.<sup>25</sup> RNAi knockdown experiments on Sema3A receptor *PlexinA1* in oculomotor neurons have induced guidance defects of oculomotor fibers. However, the trajectories of the oculomotor nerve are normal in *Sema3A* mutant embryos.<sup>26</sup> This may be due to the fact that other PlexinA1 ligands, such as Sema3C expressed in the extraocular muscles,



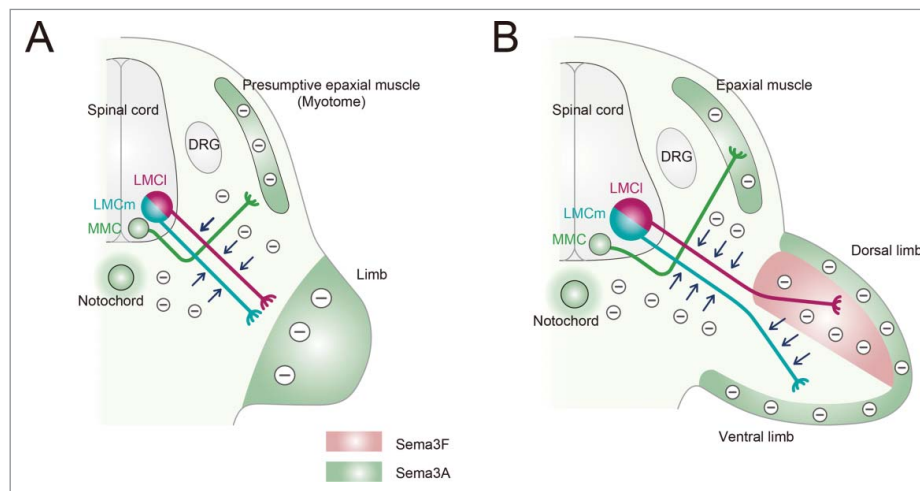
**Table 2.** *In vivo* function of semaphorins in the PNS of higher vertebrates.

Semaphorins and their receptors	PNS phenotypes of mutant mice	Refs.
Sema3A	defects in the projection of DRG TrkA-positive afferents within the spinal cord defects of segmental DRG formation defects in spinal motor fiber growth and guidance defects in sympathetic innervation and abnormal morphogenesis of the sympathetic trunk normal projections of the oculomotor nerve (III) premature projections of the trigeminal (V) and geniculate (VII) nerves to pharyngeal arches defects in sensory projections of vestibular ganglion neurons (VIII) disorganization of the trigeminal (V), facial (VII), glossopharyngeal (IX), vagus (X) and accessory nerves (XI)	17 32,33 20 24,34 26 27 30 26
Sema3C	no abnormality in projections of cranial nerves and the sympathetic nervous system	65
Sema3E	disorganization of specific sensory-motor connections in the spinal cord	66,67
Sema3F	defects in the projection of LMCm neurons in the limb severe defasciculation of the oculomotor nerve (III) absence of projections of the trochlear nerve (IV) disorganization of segmental migration patterns of NCCs	20 39 39 40
Sema5A	no abnormality in DRG and spinal motor fibers, and in cranial nerves	68
Sema6A	ectopic migration of spinal motor neurons	43
Sema6D	aberrant projections of proprioceptive DRG neurons in the dorsal spinal cord	48
Nrp1	defects in the projection of DRG TrkA-positive afferents within the spinal cord defects of segmental DRG formation defects in spinal motor fiber growth and guidance ectopic positions of sympathetic neurons normal projections of the oculomotor nerve (III) defasciculation of the trigeminal (V), facial (VII), glossopharyngeal (IX) and vagus (X) nerves defects in the projection of the vestibulocochlear nerve (VIII) defasciculation of the hypoglossal nerve (XII)	18 32,33 20 34 69 69 18 70
Nrp2	defects in the projection of LMCm neurons in the limb ectopic migration of spinal motor neurons no defects in the morphology of the sympathetic trunk aberrant projections of the oculomotor (III) and trochlear (IV) nerves similar to those in <i>Sema3F</i> mutants defasciculation of ophthalmic branches of the trigeminal nerve (V) partial defasciculation of the facial nerve (VII) normal projections of the vestibulocochlear (VIII) and vagus (X) nerves disorganization of segmental migration patterns of NCCs	20 43 38 38,39,71 71 38 38 40
PlexinA3*	defasciculation of ophthalmic branches of the trigeminal nerve (V)	72
PlexinA4	defects in DRG and sympathetic fibers aberrant projections in sympathetic fibers	45 45
PlexinD1	defasciculation of the trigeminal (V), facial (VII), glossopharyngeal (IX) and vagus (X) nerves disorganization of specific sensory-motor connections in the spinal cord	45 66,67

\*No homologue has been identified in the chick.



