

## ROLE FOR NETRIN-1 IN SENSORY AXONAL GUIDANCE IN HIGHER VERTEBRATES

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**Abstract :** During development, dorsal root ganglion (DRG) neurons in higher vertebrates extend their axons centrally to the spinal cord through the dorsal root entry zone (DREZ) and peripherally to muscle and skin targets. After entering the spinal cord, DRG axons project into the dorsal mantle layer. In this review, we focus on evidence showing the role for netrin-1 in forming sensory axonal trajectories. Netrin-1 is a diffusible axonal guidance molecule that chemorepels developing DRG axons. When DRG axons project toward the DREZ, ventral spinal cord-derived netrin-1 prevents DRG axons from projecting aberrantly toward the ventral spinal cord. At later stages, the dorsal spinal cord cells transiently express netrin-1. This dorsal spinal cord-derived netrin-1 prevents DRG axons from invading the dorsal spinal cord during the waiting period. Together, the data reviewed provide strong evidence that netrin-1 plays a crucial role in sensory axon projection during development.

**Key words :** netrin-1, dorsal root ganglion, axon guidance, spinal cord

### INTRODUCTION

Developing axons project toward their targets by attractive and repulsive guidance forces working in a contact-dependent or diffusible fashion<sup>1)</sup>. Sensory axons from dorsal root ganglion (DRG) neurons extend centrally toward the dorsolateral region of the spinal cord called the “dorsal root entry zone (DREZ)” (Fig. 1A). They enter the spinal cord exclusively through the DREZ, and never orient themselves toward the notochord or the ventral spinal cord because the notochord and the ventral spinal cord secrete unidentified molecules that have a chemorepulsive

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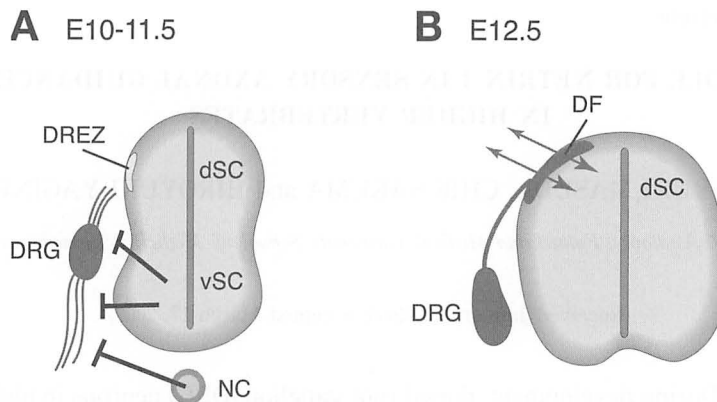


Fig. 1. Schematic transverse sections of E10-11.5 and E12.5 mouse embryos showing trajectories of sensory axons. A: At E10-11.5, the notochord (NC) and the ventral spinal cord (vSC) exert chemorepulsive activities to orient DRG axons to the DREZ in the dorsal spinal cord (dSC) and peripheral targets. B: At E12.5, DRG axons grow to the marginal zone of the dorsal spinal cord longitudinally (arrows) to form the dorsal funiculus (DF).

activity for DRG axons<sup>2</sup>).

After entering the spinal cord, DRG axons grow to the marginal zone of the spinal cord longitudinally and form the dorsal funiculus without projecting to the dorsal mantle layer for a few days, which time is called the “waiting period” (Fig. 1B)<sup>3</sup>. After this waiting period, proprioceptive DRG axons begin to send collaterals into the dorsal layers, and cutaneous axons project ventrally through the dorsal layers<sup>3</sup>. This event suggests the possibility that repulsive axonal guidance cues transiently prevent DRG collaterals from penetrating the dorsal spinal cord during the waiting period.

Netrin-1 plays a crucial role in axonal guidance events by attracting some axons via the Deleted in Colorectal Cancer (DCC) receptor and repelling others via Unc5 receptors<sup>4</sup>. However, it has not been clear whether netrin-1 plays a role in the guidance of DRG axons.

In this review, we describe recent advances in our understanding of netrin-1 as a chemorepulsive molecule for DRG axons to shape sensory axonal trajectories during development.

#### ROLE FOR NETRIN-1 IN THE VENTRAL SPINAL CORD

Loss-of-function experiments using mutant animals together with treatment with function-blocking antibodies have provided a better understanding of the molecular nature of the chemorepellents and their receptors involved in DRG axonal guidance.

