Rac1 GTPase controls myelination and demyelination

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After peripheral nerve injuries, Wallerian degeneration starts with a stereotypic fragmentation of myelin sheath into myelin ovoids, which occur near Schmidt-Lantermann incisures (SLI). This demyelination process requires a dramatic change in cytoskeletal structures in Schwann cells. We have recently shown that actin polymerization around SLI is an important step for cleavage of the myelin sheath. We described that Rac1 GTPase regulates actin polymerization in SLI after injury. It has been previously reported that Rac-dependent cytoskeletal reorganization also plays an important role in myelination during the development of peripheral nerves. Thus, our findings suggest that Rac-dependent actin polymerization controls both myelination and demyelination in the peripheral nerves. We further discuss our new findings in relation to Schwann cell dedifferentiation and segmental demyelination.

Myelination and Actin Polymerization

Peripheral axons transmit general somatic information, including motor signals, in adult vertebrates and are myelinated by Schwann cells. During development, premature Schwann cells embrace many axons, but eventually only one axon per Schwann cell is myelinated.2 This axonal sorting is followed by myelination of the selected axon by a single ensheathing Schwann cell. Both axonal sorting and myelination involve dramatic changes in Schwann cell morphology, such as the protrusion of plasma membrane processes, which is mostly regulated by the actin cytoskeleton. As expected, Rac1 GTPase, one of the central regulators of the actin cytoskeleton, is implicated in axonal sorting and myelination.2,3 The conditional ablation of Rac1 in Schwann cells around embryonic age 13 significantly delayed axonal sorting without affecting Schwann cell migration and proliferation. Myelination involves the wrapping of Schwann cell membranes, which requires extensive elongation of radial membranes. In the Rac1-deleted mutant, most Schwann cells that had completed axonal sorting were then arrested at this stage and did not go on to myelinate the axon, indicating a role of Rac1 also in myelination. Although there is no direct evidence showing that the defect in actin regulation caused the abnormalities in axonal sorting and myelination in the mutant mice, Rac1-null Schwann cells were unable to extend lamellipodia in vitro, a process that occurs during axonal ensheathment. This suggests a pivotal function of Rac-dependent actin regulation in axonal sorting and myelination.2,3 It was recently reported that another actin regulator Neural Wiskott-Aldrich syndrome protein, an effector of RhoGTPase including Rac1, is also crucial for axonal sorting and myelination.4,5 Together, these data strongly suggest that extracellular signals regulating the actin cytoskeleton are essential for myelination during development (Fig. 1).

Myelin Fragmentation: Why Is It Important for Demyelination?

Demyelination results from a degenerative process of the myelin sheath that is observed in various circumstances such as...
Wallerian degeneration after injury and in certain neuropathies. Demyelination in Wallerian degeneration is not a reverse process of myelination, like dewrapping, as it initiates the fragmentation of the myelin sheath into small ovoid-like myelin chambers (Fig. 1).6-8 The appearance of this fragmentation in the injured nerves was originally considered, by Waller,
a degeneration process after injury. The fragmentation of the myelin sheath may enhance the efficiency of myelin removal because it probably exposes myelin to lysosomal hydrolytic enzymes, demyelinating lipid metabolites and phagocytosing macrophages. We recently showed that the inhibition of myelin fragmentation indeed delayed myelin protein degradation, suggesting that this presumption might be true. Myelin fragmentation involves at least two opposite phenomena. One is the cleavage of myelin lamellae and the other is the membrane fusion or sealing of the cleaved membranes to make the enclosed myelin ovoid. Making a sealed chamber is conceptually important because the release of degenerating axoplasmic contents, such as calpain and proteasomes, into the Schwann cell cytoplasm might damage the normal function of Schwann cells. Thus the fragmentation might be an active and precisely ordered process rather than a passive and randomly occurring degradation of the myelin sheath in injured nerves. Despite the recent advances in research on myelinization, the molecular mechanism of demyelination, especially focusing on cytoskeletal proteins and their regulators, remains obscure.