**Figure 2.** Both central and peripheral projections of DRG fibers are guided by Sema3A. Transverse views through the spinal cord showing DRG fibers and their target tissues. (A) Before DRG fibers reach the dorsal spinal cord, the myotome, medial motor column (MMC) motor neurons, notochord and perinotochordal mesenchyme exert Sema3A-induced repulsion (–) against DRG fibers (peppermint green). (B) After DRG fibers reach the dorsal spinal cord, small-diameter tyrosine receptor kinase A (TrkA)-positive neurons (nociceptive neurons, red) terminate their cutaneous afferents in the superficial layers of the dorsal spinal cord (laminae I and II) due to Sema3A-induced repulsion (–) from the ventral spinal cord (peppermint green). In contrast, large-diameter TrkC-positive neurons (proprioceptive neurons, blue) project their Ia afferents ventrally into the deeper laminae of the spinal cord; muscle spindle Ia afferents terminate in the intermediate laminae and the ventral horn because they are not repelled by Sema3A.



**Figure 3.** Spinal motor fibers are guided by Sema3A and Sema3F. A schematic transverse section through the spinal cord and limb bud at the brachial/lumbar levels summarizing spinal motor columns and Sema3A/3F for axonal targeting. (A) Lateral motor column (LMC) motor neurons are divided into 2 divisions: medial (m, blue) and lateral (l, red). In the early stages, both LMCm and LMCI motor neurons extend fibers straight toward the limb and pause before further growth in response to surround-repulsion by Sema3A (—): Sema3A secreted by the limb, presumptive epaxial muscle (myotome), perinotochordal mesenchyme and notochord (peppermint green). medial motor column (MMC) motor neurons expressing *Sema3A* are located medially and send fibers (green) to the epaxial muscle because intrinsic Sema3A proteins reduce the availability of Nrp1 receptors and modulate the sensitivity of MMC fibers to Sema3A. This modulation allows MMC fibers to extend dorsally toward the myotome. (B) At later stages, the expression of *Sema3A* in the limb is decreased, and the dorsal part of the limb begins to express *Sema3F* (pink). LMCI neurons express *Nrp1* but not the Sema3F receptor *Nrp2* and enter the dorsal limb because of their insensitivity to Sema3F. In contrast, LMCm neurons express both *Nrp1* and *Nrp2* and are constrained in the ventral limb by Sema3F-induced repulsion (—).

might prevent oculomotor fibers from projecting aberrantly and help guide their stereotypical projection.<sup>25</sup>

The V<sub>3</sub> branch of the trigeminal nerve (n V) and fibers of geniculate ganglia (n VII) project to the first and second pharyngeal arches, respectively (see Fig. 1). *In vitro* studies have revealed that trigeminal and geniculate ganglion axons are repelled by Sema3A derived from pharyngeal arches.<sup>27,28</sup> Consistent with these *in vitro* results, trigeminal and geniculate sensory fibers innervate the pharyngeal arches prematurely in the absence of *Sema3A*.<sup>27</sup>

Regarding other peripheral nerves, the glossopharyngeal (n IX) and vagus (n X) nerves are highly defasciculated in *Sema3A* mutants.<sup>26</sup> Based on the fact that axons corresponding to these cranial nerves are shown to be repelled by Sema3A in culture experiments (see Table 1),<sup>19,29</sup> defasciculation of these nerves may be caused by the repulsive activities of Sema3A secreted from the surrounding mesenchymal tissues. Of note, a previous study has reported that vestibular ganglion axons (n VIII) are not repelled by Sema3A.<sup>29</sup> However, the expression patterns of *Sema3A*, *Nrp1* and *PlexinA1/3* strongly suggest the involvement of Sema3A signaling in the formation of vestibular circuits.<sup>30</sup> Indeed, analyses of *Sema3A*-deficient mutant embryos have shown that Sema3A signaling is required for the proper afferent projection of vestibular ganglia.<sup>30</sup>

In addition, the 5th–12th cranial motor neurons exclude their fibers from the floor plate of the hindbrain during development. Guthrie's group has clearly demonstrated that the motor axons of trigeminal (n V), abducent (n VI), facial (n VII) and glossopharyngeal (n IX) nerves are repelled by Sema3A derived from the floor plate of the hindbrain.<sup>19</sup> Thus, these cranial motor fibers may be guided in the proper direction partially by Sema3A.