Semaphorin 3A (Sema3A), a member of the semaphorin family, is the best-characterized axonal chemorepellent and it acts via the neuropilin-1 receptor<sup>5</sup>).

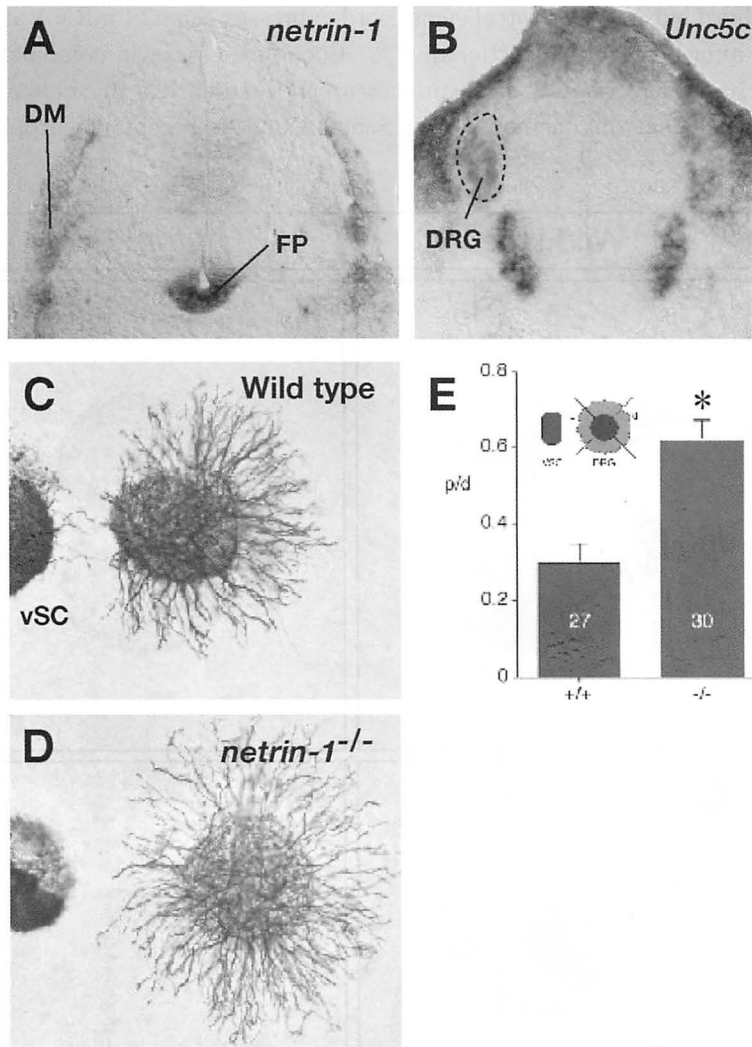


Fig. 2. Netrin-1 is an axon chemorepellent derived from the ventral spinal cord. A: At E10.5, *netrin-1* mRNA is expressed in the floor plate (FP) and dermamyotome (DM). B: At E10.5, the netrin receptor *Unc5c* is expressed in the DRG neurons (dashed circle). C, D: E11.5 mouse DRG explants were cocultured with E11.5 wild-type (C) or *netrin-1* mutant (D) ventral spinal cord explants (vSC). E: Quantification of chemorepulsive activity of *netrin-1* mutant or wild-type ventral spinal cord explants toward DRG axons. Axons from DRG explants were grouped into four quadrants: proximal, distal, and two lateral ones. The length of DRG axons in the proximal quadrant (p) was compared with that in the distal quadrant (d). The *p/d* value is a measure of repulsive activity, with a ratio of 0 and 1 indicating complete and no repulsion, respectively. The *p/d* value was significantly greater in the group cocultured with *netrin-1*-deficient ventral spinal cords. The bars represent the mean ± SEM, and the number of cocultures is shown in the bar. \**p* < 0.0001. Modified from Masuda *et al.* (2008) with permission.

