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Reelin is transiently expressed in the peripheral nerve during development and is upregulated following nerve crush

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Reelin is an extracellular matrix protein which is critical for the positioning of migrating post-mitotic neurons and the laminar organization of several brain structures during development. We investigated the expression and localization of Reelin in the rodent peripheral nerve during postnatal development and following crush injury in the adult stage. As shown with Western blotting, immunocytochemistry and RT-PCR, Schwann cells in the developing peripheral nerve and in primary cultures from neonatal nerves produce and secrete Reelin. While Reelin levels are downregulated in adult stages, they are again induced following sciatic nerve injury. A morphometric analysis of sciatic nerve sections of reeler mice suggests that Reelin is not essential for axonal ensheathment by Schwann cells, however, it influences the caliber of myelinated axons and the absolute number of fibers per unit area. This indicates that Reelin may play a role in peripheral nervous system development and repair by regulating Schwann cell–axon interactions.

Keywords: Reelin; Reeler; Schwann cell; Nerve development; Nerve injury; Morphometry

Introduction

Reelin is a large protein that is secreted into the extracellular matrix and is critical for the laminar organization of several brain structures during development (D'Arcangelo et al., 1995; Rice and Curran, 2001; Tissir and Goffinet, 2003). Reelin is secreted by Cajal–Retzius cells located in the marginal zone of the developing cerebral cortex and regulates positioning of post-mitotic migratory neurons. A well-established signaling pathway of Reelin involves binding to the lipoprotein receptors VLDLR and ApoER2 followed by tyrosine phosphorylation of the intracellular adaptor Dab1, leading to cytoskeletal rearrangements and gene expression changes in the target neurons (D'Arcangelo et al., 1999; Hiesberger et al., 1999). Reelin, in addition to its well-known function in neuronal migration, also appears to have a role in the development and synaptogenesis of hippocampal connections since its absence leads to alterations in the entorhino-hippocampal pathway, including reduced axonal branching, an increase in the number of misrouted aberrant fibers and fewer entorhino-hippocampal synapses (Del Rio et al., 1997; Borrell et al., 1999). Although most studies have focused on Reelin in the control of migration and cell positioning during central nervous system development, little is known about the role of Reelin in the development of the peripheral nervous system (PNS) (Ikeda and Terashima, 1997). Given its putative role in axonal growth and synaptogenesis, we were prompted to investigate whether Reelin is expressed in the peripheral nervous system at the early stages of postnatal development and during nerve regeneration following injury, i.e. during two stages characterized by axon growth and extracellular matrix remodeling (Kury et al., 2001; Corfas et al., 2004). Thus, in the present study, we investigated with RT-PCR, Western blotting and immunohistochemistry the expression and localization of Reelin in the mouse sciatic nerve during the early phases of postnatal development, in the adult stage and following crush injury. Our findings were extended by analysis on Schwann cell primary cultures. To investigate whether Reelin plays a role in axon–Schwann cell interactions in the peripheral nervous system, a morphometric analysis was conducted on semithin sciatic nerve sections of *reeler* mice. Our studies revealed that Reelin is expressed in the rodent developing peripheral nerve and in vitro by primary Schwann cell cultures. Levels of Reelin are downregulated in adult stages, however, these are induced again following crush injury to the sciatic nerve. While the morphometric study suggests that Reelin is not required for the myelination process, because its absence does not perturb the axon–Schwann

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cell relationship, Reelin appears to subtly influence the caliber of myelinated axons and the absolute number of myelinated axons per unit area.

Results

Reelin is expressed in early postnatal mouse sciatic nerves

To investigate the expression of Reelin mRNA in rodent peripheral nerves, we conducted an RT-PCR analysis on total RNA extracted from the sciatic nerves of young postnatal (P9) wild-type (WT), heterozygous reeler (HZ) and homozygous reeler (RL) mice. Examination of the amplified product (591 bp Reelin fragment; Fig. 1A) reveals the presence of Reelin mRNA in sciatic nerve samples prepared from HZ and WT mice, whereas the signal is absent in the sample prepared from a RL mouse of the same age. To confirm expression at the protein level and to study isoforms of Reelin expressed in the peripheral nerve, we performed Western blot experiments on sciatic nerve protein extracts of young postnatal WT and RL mice using the monoclonal antibody Ab142 that recognizes an epitope localized in the N-terminal region of Reelin (De Bergeyck et al., 1998). We found that addition of 1% SDS and 100 mM β-Mercaptoethanol to the homogenization buffer was critical for the detection of Reelin from sciatic nerve preparations. As a positive control, we used the supernatant of Reelin expressing cells (CER, Niu et al., 2004; Fig. 1B, lane 1). The experiment shows in the lane containing the cell supernatant a Reelin-specific band recognized by Ab142 at ~400 kDa (full-length protein), plus two additional bands at ~300 and 320 kDa and another Reelin isoform at ~150 kDa. In the lanes containing the sciatic nerve protein extracts from a WT mouse at P9 (Fig. 1B, lane 4), the Ab142 recognizes the full-length Reelin band at ~400 kDa and the lower isoform band at ~150 kDa, which are absent in the lane containing the sciatic nerve protein extracts from a RL mouse at P9 (Fig. 1B, lane 5). The results also indicate that the ~400 kDa and the ~150 kDa isoforms detected in the WT P9 sciatic nerve are also present in the extracts of the WT P9 brain (Fig. 1B, lane 2), but not in the extracts of the RL P9 brain (Fig. 1B, lane 3). The smear observed in the high MW range of the WT brain extract lane is due perhaps to an insufficient separation of the full-length glycosylated Reelin isoforms which