**Actin Polymerization in Schmidt-Lantermann Incisures Regulates Myelin Fragmentation**

Schmidt-Lantermann incisures (SLI) are cytoplasmic channels, regularly spaced in compact myelin, that allows the transport of materials between periaxial areas and Schwann cell cytoplasm in myelinated nerves. It has been suggested that the fragmentation of the myelin sheath occurs at SLI, thereby the products of myelin fragmentation, myelin ovoids, are delimited by SLI (Fig. 1). Although the morphological changes in SLI during myelin fragmentation have long been described by researchers, it is still unknown why myelin cleavage occurs specifically near SLI. We recently reported that new actin polymerization occurs in SLI (Fig. 1) and that myelin fragmentation does not happen when actin polymerization is inhibited in ex vivo explant cultures. The role of actin filaments is specific since the inhibition of microtubule polymerization and the deletion of glial fibrillary acidic protein, the major intermediate filament induced in Schwann cells after injury, did not alter myelin ovoid formation (unpublished observation). The SLI contains atypical adherens junctions (AJ), namely autotypic, because both membranes from the same Schwann cells contact each other to form the junction. The molecular components comprising the junction in SLI are very similar to that of epithelial, heterotypic AJ. For example, E-cadherin/catenin complexes are abundantly expressed in SLI and F-actin provides a cytoskeletal framework for the maintenance of this junction, as in an AJ. During the early phases of Wallerian degeneration, the separation of myelin lamellae and widening of cytoplasmic areas occurs within SLI, indicating the destruction of junctional structures. The elimination of E-cadherin signaling disrupted SLI in normal inter-nodes, therefore the downregulation of E-cadherin in the injured peripheral nerves may represent a biochemical sign for SLI destruction. We found that the inhibition of actin polymerization prevents the degradation of E-cadherin in SLI and subsequent myelin fragmentation. Thus, an actin polymerization-dependent destruction of molecular structures in SLI may be a prerequisite for myelin cleavage.

The mechanism of SLI destruction appears to be similar to that of destruction of AJ during epithelio-mesenchymal transition (EMT). Actin polymerization is required for E-cadherin destruction in AJ and the disassembly of E-cadherin/catenin complexes results in the loss of membrane contact in AJ. In EMT, beta-catenin is released from AJ and is transported to the nucleus. Interestingly, the nuclear translocation of beta-catenin from SLI in an actin polymerization-dependent manner was also observed in the injured nerves. It may be possible that the beta-catenin in the Schwann cell nucleus regulates genes that are implicated in Schwann cell proliferation as it was reported that the inhibition of beta-catenin reduced the proliferation of Schwann cells. Cyclin D, a target of beta-catenin in many cells, may be under the control of beta-catenin in Schwann cells during Wallerian degeneration. Collectively, these findings may indicate that actin-polymerization-dependent disruption of SLI provides both mechanical and molecular signals for reactive changes in Schwann cells during the early period of Wallerian degeneration.

**Rac GTPases Regulate Actin Polymerization in SLI**

Since Rho GTPases play a central role in the regulation of actin dynamics, we systematically investigated the distribution and activity of Rac1 and cdc42, two major Rho GTPases found in peripheral nerves. An interesting result was the specific recruitment of Rac1, but not cdc42, into the SLI after nerve injury (Fig. 1). The recruitment of Rac1 into incisures does not seem to require the activity of Rac GTPases as we demonstrated that a dominant negative mutant of Rac1 GTPase was also transported into SLI in the injured nerves (unpublished observation). The dilatation of incisures and the widening of Schwann cell cytoplasm between major dense lines, may participate in Rac1 recruitment. In parallel with the localization of Rac1 in SLI, the activity of Rac1, but not cdc42, was increased in the injured nerves, and inhibition of Rac activity with a specific inhibitor and dominant negative mutant suppressed actin polymerization and subsequent myelin fragmentation. These results suggest that Rac1 GTPase controls actin polymerization in SLI after nerve injury.

We also demonstrated that neuregulin is one of the regulating factors for Rac1 GTPase activation in the injured nerves. Neuregulin is a glial growth factor that bidirectionally regulates both myelination and demyelination. Neuregulin-induced demyelination involves myelin ovoid formation and our data suggest that Rac GTPase is a signaling mediator for the demyelination induced by neuregulin. Because both neuregulin and Rac GTPases are important for myelination during development, our findings give new insight that actin polymerization, through the activation of the neuregulin-Rac pathway, might regulate both myelination and demyelination.

**Unresolved Issues**

One of the remaining issues on the role of Rac GTPases in demyelination is a
possible involvement of Rac GTPases in Schwann cell dedifferentiation. In injured nerves, myelinated Schwann cells actively dedifferentiate into an immature Schwann cell phenotype. These dedifferentiated Schwann cells no longer express myelin proteins, but re-express several proteins found in immature Schwann cells such as p75 neurotrophin receptor. We observed that the inhibition of myelin fragmentation with actin polymerization inhibitors did not affect Schwann cell dedifferentiation. This finding indicates that myelin fragmentation is not a crucial factor for determining Schwann cell dedifferentiation.

How about Rac GTPases? Rac GTPases regulate many signaling pathways, including MAP kinases and c-jun, which are known to be implicated in segmental demyelination, probably due to intact cytoskeletal dynamics, normal myelin gene expression, and peripheral nerve myelination. This regulation is not a crucial factor for determining whether Rac-dependent actin polymerization in SLI also contributes to the process. The observation that small myelin ovoids are rarely found in segmental demyelination, probably due to intact axons, may suggest that other molecular changes may occur in this case. Further studies to elucidate these questions will provide important insight into the pathogenesis of peripheral neuropathies.

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