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Neuropilin-mediated neural crest cell guidance is essential to organise sensory neurons into segmented dorsal root ganglia

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The peripheral nervous system (PNS) of higher vertebrates is segmented to align the spinal nerve roots with the vertebrae. This co-patterning is set up during embryogenesis, when vertebrae develop from the sclerotome layer of the metameric somites, and PNS neurons and glia differentiate from neural crest cells (NCCs) that preferentially migrate into the anterior sclerotome halves. Previous analyses of mice deficient in the class 3 semaphorin (SEMA3) receptors neuropilin (NRP) 1 or 2 raised the possibility that each controlled a distinct aspect of trunk NCC migration. We now demonstrate that both pathways act sequentially in distinct NCC subpopulations and thereby cooperate to enforce segmental NCC migration. Specifically, SEMA3A/NRP1 signalling first directs one population of NCCs from the intersomitic path into the sclerotome, and SEMA3F/NRP2 signalling acts subsequently to restrict a second population to the anterior half of the sclerotome. NCC exclusion from either the posterior sclerotome or the intersomitic boundary is sufficient to enforce the separation of neighbouring NCC streams and the segregation of sensory NCC progeny into metameric dorsal root ganglia (DRG). By contrast, the combined loss of both guidance pathways leads to ectopic invasion of the intersomitic furrows and posterior sclerotome halves, disrupting metameric NCC streaming and DRG segmentation.

KEY WORDS: Dorsal root ganglia, Neural crest cell, Neuropilin, Segmentation, Semaphorin, Sensory neurons, Mouse

INTRODUCTION

The division of embryonic tissues into segments is a fundamental patterning process in early vertebrate development. It is most obvious in the trunk, where mesoderm on either side of the neural tube forms metameric epithelial spheres termed somites. Whereas the sclerotome layer of the somites gives rise to the spinal vertebrae, the dermomyotome layer differentiates into dermis and epaxial muscle (reviewed by Brent and Tabin, 2002). In higher vertebrates, the posterior half of each sclerotome is repulsive for motor axons and the neural crest cell (NCC) precursors of trunk sensory and sympathetic ganglia, forcing them to migrate preferentially through the anterior sclerotome (reviewed by Le Douarin and Kalcheim, 1999). This pattern of NCC migration was previously thought to lay the foundation for the segmental organisation of their neuronal progeny (e.g. Kalcheim and Teillet, 1989; Kuan et al., 2004). However, loss of the repulsive guidance cue SEMA3F or its receptor NRP2 in the mouse causes NCC invasion into both anterior and posterior sclerotome without disrupting the metameric pattern of the sympathetic or dorsal root ganglia (DRG) (Gammill et al., 2006; Waimey et al., 2008). We have since shown that the related SEMA3A/NRP1 pathway provides an alternative mechanism for trunk NCC guidance (Schwarz et al., 2009). Specifically, loss of SEMA3A or NRP1 leads to excessive NCC migration along intersomitic and perisomitic routes and consequently to ectopic sensory and sympathetic neuron differentiation. However, this defect does not abolish metameric NCC migration and therefore perturbs DRG segmentation only mildly. The presence of either SEMA3A/NRP1 or SEMA3F/NRP2 signalling is therefore sufficient for the formation of segmented DRG. We now show that the simultaneous loss of both signalling pathways severely disrupts metameric NCC streaming and DRG segmentation. Thus, trunk NCCs in compound mutants lacking either the two ligands or the two receptors invade both intersomitic furrows and the posterior sclerotome halves, and the sensory NCC progeny consequently coalesce into a continuous neuronal band adjacent to the neural tube, rather than forming separate DRG. These findings are of great significance for our understanding of peripheral nervous system organisation and will help to elucidate the developmental mechanisms that coordinate the organisation of NCC-derived sensory neurons with the sclerotome-derived vertebrae.

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MATERIALS AND METHODS

Mice carrying a *Sema3a-* (Taniguchi et al., 1997) or *Sema3f-* (Sahay et al., 2003) null allele or mutations that disrupt semaphorin signalling through both neuropilins (Gu et al., 2003) have been described. In situ hybridisation was performed with digoxigenin-labelled riboprobes transcribed from plasmids containing cDNA for *Isl1* (Pfaff et al., 1996), *Nrp1* or *Sox10* (Schwarz et al., 2008a). For immunolabelling, we used goat anti-NRP1 and goat anti-NRP2 (R&D Systems), rabbit anti-p75, rabbit anti-neurofilament and rat anti-endomucin (Schwarz et al., 2008a).

