Developmental Biology 324 (2008) 1–9

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Plexin A3 and plexin A4 convey semaphorin signals during facial nerve development

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ARTICLE INFO

Article history:
Received for publication 4 May 2008
Revised 16 August 2008
Accepted 18 August 2008
Available online 3 September 2008

Keywords:
Facial nerve
Facial branchiomotor neuron
Facial visceromotor neuron
Greater superficial petrosal nerve
Axon guidance
Neuropilin
Plexin
Semaphorin
SEMA3A
SEMA3F

ABSTRACT

In vertebrates, class 3 semaphorins (SEMA3) control axon behaviour by binding to neuronal cell surface receptors composed of a ligand binding subunit termed neuropilin (NRP) and a signal transduction subunit of the A-type plexin family (PLXNA). We have determined the requirement for SEMA3/NRP/PLXN signalling in the development of the facial nerve, which contains axons from two motor neuron populations, branchiomotor and visceromotor neurons. Loss of either SEMA3A/NRP1 or SEMA3F/NRP2 caused defasciculation and ectopic projection of facial branchiomotor axons. In contrast, facial visceromotor axons selectively required SEMA3A/NRP1. Thus, the greater superficial petrosal nerve was defasciculated, formed ectopic projections and failed to branch in its target area when either SEMA3A or NRP1 were lost. To examine which A-type plexin conveyed SEMA3/neuropilin signals during facial nerve development, we combined an expression analysis with loss of function studies. Even though all four A-type plexins were expressed in embryonic motor neurons, PLXNA1 and PLXNA2 were not essential for facial nerve development. In contrast, loss of PLXNA4 phenocopied the defects of SEMA3A and NRP1 mutants, and loss of PLXNA3 phenocopied the defects of SEMA3F and NRP2 mutants. The combined loss of PLXNA3 and PLXNA4 impaired facial branchiomotor axon guidance more severely than loss of either plexin alone, suggesting that SEMA3A and SEMA3F signals, even though both essential, are partially redundant.

Introduction

The brainstem of the adult vertebrate brain contains several different types of motor neurons that control important physiological processes, for example feeding, speech and eye movement. These motor neurons can be classified into subtypes according to their synaptic targets and the projection pattern of their axons as they leave the brain. The axons of branchiomotor neurons innervate muscles within the branchial arches, whilst the axons of visceromotor neurons innervate parasympathetic ganglia and smooth muscles. The somatic motor neurons, on the other hand, innervate muscles derived from paraxial or prechordal mesoderm. As they leave the hindbrain, the axons of visceromotor and branchiomotor neurons converge on shared dorsal exit points, whereas the axons of most somatic motor neurons exit ventrally. The anatomical organisation and function of these brainstem nerves is set up during embryonic development, when hindbrain motor neurons develop in lineage-restricted compartments termed rhombomeres and express selective subsets of transcription factors to control the responsiveness of their axons to environmental guidance cues (reviewed by Cordes, 2001). Thus, axon migration within the hindbrain is governed by anteroposterior and dorsoventral cues that guide axons to defined exit points, whilst axon migration outside the hindbrain is controlled by repulsive cues that surround the nerve path in combination with attractive target-derived cues. In combination, these patterning mechanisms ensure that the axons of hindbrain motor neurons are wired appropriately to perform their adult functions. The identification of genes that control axonal patterning of the cranial nerves during embryogenesis therefore enhances our understanding of congenital abnormalities such as Duane syndrome and congenital facial nerve palsy, and in the future may also contribute to improved diagnosis of these conditions (reviewed by Traboulsi, 2007). In addition, the brainstem motor neurons present particularly good model systems to study the molecular mechanisms of axon guidance, as the anatomically well-defined arrangement of their cell bodies within the rhombomeres of the developing brain facilitates the identification of axon guidance receptors that are used by specific cranial motor nerves (Fig. 1B; see, for example, Auclair et al., 1996; Jacob and Guthrie, 2000; Lumsden and Keynes, 1989; Studer et al., 1996).