The notochord but not the ventral spinal cord expresses *Sema3A* mRNA at the time that DRG axons extend bidirectionally<sup>6-8</sup>). Coculture assays in combination with tissues derived from *Sema3A*-deficient or *neuropilin-1*-deficient mice have provided direct evidence that the chemorepellent *Sema3A* and its receptor neuropilin-1 are

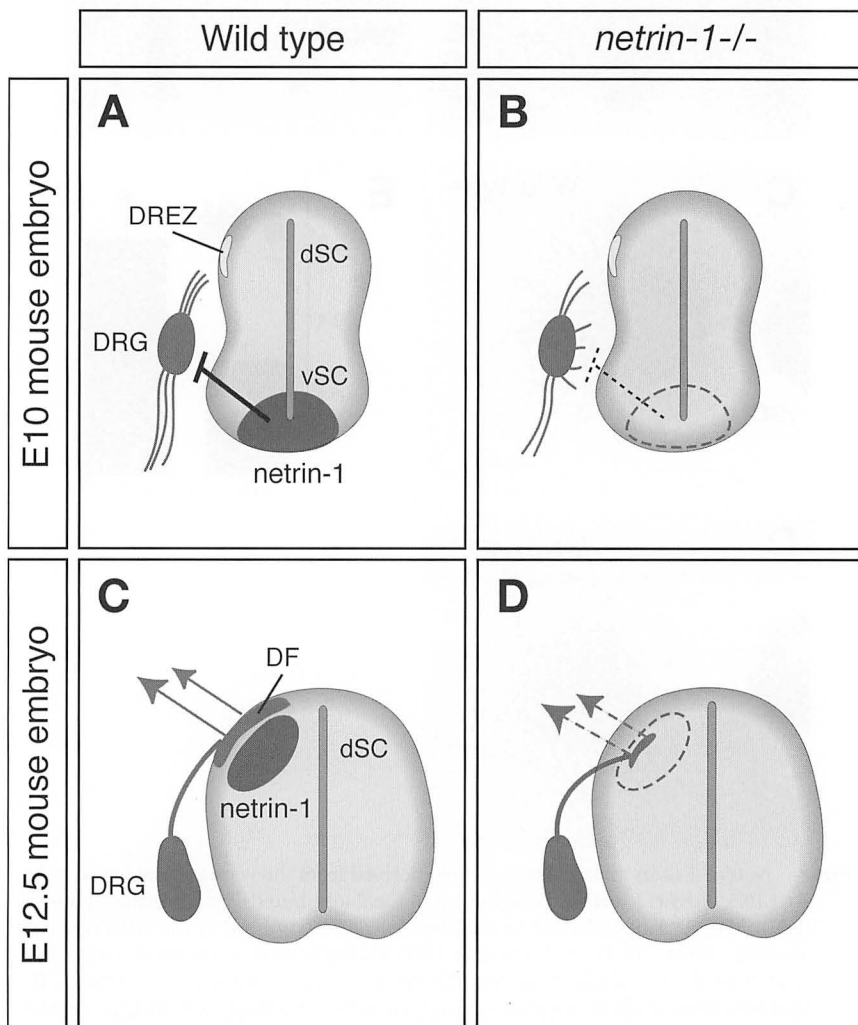


Fig. 3. Schematic diagrams of defects in trajectories of DRG axonal projection in *netrin-1* mutants. **A**: When DRG axons project toward the DREZ in the dorsal spinal cord (dSC), ventral spinal cord-derived netrin-1 chemorepels DRG axons to prevent them from orienting aberrantly toward the ventral spinal cord (vSC). **B**: In *netrin-1*-deficient embryos, some DRG axons become misoriented toward the ventral spinal cord because of the absence of netrin-1 there. **C**: At E12.5 when DRG axons grow to the marginal zone of the spinal cord longitudinally, *netrin-1* is transiently expressed in the dorsal spinal cord cells adjacent to the dorsal funiculus (DF). **D**: In *netrin-1*-deficient embryos, the dorsal funiculus is disorganized because DRG axons do not wait to invade the dorsal mantle layer.

required for mediating the notochord-derived chemorepulsion for DRG axons<sup>8</sup>.