RESULTS AND DISCUSSION

Distinct contribution of NRP1 and NRP2 to NCC guidance

Trunk NCCs form by delamination from the embryonic neuroectoderm and disseminate into the body along specific pathways. These pathways are characterised by their relationship to the emerging somites, which are added to the elongating embryo in a rostrocaudal fashion (reviewed by Le Douarin and Kalcheim, 1999). In the mouse, a small population of early-born, NRP1-negative NCCs normally travels ventrally in the space between newly formed somites towards the dorsal aorta to seed the sympathetic ganglia (see Fig. S1 in the supplementary material)

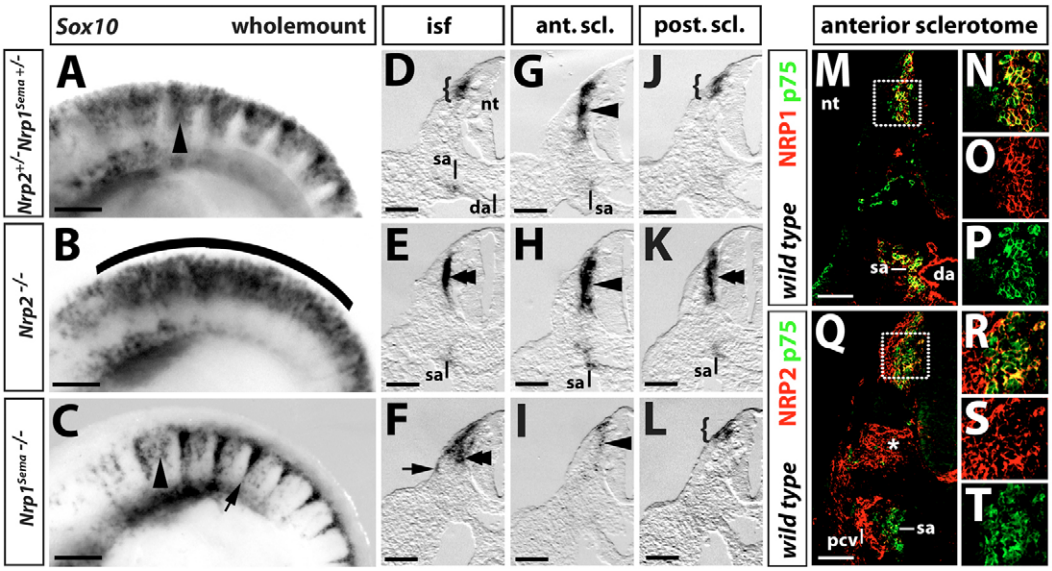

Fig. 1. Different roles for NRP1 and NRP2 in trunk NCC migration. (**A-L**) In situ hybridisation at 9.5 dpc for *Sox10*. (A-C) Lateral view of wholemount mouse embryo trunks (rostral to the left). (D-L) Transverse sections at the level of the intersomitic furrow (isf), anterior and posterior sclerotome (scl). NCCs migrated normally into the anterior sclerotome (arrowheads in A,G), but accumulated in the migration staging area next to the neural tube (nt) at the level of the intersomitic furrow and posterior sclerotome (brackets in D,J). NCCs clustered into sympathetic anlagen (sa) at the dorsal aorta (da). In *Nrp2*-null mutants, NCCs migrated in an almost continuous band along the rostrocaudal axis (curved line in B), but maintained a ventromedial path (arrowhead in H, double arrowheads in E,K). In *Nrp1*^{Sema} mutants, excess NCCs travelled through the intersomitic furrow (arrow in C) on dorsolateral and ventromedial paths (arrow and double arrowhead, respectively, in F); few NCCs migrated into the anterior sclerotome at this stage (arrowhead in I). (**M-T**) Transverse sections at the level of the anterior sclerotome immunolabelled for p75 and NRP1 (M-P) or NRP2 (Q-T); yellow indicates colocalisation. NRP1 and NRP2 were co-expressed with p75 in the DRG anlagen (boxed areas, which are shown at higher magnification in N-P and R-T). NRP1, but not NRP2, was prominent in the sympathetic anlagen. NRP2 was also prominent in the anterior sclerotome (asterisk) and posterior cardinal vein (pcv), whereas NRP1 was also expressed by the dorsal aorta (da). Scale bars: 250 μm in A-C; 100 μm in D-L,M,Q.