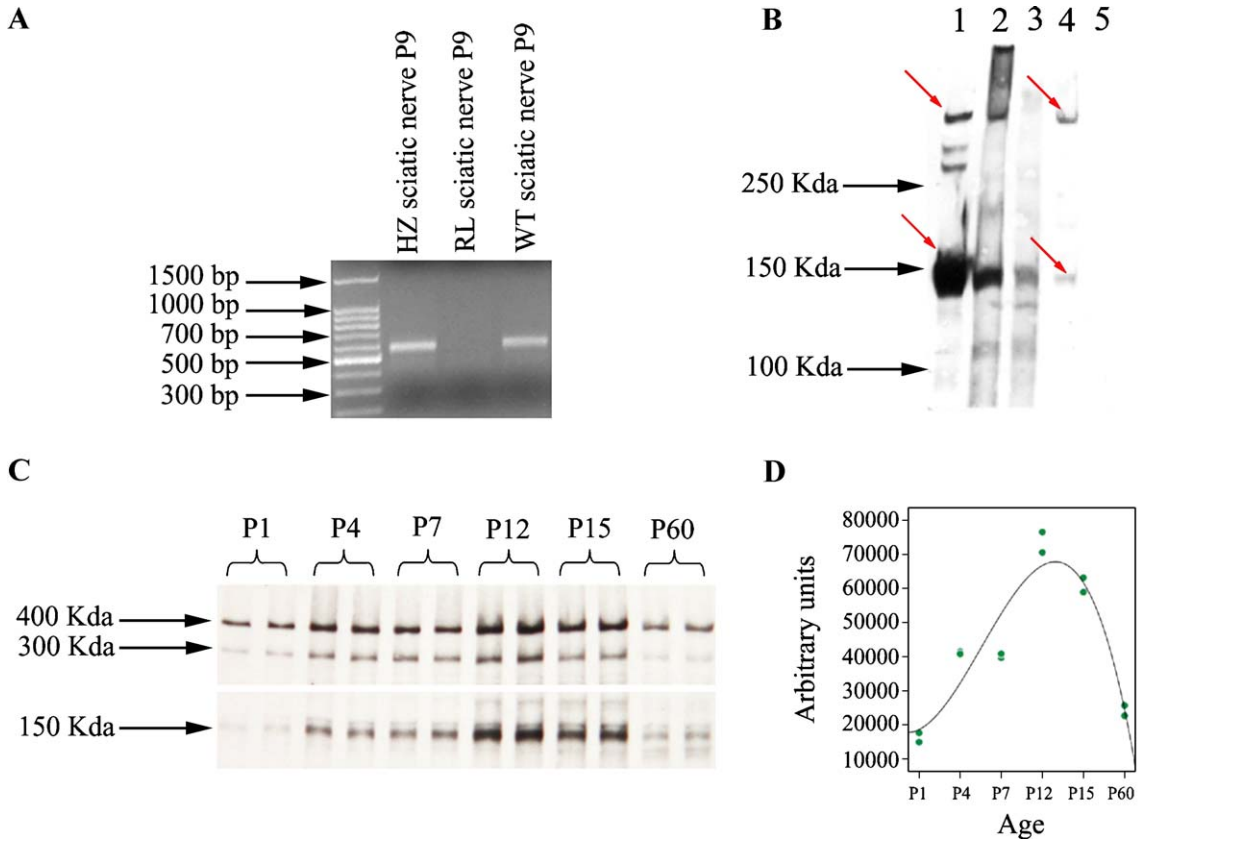

Fig. 1. (A) Expression of Reelin mRNA in early postnatal mouse sciatic nerve. The expected molecular weight of the RT-PCR product is 591 bp. Reelin mRNA is present in extracts of WT (wild-type) and HZ (heterozygous) mice sciatic nerves at postnatal day 9 and absent in extracts of RL (reeler) mice of the same age. (B) Western blot on WT/RL sciatic nerve protein extracts indicates Reelin expression in early postnatal mouse sciatic nerves. Lane 1, supernatant of Reelin expressing cells (CER); lane 2, brain extracts from WT at P9; lane 3, brain extracts from RL at P9; lane 4, sciatic nerve extracts from WT at P9; lane 5, sciatic nerve extracts from RL at P9. Arrows indicate the two Reelin isoforms at ~400 kDa and ~150 kDa present in the supernatant of Reelin expressing cells as well as in the sciatic nerve extracts of WT mice at P9. Molecular weight protein markers are indicated on the left. Approximately 35 μg of total protein was loaded for the brain extracts and 15 μg for the sciatic extracts. (C) Developmental time course of Reelin expression in the sciatic nerve. Western blot on sciatic nerve extracts from two different WT mice at P1 (lanes 1, 2), at P4 (lanes 3, 4), at P7 (lanes 5, 6), at P12 (lanes 7, 8), P15 (lanes 9, 10) and P60 (lanes 11, 12). Approximately 10 μg of total protein was loaded per lane. (D) The net optical intensity values were measured for the bands of Reelin isoforms obtained in panel C and plotted against developmental time.