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Schwannomin/merlin promotes Schwann cell elongation and influences myelin segment length

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ABSTRACT

The Neurofibromatosis type 2 tumor suppressor, schwannomin (Sch) is a plasma membrane–cytoskeleton linking protein that regulates receptor signaling and actin dynamics. We examined Sch's role in specifying morphological changes needed for Schwann cell (SC) function in vitro. Isolated Sch-GFP-expressing SCs extended bipolar processes 82% longer than those formed by GFP-expressing cells. In contrast, SCs expressing dominant negative Sch-BBA-GFP extended bipolar processes 16% shorter than controls and 64% shorter than Sch-GFP-expressing SCs. *nf2* gene inactivation caused isolated mouse SCs to transition from bipolar to multipolar cells. Live imaging revealed that SCs co-expressing Sch-GFP and dominant negative RacN17 behaved similarly in dorsal root ganglion explant cultures; they quickly aligned on axons and slowly elongated bipolar processes. In contrast, SCs expressing constitutively active RacV12 underwent continuous transitions in morphology that interfered with axon alignment. When co-cultured with neurons under myelin-promoting conditions, Sch-GFP-expressing SCs elaborated longer myelin segments than GFP-expressing SCs. In contrast, Sch-BBA-GFP-expressing SCs failed to align on or myelinate axons. Together, these results demonstrate that Sch plays an essential role in inducing and/or maintaining the SC's spindle shape and suggest that the mechanism involves Sch-dependent inhibition of Rac activity. By stabilizing the bipolar morphology, Sch promotes the alignment of SCs with axons and ultimately influences myelin segment length.

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Introduction

Dynamic control of Schwann cell (SC) morphology is critical at all stages of development of the peripheral nervous system. SCs undergo morphological transitions as they develop from immature to myelinating glial cells. The major stages of SC development requiring extensive cytoskeletal remodeling include: 1) radial sorting of axons by extension of multiple processes into axon fascicles, 2) bipolar elongation of processes along axons to establish the presumptive myelin internode, and 3) active myelination by elaboration of a large plasma membrane sheath around axons. The transition from axial elongation along an axon to radial expansion of membrane around an axon represents the most pronounced reorganization of the SC cytoskeleton. How actin polymerization is regulated in SCs during these stages of development is not well understood. However, it must be linked to receptor-dependent mechanisms activated by a contact of SCs with axons and extracellular matrix.

The Neurofibromatosis Type 2 (NF2) tumor suppressor, known as merlin and schwannomin (Sch) was identified as a plasma membrane–cytoskeleton linking protein based on its homology to the FERM family of proteins (Rouleau et al. 1993; Trofatter et al. 1993). SCs with inactivation of the *nf2* gene form benign slow growing schwannomas. When cultured, schwannoma cells do not assume the typical bipolar shape of SCs, but rather spread into large round flat cells with abundant ruffling membranes (Pelton et al. 1998). This altered morphology has been attributed at least in part to an increased Rac, PAK and JNK activity which inhibits their ability to extend processes onto axons (Kaempchen et al. 2003; Nakai et al. 2006). Transgenic modification of *nf2* in mice perturbs peripheral nerve development (Giovannini et al. 2000; Denisenko et al. 2008). The abnormalities observed include axonal loss, aberrant myelination and disorganization of axoglial contacts. These results suggest that Sch plays a role in myelination, yet the mechanism(s) are unknown.

Abbreviations: SC, Schwann cell; NF2, Neurofibromatosis Type 2; PAK, p21-activated kinase; PKA, protein kinase A; DRG, dorsal root ganglion, NGF, nerve growth factor; NRG, neuregulin beta-1.

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Sch regulates many signaling pathways initiated from multiple receptors to control proliferation, apoptosis and morphology (reviewed in Okada et al. 2007; Lallemand et al. 2009). A well-established mechanism by which Sch exercises its tumor suppressor function involves the inhibition of Cdc42/Rac activation of p21-activated kinase (PAK) (Hirokawa et al. 2004; Kissil et al. 2003; Okada et al. 2005). This ability is inactivated by phosphorylation of Sch at serine 518 (S518) by protein kinase A (PKA) and Cdc42/Rac-PAK (Alfthan et al. 2004; Kissil et al. 2002; Xiao et al. 2002). We have demonstrated that activation of β1 integrin and ErbB2 receptors promotes Sch-S518 phosphorylation in PAK and PKA dependent manners, respectively (Thaxton et al. 2008). Moreover, we found that β1 integrin and ErbB2 receptors are enriched with Sch, Cdc42 and PAK at the distal tips of the SC processes (Thaxton et al. 2008). These tips are highly motile structures similar to axonal growth cones and pathways initiated there mediate alignment and motility of SCs on axons (Gatto et al. 2003; Gatto et al. 2007). β1 integrin and ErbB2 receptors transduce signals from the extracellular matrix and axons, respectively and are essential for SC function (Berti et al. 2006; Britsch 2007).

Sch also indirectly controls activation of Rac (Morrison et al. 2007) by controlling its translocation to the plasma membrane (Okada et al. 2005). Rac and Cdc42 GTPases have been reported to have essential but distinct roles during SC development (Feltri et al. 2008) but act synergistically in oligodendrocytes to regulate myelin sheath formation (Thurnherr et al. 2006). Sch is thus well-positioned to integrate signals from ErbB2 and β1 integrin to regulate Cdc42/Rac-dependent changes in SC morphology during peripheral nerve development.