(Schwarz et al., 2009). By contrast, most other NCCs that give rise to neurons and glia in the trunk migrate through the anterior half of the somite, after it has segregated into sclerotome and dermomyotome layers. The onset of this migration pattern correlates with NRP1 expression by NCCs and SEMA3A expression in their environment (Schwarz et al., 2009). Moreover, NRP1 and its ligand SEMA3A are essential to prevent trunk NCC migration through the intersomitic furrow and to keep NCCs on a ventromedial path (Schwarz et al., 2009). Consistent with the phenotype of *Sema3a*- and *Nrp1*-null mutants, a mutation that reduces semaphorin signalling through NRP1 (Gu et al., 2003) leads to excessive intersomitic NCC migration, with entry of NCCs into the dorsolateral path (Fig. 1C,F) (Schwarz et al., 2009). By contrast, SEMA3F and its receptor NRP2 exclude NCCs from the posterior sclerotome (Fig. 1B,H) (Gammill et al., 2006). Significantly, we found that NRP2 signalling is not essential to confine NCC migration to the correct dorsoventral plane (Fig. 1E,H,K). Together, these observations raise the possibility that SEMA3A/NRP1 and SEMA3F/NRP2 synergise to control distinct aspects of trunk NCC guidance, perhaps by affecting different NCC subpopulations.

To address whether NRP1 and NRP2 preferentially guide different trunk NCC subpopulations, we compared their expression pattern to that of the nerve growth factor receptor p75 (NGFR), a good marker for NCCs with neuronal or glial potential (Fig. 1M-T; the plane of sectioning is illustrated in Fig. S1A in the supplementary material). We observed that sympathetic progenitors preferentially expressed NRP1 over NRP2 (compare Fig. 1M with 1Q). By contrast, both neuropilins appeared to be expressed in NCCs in the anterior sclerotome (Fig. 1N-P,R-T). However, the high level of NRP2 in the anterior sclerotome (Fig. 1Q; Fig. 2B) precluded the unequivocal assignment of NRP2 expression to trunk NCCs in situ, as previously discussed (Gammill et al., 2006). We therefore examined whether NRP2 colocalised with p75 in *Sema3f*-null embryos, which contain ectopic NCCs in the NRP2-negative posterior sclerotome (Fig. 2B,H). Using this genetic manipulation, we could clearly identify NRP2 in NCCs (Fig. 2I-L). Interestingly, NCCs within the sclerotome appeared to be heterogeneous with respect to NRP2 levels, as cells were stained with variable intensity in the anterior sclerotome of wild types and in the posterior sclerotome of *Sema3f*-null mutants (Fig. 2F,L). It is possible that the intermixing of high- and low-level NRP2-expressing NCC populations is due to a community effect, in which subtypes of NCCs remain in physical contact while they migrate within the sclerotome (Kulesa and Fraser, 1998).

We next examined NRP2 expression in *Sema3a*-null mutants, which display excessive NCC migration on the intersomitic route that is normally taken only by the early wave of sympathetic NCC precursors (Schwarz et al., 2009). We found that the mutant intersomitic stream predominantly contained NCCs with undetectable NRP2 expression (Fig. 2M-R). By contrast, the ectopic intersomitic stream prominently expressed NRP1 (Fig. 2S-X), suggesting that NRP1, but not NRP2, is a marker for intermediate wave NCC precursors with sympathetic potential (see Fig. S1B in the supplementary material). In support of this idea, *Nrp1*-null, but not *Nrp2*-null mutants contain ectopic sympathetic neurons (Kawasaki et al., 2002; Waimey et al., 2008).