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doi:10.1016/j.ydbio.2008.08.020

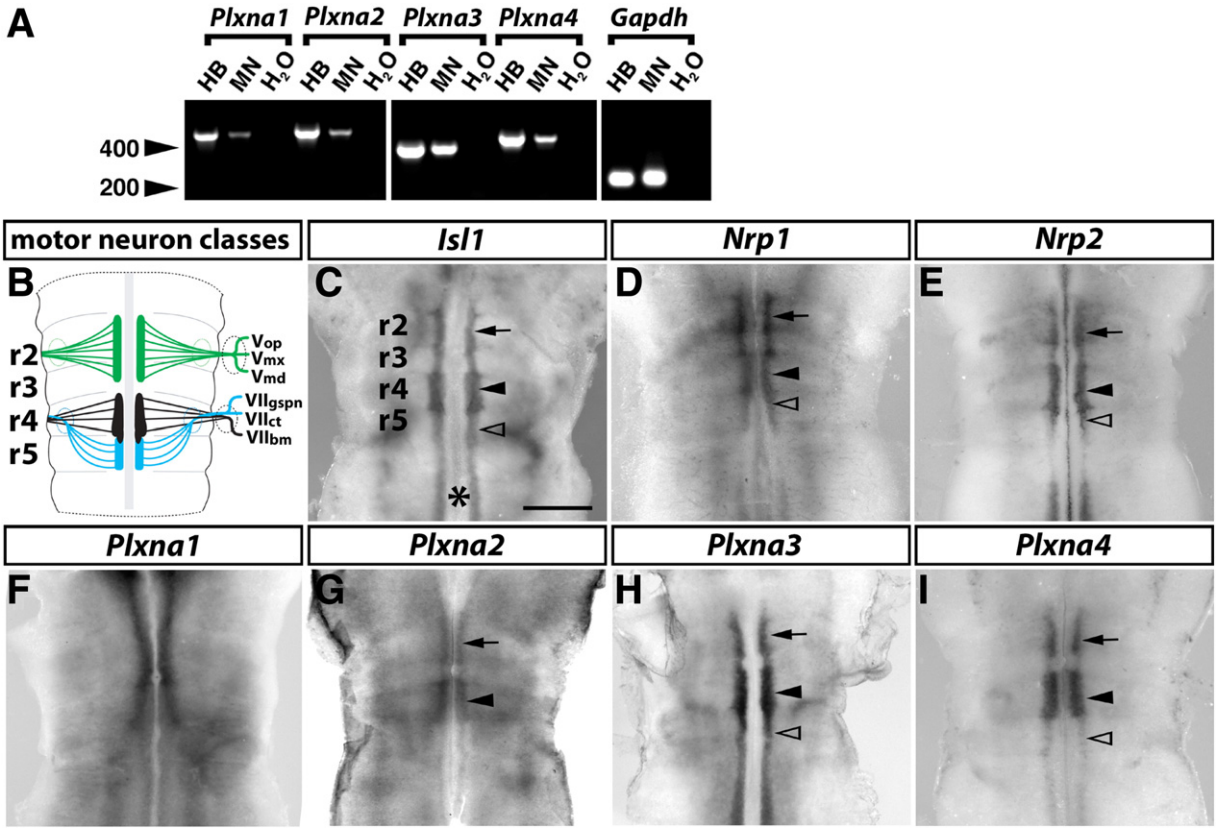

Fig. 1. A-type plexin expression in motor neurons. (A) RT-PCR analysis of mouse hindbrain tissue (HB), primary rat embryonic motor neurons (MN) and a negative control (H_2O) for *Plxna1-a4* and a *Gapdh* control; molecular weight marker: 200 and 400 bp (arrowheads). (B) Motor neuron populations in the mouse hindbrain at 10.5 dpc; r2-derived neurons are shown in green, r4-derived neurons in black and r5-derived neurons in blue; abbreviations: V_{op}, V_{max}, V_{md} — ophthalmic, maxillary and mandibular branches of the Vth cranial (trigeminal) nerve; VII_{gspn}, VII_{ct}, VII_{bm} — greater superficial petrosal nerve, chorda tympani and branchiomotor nerve of the VIIth cranial (facial) nerve. (C–I) Wholemount in situ hybridisation of 10.5 dpc mouse hindbrains. (C) A probe specific for the motor neuron marker *Isl1* reveals the nascent motor neuron column on each side of the midline (asterisk); focal thickenings at the level of r2 and r4 contain the trigeminal (arrow) and facial branchiomotor neurons (black arrowhead), respectively. Facial visceromotor neurons are born in r5 (clear arrowhead); r5 also contains caudally migrating facial branchiomotor neurons. (D, E) Hindbrain motor neurons express *Nrp1* (D) and *Nrp2* (E). *Plxna1* is expressed near the midline in all rhombomeres anterior to r5 (F); the expression patterns of *Plxna2* (G), *Plxna3* (H) and *Plxna4* (I) are consistent with a role in trigeminal (arrow) and facial (arrowhead) branchiomotor neurons; *Plxna3* (H) and *Plxna4* (I) are also expressed in posterior hindbrain motor neurons, including those in r5 (clear arrowhead). Scale bar (C–I): 500 μm.