#### Neural crest cell migration

Neural crest cells (NCCs) are known to migrate from the dorsal neural tube to specific peripheral districts. NCCs are precursors of DRG neurons which migrate into the anterior sclerotome. *Sema3A* is expressed in the posterior halves of sclerotomes, and *Nrp1* is expressed in NCCs in the trunk.<sup>31</sup> Detailed analyses demonstrate that Sema3A expressed in posterior sclerotomes plays an important role not only in guiding NCC migration through anterior sclerotomes by preventing NCCs from entering posterior sclerotomes but in the segmental neurogenesis of DRG neurons.<sup>32,33</sup> NCCs are also a precursor for sympathetic neurons. In both *Sema3A* and *Nrp1* mutants, many sympathetic neuron precursors are located at ectopic positions.<sup>34</sup> Culture experiments have also shown that Sema3A suppresses the cell migration of sympathetic neurons.<sup>34</sup> Thus, Sema3A expressed around the

migratory path of NCCs/sympathetic neuron precursors further plays a crucial role in regulating the migration and arrest of NCCs and consequently contributes to the proper innervation of sympathetic fibers.

### **Sema3F in the formation of the PNS**

Receptors for Sema3F are PlexinA1–PlexinA4, functioning together with co-receptors Nrp1/2 and NrCAM.<sup>12,35</sup>

### **Sympathetic fibers**

Sema3F exerts repulsive activity toward sympathetic but not DRG axons.<sup>4,36</sup> Additionally, close *in vitro* examinations using knockout mouse tissues have shown that Sema3F/Nrp2 signaling in sympathetic axons is mediated principally by PlexinA3 and partially by PlexinA4.<sup>37</sup> However, *Nrp2*-deficient mice are observed to display no gross defects in the morphology of their sympathetic trunk.<sup>38</sup> This result is consistent with the fact that *Sema3F* is absent in the migratory path of sympathetic neuron precursors and in the pathway of sympathetic fibers.

### **Spinal motor fibers**

As mentioned above, LMCl motor fibers project into the dorsal limb, whereas LMCm motor ones project into the ventral limb at later stages (Fig. 3B). The dorsal part of the limb begins to express *Sema3F* at later stages (Fig. 3B).<sup>20</sup> As LMCm neurons express the Sema3F receptor *Nrp2*, Sema3F blocks the projection of LMCm fibers to the dorsal limb and guides them to the ventral limb (Fig. 3B).<sup>20</sup> Conversely, the fibers of LMCl neurons that do not express *Nrp2* enter the dorsal limb because they are not affected by Sema3F-induced repulsion (Fig. 3B).<sup>20</sup> Thus, Sema3F/Nrp2 signaling plays a critical role in guiding LMC motor fibers to the limb.

### **Cranial nerves**

The oculomotor (n III) and trochlear (n IV) motor neurons express the Sema3F receptor *Nrp2* during early prenatal stages.<sup>39</sup> Interestingly, *Sema3F* expression is abundant in the caudal midbrain and the rostral hindbrain, but is absolutely absent in the hindbrain-midbrain junction where the trochlear nerve extends toward the eye.<sup>38</sup> Taking into account the fact that the trochlear motor axons are repelled by Sema3F *in vitro*,<sup>38</sup> these findings suggest that *Sema3F* expression flanking the path of the trochlear nerve may prevent trochlear fibers from projecting aberrantly and serve to promote their fasciculation. Consistent with this speculation, the trajectories of the trochlear nerve are absent in the absence of *Sema3F*. This phenotype in mutants further indicates an indispensable role for Sema3F in the normal development of the trochlear nerve.<sup>38,39</sup> In contrast, the trajectories of the

oculomotor nerve are severe defasciculated, but they still project into their normal target fields in *Sema3F* mutants.<sup>39</sup>

### **Neural crest cell migration**

*Sema3F* and *Nrp2* are expressed in the posterior sclerotome and in NCCs in the trunk, respectively.<sup>40</sup> As is the case in Sema3A, Sema3F/Nrp2 signaling plays an important role in guiding segmental NCC migration through sclerotomes.<sup>40</sup> However, this signaling is not required for segmental neurogenesis of DRG neurons.<sup>40</sup>