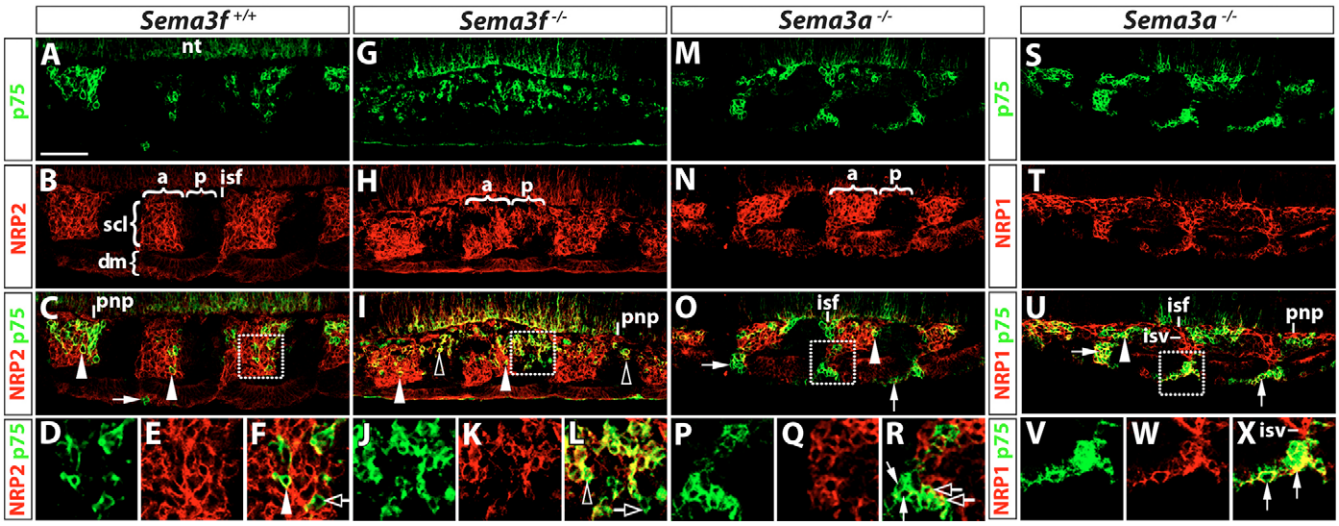

Fig. 2. Neuropilin expression during trunk NCC migration in semaphorin mutants. Longitudinal sections of mouse embryo trunks at 9.5 dpc, immunolabelled for p75 and NRP2 (A-R) or NRP1 (S-X); yellow indicates colocalisation. The boxed areas are shown at higher magnification below the corresponding panel. (**A-F**) NCCs in the anterior sclerotome appeared positive for NRP2 (arrowheads in C), but staining was more prominent in some NCCs than others (compare cells indicated by the arrowhead and arrow in F). Rare NCCs in the perisomitic space were NRP2 negative (arrow in C). (**G-L**) In *Sema3f* mutants, NCCs migrated into both anterior and posterior sclerotome (white and open arrowheads, respectively, in I); ectopic NRP2 staining in the posterior sclerotome localised to NCCs (open arrowheads in I; see also J-L). In both sclerotome halves, there were NRP2-positive NCCs (white and open arrowheads in I). Staining was prominent in some NCCs, but faint in others (indicated by an open arrowhead and arrow, respectively, in L). (**M-X**) In *Sema3a* mutants, few NCCs migrated into the anterior sclerotome (arrowheads in O,U), as most NCCs were rerouted into the intersomitic/perisomitic space (boxed areas; arrows in O,U). NCCs in the anterior sclerotome were NRP2 positive (open arrows in R), but ectopic NCCs in the intersomitic furrow did not express NRP2 at detectable levels (white arrows in R). By contrast, ectopic NCCs were positive for NRP1 (arrows in X). Intersomitic vessels were also NRP1 positive. a, anterior; p, posterior; scl, sclerotome; dm, dermomyotome; pnp, perineural vessel plexus; isv, intersomitic vessels. Scale bar: 100 μm.

NRP1 and NRP2 pathways cooperate to guide different subpopulations of trunk NCCs

Taking advantage of a mouse strain that carries the *Nrp1*^{Sema} mutation on a *Nrp2*-null background and preserves VEGF signalling through NRP1 (Gu et al., 2003) and hence does not disrupt vascular patterning (Vieira et al., 2007), we next asked how the simultaneous loss of both neuropilins affected trunk NCC migration. In these compound *Nrp1*^{Sema} *Nrp2* null mutants, neither intersomitic migration nor posterior sclerotome invasion was aggravated relative to single mutants (Fig. 3). Rather, p75 labelling demonstrated an excessive migration of NCCs alongside blood vessels in the intersomitic furrow and the perisomitic space, as seen in single *Sema3a*- and *Nrp1*-null mutants, but the remaining NCCs entered the sclerotome with no preference for the anterior or posterior half, as in single *Sema3f*- and *Nrp2*-null mutants (Fig. 3A,B).