The facial nerve contains axons from both branchiomotor and visceromotor neurons, which are born in two different rhombomeres (r) of the developing hindbrain. Whilst facial branchiomotor neurons are born in r4, facial visceromotor neurons are born in r5 (Figs. 1B, C; Auclair et al., 1996; Jacob and Guthrie, 2000; Lumsden and Keynes, 1989; Studer et al., 1996). Having left the hindbrain through a shared exit point in r4, their axons pass through the geniculate ganglion complex and then segregate again to innervate specific targets in the head and neck. The facial branchiomotor neurons (FBM) innervate the muscles of the second branchial arch, whereas the facial visceromotor neurons innervate the submandibular ganglion as the chorda tympani (CT) and the sphenopalatine ganglion as the greater superficial petrosal nerve (GSPN) (Fig. 1B).

The transmembrane protein neuropilin 1 (NRP1) is essential for the patterning of the facial nerve in the mouse, as it binds the secreted class 3 semaphorin SEMA3A to guide facial branchiomotor axons in the second branchial arch and the vascular endothelial growth factor isoform VEGF164 to control the position of facial branchiomotor neuron cell bodies within the hindbrain (Kitsukawa et al., 1997; Schwarz et al., 2004; Taniguchi et al., 1997). Mouse embryos lacking NRP1 or SEMA3A also show defasciculation of the trigeminal, glossopharyngeal and vagus cranial nerves (Kitsukawa et al., 1997; Taniguchi et al., 1997). Neuropilin 2 (NRP2) binds a different subset of class 3 semaphorins, and its principal ligand during axon guidance is SEMA3F (Chen et al., 1997). Loss of NRP2 or SEMA3F causes partial defasciculation of the facial branchiomotor and ophthalmic trigeminal nerves and severe defasciculation of the oculomotor nerve; in addition, the trochlear nerve fails to project to its target in these mutants (Chen et al., 2000; Giger et al., 2000; Sahay et al., 2003).

Neither NRP1 nor NRP2 are able to convey semaphorin signals on their own (Feiner et al., 1997). Rather, they recruit a member of the plexin family to control cytoskeletal behaviour in neurons (Rohm et al., 2000; Tamagnone et al., 1999). The neuropilins can associate with one of four different A-type plexins (PLXNA) in vitro (Rohm et al., 2000; Suto et al., 2003; Takahashi et al., 1999; Takahashi and Strittmatter, 2001; Tamagnone et al., 1999). However, targeted mouse mutations demonstrated plexin selectivity during semaphorin/neuropilin signalling in vivo. Thus, knockout studies did not confirm a direct role for PLXNA1 in sensory nerve axon guidance (Takegahara et al., 2006), even though truncated PLXNA1 protein blocks SEMA3A-induced growth cone turning in cultured sensory neurons (for example Rohm et al., 2000; Takahashi et al., 1999). Instead, specific combinations of the other three A-type plexins have been implicated in different axon guidance pathways both in vitro and in vivo (Bagri et al., 2003; Cheng et al., 2001; Palaisa and Granato, 2007; Suto et al., 2005; Tanaka et al., 2007; Waimey et al., 2008; Yaron et al., 2005). For example, PLXNA4 and, to a lesser extent, PLXNA3 are involved in the patterning of SEMA3A-responsive sensory and sympathetic axons, whilst PLXNA3, but not PLXNA4, is essential for the guidance of SEMA3F-responsive trochlear axons (Cheng et al., 2001; Suto et al., 2005; Yaron et al., 2005). In the mouse, PLXNA4 also cooperates with PLXNA2 to control the projection of hippocampal mossy fibres, but this mechanism depends