Results

Sch promotes elongation of bipolar processes in isolated SCs

To assess Sch's role in regulating SC morphology, we transiently transfected primary rat SCs with GFP, Sch-GFP, and the dominant negative variant, Sch-BBA-GFP (LaJeunesse et al. 1998; Johnson et al. 2002), and compared cell shape and process length (Fig. 1A–C, Table 1). SCs expressing GFP alone were typically bipolar, similar to normal confluent SCs. The lengths of their processes ranged from 21 μm to 520 μm, with the highest percentage of processes falling

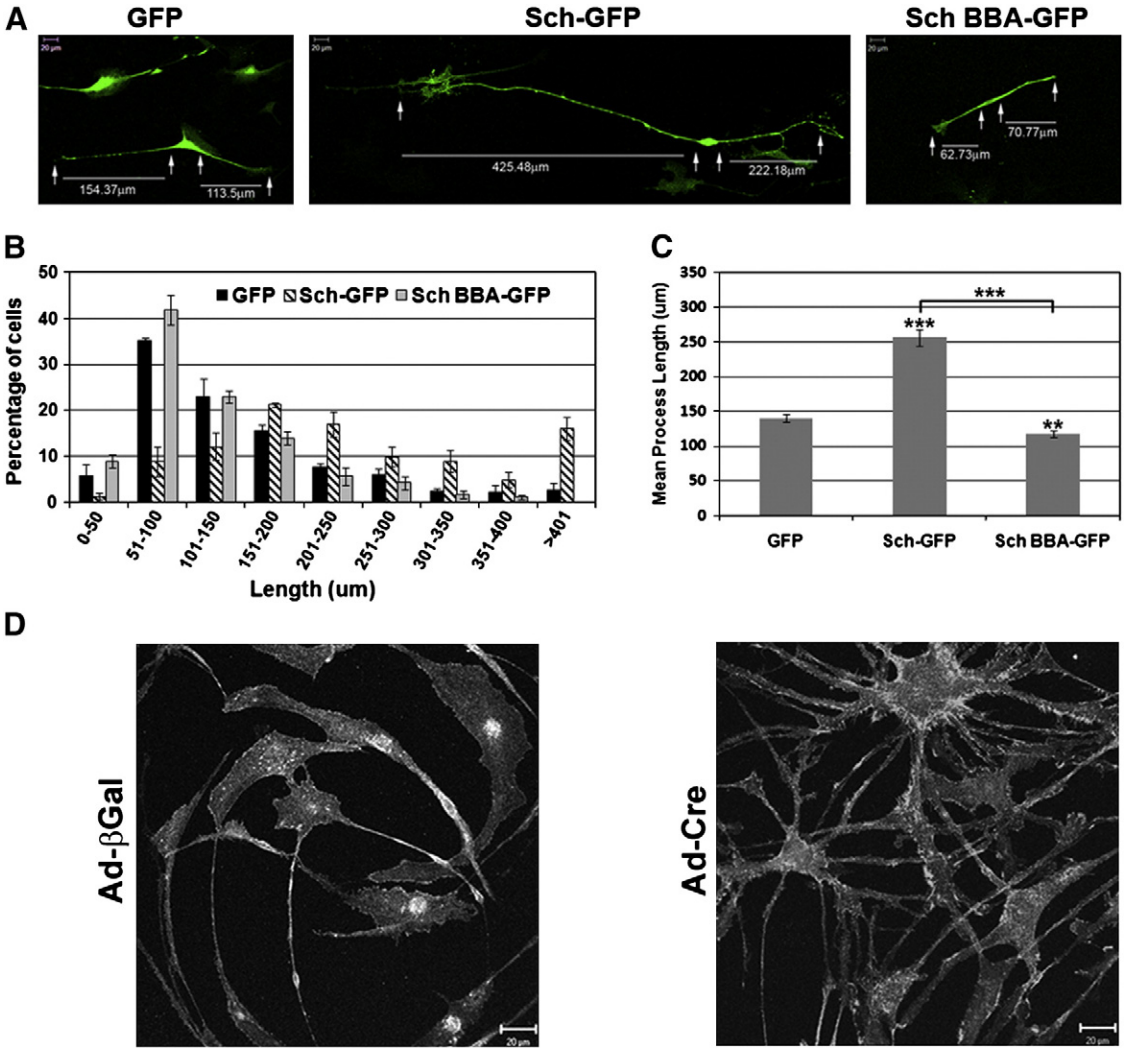

Fig. 1. Sch promotes bipolar elongation and maintenance in isolated rat SCs. (A) Purified neonatal rat SCs were transiently transfected with GFP, Sch-GFP and Sch BBA-GFP in three experiments. GFP fluorescence was imaged three days after transfection. The length of each bipolar process was measured (arrows). Scale bars indicate 20 μm. (B) The frequency histogram represents the mean percent and SEM of processes within the indicated range of the 3 experiments. (C) The mean process length shown is the mean of all processes measured over 3 experiments. The error bar represents the standard error of the mean (SEM). GFP ($n=227$), Sch-GFP ($n=183$), and Sch BBA-GFP ($n=203$). Asterisks denote statistical significance with p-values as follow: (*) ≤ 0.05, (**) ≤ 0.005, (***) ≤ 0.0001. (D) $Nf2^{flox2/flox2}$ adult mouse SCs were infected with an adenovirus directing expression of β-galactosidase (*Ad-βgal*) or Cre recombinase (*Ad-Cre*). Nine days after infection, SCs were immunostained for ErbB2 receptors to visualize cells. SCs expressing Cre become multipolar, whereas those expressing β-galactosidase remain bipolar.