The analysis of compound *Nrp1*^{Sema} *Nrp2* null mutants by wholemount *Sox10* in situ hybridisation further revealed a consistent heterogeneity of NCC defects along the rostrocaudal axis (Fig. 3C-F). Thus, excessive NCC migration in the intersomitic furrow and alongside perisomitic vessels, typical of *Nrp1*-null mutants, was prominent in the caudal trunk (arrows in Fig. 3C). By contrast, the indiscriminate migration through both anterior and posterior sclerotome, characteristic of *Nrp2*-null mutants, was obvious in the rostral trunk (curved line in Fig. 3C). Transverse sections at forelimb level, where both migratory patterns overlapped at 9.5 dpc, established that the ectopic NCC streams followed the same dorsoventral trajectory as in single mutants; thus, the intersomitic stream travelled on both dorsolateral and ventromedial paths, as in single *Nrp1*- and *Sema3a*-null mutants (Fig. 3D), whereas NCCs in the anterior and posterior sclerotome were confined to a ventromedial path, as in single *Nrp2*- and *Sema3f*-null mutants (compare Fig. 3I,J with Fig. 3E,F). Consistent with previous studies on ligand-receptor pairings in axon guidance (Schwarz et al., 2008b; Yaron et al., 2005), compound *Sema3a Sema3f* null mutants precisely phenocopied the defects of compound *Nrp1*^{Sema} *Nrp2* null mutants (compare Fig. 3C-F with Fig. 3G-J). Because the caudal trunk is younger than the rostral trunk, the variation of NCC phenotype across the rostrocaudal axis in compound mutants implies that SEMA3A/NRP1 signalling acts first to direct NCCs from the intersomitic path into the sclerotome, and that SEMA3F/NRP2 signalling subsequently restricts NCC migration to the anterior sclerotome. We therefore conclude that NRP1 and NRP2 do not act redundantly at the level of individual NCCs, but cooperate to pattern NCC migration because of their functional requirement in distinct NCC subpopulations (see Fig. S1B in the supplementary material).

Loss of semaphorin signalling through NRP1 and NRP2 abolishes DRG segmentation

Even though NCC migration is severely affected in *Nrp1*- and *Nrp2*-null mutants, and *Nrp1*-null mutants have ectopic sensory neurons, DRG segmentation is preserved in both types of single mutants (Gammill et al., 2006; Schwarz et al., 2009). These findings suggest that the maintenance of a NCC-free region either in the intersomitic furrow or the posterior sclerotome is sufficient to ensure the segmentation of the sensory NCC progeny. To test whether DRG separation is compromised if both neuropilin-mediated mechanisms of NCC guidance are lost in tandem, we examined compound *Nrp1*^{Sema} *Nrp2* null mutants by in situ hybridisation with probes for