In contrast to the chemorepulsion by the notochord, that by the ventral spinal cord for DRG axons is less clear in terms of its molecular nature. Cocultures using *Sema3A*-deficient ventral spinal cords or *neuropilin-1*-deficient DRG explants showed that chemorepulsion from the ventral spinal cord is independent of *Sema3A*/*neuropilin-1*<sup>8</sup>. So what molecules are responsible for the ventral spinal cord-derived repulsion of DRG axons? In the mouse embryo at embryonic day (E) 10–11.5, when DRG axons orient themselves toward and then reach the DREZ, *netrin-1* is strongly expressed in the floor plate of the ventral spinal cord (Fig. 2A)<sup>9</sup>. In addition, the repulsive netrin-1 receptor *Unc5c* is expressed in DRG neurons at the period between E10 and E13.5 (Fig. 2B)<sup>9,10</sup>. Cell and tissue cultures combined with tissues from *netrin-1*-deficient mice provide evidence that netrin-1 exerts a chemorepulsive activity for developing DRG axons and that the ventral spinal cord-derived repulsive activity depends on netrin-1 *in vitro* (Fig. 2C–E)<sup>9</sup>. Additional evidence for a chemorepulsive role of netrin-1 comes from the observation of DRG axons in *netrin-1*-deficient mice. In *netrin-1*-deficient embryos at E10, we found that some DRG axons misorient themselves toward the ventral spinal cord because of the absence of netrin-1 in the ventral spinal cord (Fig. 3A and B)<sup>9,10</sup>. The above findings lead us to the conclusion that ventrally derived netrin-1 prevents sensory axons from entering the ventral spinal cord.

#### ROLE FOR NETRIN-1 IN THE DORSAL SPINAL CORD

At E12.5 when DRG neurons extend their axons longitudinally along the dorsolateral margin of the spinal cord, *netrin-1* is expressed in the dorsolateral region adjacent to the DREZ (Fig. 3C), but the *netrin-1* expression is down-regulated in the dorsal spinal cord at E13.5 when many collaterals have entered the mantle layer<sup>10</sup>.

In *netrin-1*-deficient embryos at E12.5, the dorsal funiculus is disorganized because DRG axons do not wait to invade the dorsal mantle layer in the absence of netrin-1 adjacent to the dorsal funiculus (Fig. 3D)<sup>10</sup>. These results clearly show that netrin-1 in the dorsal spinal cord plays an important role to prevent DRG axons from penetrating the dorsal spinal cord during the waiting period.

#### CONCLUSION

At the beginning of this century, there was no evidence to show that netrin-1 has an influence on the extension of sensory axons. Recently, Ono's group and ours clearly showed that netrin-1 is an axon chemorepellent for DRG axons and plays a crucial role in their guidance during development. Discovering what molecules guide axons might offer novel therapeutic opportunities. The analyses of precise expression patterns of *netrin-1* before and after a spinal cord injury will reveal the therapeutic potential of netrin-1 in the axonal regeneration.

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## REFERENCES

1. Tessier-Lavigne M, Goodman CS. The molecular biology of axon guidance. *Science*, **274**: 1123-1133, 1996.
2. Masuda T, Shiga T. Chemorepulsion and cell adhesion molecules in patterning initial trajectories of sensory axons. *Neurosci Res*, **51**: 337-347, 2005.
3. Ozaki S, Snider WD. Initial trajectories of sensory axons toward laminar targets in the developing mouse spinal cord. *J Comp Neurol*, **380**: 215-229, 1997.
4. Moore SW, Tessier-Lavigne M, Kennedy TE. Netrins and their receptors. *Adv Exp Med Biol*, **621**: 17-31, 2007.
5. Raper JA. Semaphorins and their receptors in vertebrates and invertebrates. *Curr Opin Neurobiol*, **10**: 88-94, 2000.
6. Wright DE, White FA, Gerfen RW, Silos-Santiago I, Snider WD. The guidance molecule Semaphorin III is expressed in regions of spinal cord and periphery avoided by growing sensory axons. *J Comp Neurol*, **361**: 321-333, 1995.
7. Shepherd I, Luo Y, Raper JA, Chang S. The distribution of collapsin-1 mRNA in the developing chick nervous system. *Dev Biol*, **173**: 185-199, 1996.
8. Masuda T, Tsuji H, Taniguchi M, Yagi T, Tessier-Lavigne M, Fujisawa H, Okado N, Shiga T. Differential non-target-derived repulsive signals play a critical role in shaping initial axonal growth of dorsal root ganglion neurons. *Dev Biol*, **254**: 289-302, 2003.
9. Masuda T, Watanabe K, Sakuma C, Ikenaka K, Ono K, Yaginuma H. Netrin-1 acts as a repulsive guidance cue for sensory axonal projections toward the spinal cord. *J Neurosci*, **28**: 10380-10385, 2008.
10. Watanabe K, Tamamaki N, Furuta T, Ackerman SL, Ikenaka K, Ono K. Dorsally derived netrin 1 provides an inhibitory cue and elaborates the 'waiting period' for primary sensory axons in the developing spinal cord. *Development*, **133**: 1379-1387, 2006.