### **Sema6A**

Receptors of Sema6A are PlexinA1, PlexinA2 and PlexinA4.<sup>12,41</sup> *Sema6A* expression overlaps with the expression of *PlexinA4* and an effector molecule *FARP-1* (*FERM*, *RhoGEF* and *pleckstrin domain-containing protein 1*) in LMC neurons of the spinal cord at the forelimb level.<sup>42</sup> Both *in vitro* and *in vivo* analyses have revealed that Sema6A/PlexinA4 signaling regulates dendritic growth of LMC neurons in the spinal cord via FARP-1.<sup>42</sup>

In the developing spinal cord, boundary cap cells located at the motor exit points express *Sema6A*.<sup>41,43</sup> On the other hand, the Sema6A receptors *PlexinA1* and *PlexinA2* are expressed in spinal motor neurons. *In ovo* experiments by RNAi in chicks have shown that the loss of *Sema6A* in boundary cap cells and the loss of *PlexinA2* on spinal motor neurons result in the ectopic migration of spinal motor neurons in the spinal cord. Thus, Sema6A/PlexinA2 signaling regulates the location of immature spinal motor neurons within the spinal cord.<sup>43</sup> Furthermore, the loss of *Sema6A* in boundary cap cells or the loss of *PlexinA1* on spinal motor neurons pushes spinal motor neurons out of the spinal cord along the ventral roots, resulting in failure to form the dorsal roots properly.<sup>41</sup> These results suggest that Sema6A produced by boundary cap cells functions as a separator between the central and peripheral nervous systems by gatekeeping the dorsal root entry and spinal motor axon exit sites.

*Sema6A* and *PlexinA4* are co-expressed in DRG neurons, whereas sympathetic neurons express *PlexinA4*, but not *Sema6A*.<sup>44</sup> *In vitro* studies have demonstrated that Sema6A can not only collapse the growth cones of sympathetic neurons, but also repel sympathetic axons via PlexinA4.<sup>45,46</sup> In contrast, DRG neurons show little response to Sema6A-induced repulsive activities.<sup>46</sup> This discrepancy may be due to the *cis* interaction between Sema6A and PlexinA4. Haklai-Topper et al.<sup>44</sup> have shown that the *cis* interaction between Sema6A and PlexinA4 perturbs the *cis-trans* binding between *trans* Sema6A and *cis* PlexinA4. Namely, the DRG axonal response to extrinsic Sema6A might be diminished by intrinsic Sema6A.



## Sema6C and Sema6D

The receptor of Sema6C and Sema6D is PlexinA1.<sup>12</sup> Both *Sema6C* and *Sema6D* are expressed in the embryonic dorsal spinal cord.<sup>47</sup> Leslie et al.<sup>48</sup> have examined proprioceptive projections of DRG fibers in the absence of *Sema6C* and *Sema6D*. *Sema6C*-deficient mice show no obvious defects. In contrast, *Sema6D*-deficient mice show defects in the trajectories of the proprioceptive fibers similar to those in *PlexinA1*-deficient mice. *Sema6C/Sema6D* double-deficient mice are defective in the trajectories of the proprioceptive fibers to the same degree as *Sema6D* mutants.<sup>48</sup> Taken together, the findings from analyses of these mutant phenotypes clearly show that Sema6D is a ligand for PlexinA1 in the dorsal spinal cord. Based on the fact that Sema6D is known to collapse growth cones of DRG axons,<sup>49</sup> the ectopic placement of proprioceptive fibers in *Sema6D* mutants might be due to the loss of Sema6D repulsion in the dorsal spinal cord.

## Conclusions

In this review, we regarded the relationship between semaphorins and plexins as a one-to-one correspondence. However, previous observations on *plexinA1* and *plexinB1* mutants strongly suggest that among their family members plexins play some redundant roles during development.<sup>50,51</sup> To date, little is known about these functional redundancies, but future studies will reveal in more detail the redundant roles of individual plexins. At present, whether or not the epigenetic regulation of semaphorin genes plays a role in the axon guidance events or in the formation of the PNS during the fetal period remains unclear. We hope that novel findings regarding epigenetic regulation will emerge from future studies, thereby helping to further clarify the mechanism of the pathogenesis of neurodegenerative diseases caused by fetal developmental abnormalities.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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