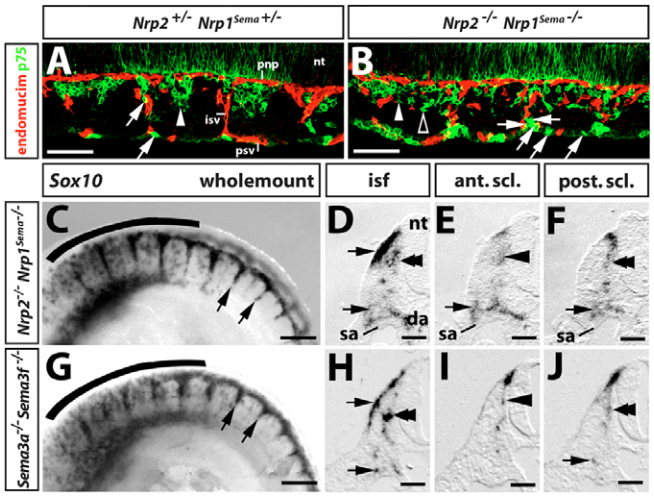

Fig. 3. NRP1 and NRP2 cooperate to guide different populations of trunk NCCs. (**A**,**B**) Longitudinal sections of 9.5 dpc mouse embryo trunks were immunolabelled for p75 and endomucin. (A) Most NCCs migrated in the anterior sclerotome (arrowhead) and few in the intersomitic/perisomitic space (arrows). (B) In compound *Nrp1*^{Sema} *Nrp2* mutants, many NCCs migrated in the intersomitic/perisomitic space (arrows), and the remaining NCCs invaded both anterior and posterior sclerotome (white and open arrowheads, respectively). (**C-J**) *Sox10* in situ hybridisation in wholemounts (rostral to the left) and transverse sections at limb level. In compound null mutants, NCCs preferentially migrated through the intersomitic furrow at caudal levels, but through both anterior and posterior sclerotome at rostral levels (arrows and curved lines, respectively, in C,G). At forelimb level, both ectopic migratory patterns overlapped, with excess NCCs in the intersomitic furrow (arrows and double arrowhead in D,H) and invasion of both anterior and posterior sclerotome halves (arrowheads in E,I and double arrowheads in F,J). pnp, perineural vessel plexus; isv and psv, intersomitic and perisomitic vessels; isf, intersomitic furrow; sa, sympathetic anlage; da, dorsal aorta; nt, neural tube; fl, forelimb. Scale bars: 100 μm, except 250 μm in C,G.

the sensory neuron markers *Isl1* (Ericson et al., 1992) and *Brn3a* (*Pou4f1* – Mouse Genome Informatics) (Eng et al., 2004). Whereas DRG neurons were organised into metameric ganglia in single mutants (Fig. 4B,C), they were arranged in a continuous band in compound mutants, and DRG therefore appeared fused (Fig. 4D,H and data not shown). There were a few sensory-neuron-free spaces along the rostrocaudal axis in compound mutants, and these correlated with the position of blood vessels; however, their presence was not sufficient to enforce proper segmentation of the DRG (Fig. 4D,H). Wholemount neurofilament labelling further confirmed that the DRG were not segmented (see Fig. S2 in the supplementary material).

In contrast to the DRG, the spinal nerves of compound mutants did not lose their segmentation. Thus, the spinal nerves between the fore- and hindlimbs maintained a metameric pattern, even though they were severely defasciculated (Fig. 4L), as previously shown [fig. 3S in Huber et al. (Huber et al., 2005)]. Similarly, sensory axons projecting from the ventral and dorsal rami of the spinal nerves towards the skin emerged in a segmental pattern, despite prominent defasciculation and precocious growth (see Fig. S2 in the supplementary material). The segmentation of spinal nerve axons is thought to be achieved through a combination of contact repulsion by posterior somite cells and chemoattractive cues from anterior somite cells (reviewed by Kuan et al., 2004). The preservation of

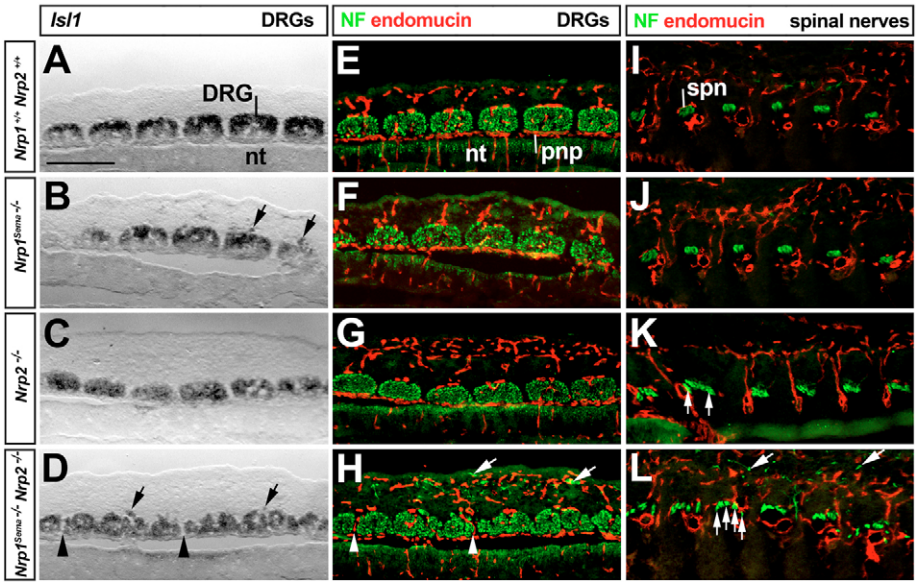

Fig. 4. Peripheral nervous system organisation in the absence of semaphorin signalling through neuropilins. Longitudinal sections of 11.5 dpc mouse embryo trunks labelled by in situ hybridisation for *Isl1*, followed by immunolabelling for neurofilaments (NF) and endomucin. (**A-H**) In wild types and single mutants, DRG were clearly separated, but appeared fused along the rostrocaudal axis in compound null mutants; the occasional separation of neighbouring DRG (arrowheads in D) correlated with the presence of vessels (arrowheads in H). In *Nrp1*^{Sema} and compound null mutants, a few neurons were positioned more laterally than normal (arrows in B,D), and many neurofilament-positive ectopic axons were present in the periphery of compound null mutants (arrows in H,L). (**I-L**) The spinal nerves appeared segmented, but were slightly defasciculated in *Nrp2*-null mutants and severely defasciculated in compound null mutants (arrows in K and L, respectively). pnp, perineural plexus; nt, neural tube; spn, spinal nerve. Scale bar: 250 μm.

spinal nerve segmentation is therefore consistent with the finding that rostrocaudal somite polarity is not lost in semaphorin or neuropilin mutants (Fig. 2N) (Gammill et al., 2006).

Importantly, the compound *Nrp1*^{Sema} *Nrp2* null mouse is the first model in which it has been shown directly that loss of DRG segmentation is due to defective NCC patterning. Previously, loss of DRG segmentation has been observed in mouse mutants with defects in somite polarity. For example, mice lacking the transcription factor UNCX (previously known as UNC4.1) show defects in specific aspects of rostrocaudal somite polarity that are crucial for sclerotome patterning (Leitges et al., 2000; Mansouri et al., 2000). The observation that somitic *Sema3a* expression is perturbed in these mutants (Mansouri et al., 2000) raises the possibility that UNCX controls semaphorin expression to coordinate the position of NCCs with sclerotome derivatives. It would therefore be interesting to examine whether *Sema3f* expression is also affected in *Uncx*-null mutants, because loss of SEMA3A/NRP1 signalling disrupts DRG segmentation only when SEMA3F/NRP2 is lost in tandem (Fig. 3).

Loss of DRG segmentation has also been observed in chick embryos, in which caudal half-somites were replaced with rostral half-somites (Kalcheim and Teillet, 1989). Because this experiment disrupted the rostrocaudal distribution of NCCs within the somites (Stern and Keynes, 1987), it led to the hypothesis that the rostrocaudal polarity of NCC migration lays the foundation for DRG segmentation. However, the intrasomitic patterning of NCC migration is not essential for DRG segmentation (Gammill et al., 2006). Because the juxtaposition of two rostral or caudal half-somites also perturbs intersomitic boundary formation (Stern and Keynes, 1987), we propose that the DRG phenotype exhibited upon transplantation of multiple rostral half-somites is similar to that of compound *Nrp1*^{Sema} *Nrp2* null mutants, as intra- and intersomitic NCC guidance pathways are simultaneously disrupted in both experimental models.

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Supplementary material

Supplementary material available online at
http://dev.biologists.org/cgi/content/full/136/11/1785/DC1

References

Brent, A. E. and Tabin, C. J. (2002). Developmental regulation of somite derivatives: muscle, cartilage and tendon. *Curr. Opin. Genet. Dev.* **12**, 548-557.

Eng, S. R., Lanier, J., Fedtsova, N. and Turner, E. E. (2004). Coordinated regulation of gene expression by Brn3a in developing sensory ganglia. *Development* **131**, 3859-3870.

Ericson, J., Thor, S., Edlund, T., Jessell, T. M. and Yamada, T. (1992). Early stages of motor neuron differentiation revealed by expression of homeobox gene Islet-1. *Science* **256**, 1555-1560.

Gammill, L. S., Gonzalez, C., Gu, C. and Bronner-Fraser, M. (2006). Guidance of trunk neural crest migration requires neuropilin 2/semaphorin 3F signaling. *Development* **133**, 99-106.

Gu, C., Rodriguez, E. R., Reimert, D. V., Shu, T., Fritzsch, B., Richards, L. J., Kolodkin, A. L. and Ginty, D. D. (2003). Neuropilin-1 conveys semaphorin and VEGF signaling during neural and cardiovascular development. *Dev. Cell* **5**, 45-57.

Huber, A. B., Kania, A., Tran, T. S., Gu, C., De Marco Garcia, N., Lieberam, I., Johnson, D., Jessell, T. M., Ginty, D. D. and Kolodkin, A. L. (2005). Distinct roles for secreted semaphorin signaling in spinal motor axon guidance. *Neuron* **48**, 949-964.

Kalcheim, C. and Teillet, M. A. (1989). Consequences of somite manipulation on the pattern of dorsal root ganglion development. *Development* **106**, 85-93.

Kawasaki, T., Bekku, Y., Suto, F., Kitsukawa, T., Taniguchi, M., Nagatsu, I., Nagatsu, T., Itoh, K., Yagi, T. and Fujisawa, H. (2002). Requirement of neuropilin 1-mediated Sema3A signals in patterning of the sympathetic nervous system. *Development* **129**, 671-680.

Kuan, C. Y., Tannahill, D., Cook, G. M. and Keynes, R. J. (2004). Somite polarity and segmental patterning of the peripheral nervous system. *Mech. Dev.* **121**, 1055-1068.

Kulesa, P. M. and Fraser, S. E. (1998). Neural crest cell dynamics revealed by time-lapse video microscopy of whole embryo chick explant cultures. *Dev. Biol.* **204**, 327-344.

Le Douarin, N. M. and Kalcheim, C. (1999). *The Neural Crest*. New York: Cambridge University Press.

Leitges, M., Neidhardt, L., Haenig, B., Herrmann, B. G. and Kispert, A. (2000). The paired homeobox gene Uncx4.1 specifies pedicles, transverse processes and proximal ribs of the vertebral column. *Development* **127**, 2259-2267.

Mansouri, A., Voss, A. K., Thomas, T., Yokota, Y. and Gruss, P. (2000). Uncx4.1 is required for the formation of the pedicles and proximal ribs and acts upstream of Pax9. *Development* **127**, 2251-2258.

Pfaff, S. L., Mendelsohn, M., Stewart, C. L., Edlund, T. and Jessell, T. M. (1996). Requirement for LIM homeobox gene Isl1 in motor neuron generation reveals a motor neuron-dependent step in interneuron differentiation. *Cell* **84**, 309-320.

Sahay, A., Molliver, M. E., Ginty, D. D. and Kolodkin, A. L. (2003). Semaphorin 3F is critical for development of limbic system circuitry and is required in neurons for selective CNS axon guidance events. *J. Neurosci.* **23**, 6671-6680.

Schwarz, Q., Vieira, J. M., Howard, B., Eickholt, B. J. and Ruhrberg, C. (2008a). Neuropilin 1 and 2 control cranial gangliogenesis and axon guidance through neural crest cells. *Development* **135**, 1605-1613.

Schwarz, Q., Waimey, K. E., Golding, M., Takamatsu, H., Kumanogoh, A., Fujisawa, H., Cheng, H. J. and Ruhrberg, C. (2008b). Plexin A3 and plexin A4 convey semaphorin signals during facial nerve development. *Dev. Biol.* **324**, 1-9.

Schwarz, Q., Maden, C., Vieira, J. M. and Ruhrberg, C. (2009). Neuropilin 1 signaling guides neural crest cells to coordinate pathway choice with cell specification. *Proc. Natl. Acad. Sci. USA* doi:10.1073/pnas.0811521106.

Stern, C. D. and Keynes, R. J. (1987). Interactions between somite cells: the formation and maintenance of segment boundaries in the chick embryo. *Development* **99**, 261-272.

Taniguchi, M., Yuasa, S., Fujisawa, H., Naruse, I., Saga, S., Mishina, M. and Yagi, T. (1997). Disruption of semaphorin III/D gene causes severe abnormality in peripheral nerve projection. *Neuron* **19**, 519-530.

Vieira, J. M., Schwarz, Q. and Ruhrberg, C. (2007). Selective requirements for neuropilin ligands in neurovascular development. *Development* **134**, 1833-1843.

Waimey, K. E., Huang, P. H., Chen, M. and Cheng, H. J. (2008). Plexin-A3 and plexin-A4 restrict the migration of sympathetic neurons but not their neural crest precursors. *Dev. Biol.* **315**, 448-458.

Yaron, A., Huang, P. H., Cheng, H. J. and Tessier-Lavigne, M. (2005). Differential requirement for Plexin-A3 and -A4 in mediating responses of sensory and sympathetic neurons to distinct class 3 Semaphorins. *Neuron* **45**, 513-523.