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PERSPECTIVE

Subcellular localization of Rho GTPases: implications for axon regeneration

Damage to neurons in the central nervous system often leads to a permanent loss of function due to several factors, including reduced capacity of axons to regenerate and an environment that inhibits axon regeneration because of disruption of myelin and the formation of a growth-refractory glial scar around the injury (Yoon and Tuszynski, 2012). Strategies to promote axon regeneration include both increasing the capacity of central nervous system axons to regenerate, as well as limiting the post-insult inhibitory environment (Gordon-Weeks and Fournier, 2014). Damaged central nervous system neurons do retain some intrinsic capacity to regenerate. If this ability could be enhanced, axon regeneration and re-connection to appropriate targets could lead to enhanced functional recovery in patients with brain or spinal cord injury.

A class of proteins that is highly important for regulating axon regeneration is the Rho family of guanosine triphosphatases (GTPases). These ubiquitous proteins regulate dynamic rearrangements of the actin cytoskeleton in growth cones located at the leading edge of extending axons (Samuel and Hynds, 2010). Rho GTPases act as molecular switches, being active and available to interact with their effectors when they are bound to guanosine triphosphate (GTP) and inactive when bound to guanosine diphosphate (GDP). The prototypical members of this family (RhoA, Rac1 and Cdc42) have received the most attention and have well-defined roles in axon growth. Studies employing expression of dominant active or dominant negative mutant forms of Rho GTPases collectively suggest that activation of RhoA leads to growth cone collapse and process retraction, while activation of Rac1 or Cdc42 is associated with increased process extension (Samuel and Hynds, 2010).

The activation of Rho GTPases by loading with GTP is regulated by three classes of proteins: guanine exchange factors (GEFs) that facilitate the binding of GTP to GTPases; GTPase activating proteins (GAPs) that promote GTP hydrolysis to GDP and inactivate Rho GTPases; and guanosine dissociation inhibitors (GDIs) that sequester Rho GTPases in the cytosol in a form that is inactive (Samuel and Hynds, 2010). The action of GEFs, GAPs and GDIs is affected by the subcellular localization of Rho GTPases. It is generally thought that the major determinant for Rho GTPase subcellular localization is whether covalently attached post-translational lipid moieties are present to direct the attachment of Rho GTPases to the plasma membrane (Liu et al., 2012). Most members of the Rho GTPase family are modified at their carboxy termini by the addition of a 20 carbon isoprenoid group (geranylgeranyl), a process called geranylgeranylation (or more generally, prenylation). For Rho GTPases, geranylgeranyltransferase I (GGTase I) facilitates transfer of the geranylgeranyl moiety from geranylgeranyl pyrophosphate (GGPP) to the cysteine at the CaaX motif, where C is a cysteine, a is an aliphatic amino acid and X is generally leucine, but may also be isoleucine, methionine, valine or phenylalanine (Roberts et al., 2008). Following the addition of the isoprenoid, the GTPase is further processed through peptidase cleavage of the aaX and methylation at the site of geranylgeranylation. Together, these ideas suggest the current model of Rho GTPase activation (**Figure 1A**) where the GTPase is geranylgeranylated and targeted to the plasma membrane, where it can interact with GEFs. The GEFs place the GTPase in the active, GTP-bound form and the membrane-bound GTPase can interact with and activate effectors. Inactive GTPases can be sequestered in the cytosol through binding with GDIs, an action that requires the presence of the geranylgeranyl group (**Figure 1A**).

According to this classical view of Rho GTPase activation, Rho GTPases localized to the cytosol should be inactive. However, we have recently developed constructs of RhoA and Rac1 that are not prenylatable by mutating the cysteine in the CaaX motif to an alanine (Reddy et al., 2015). When these constructs are expressed in neuron-like cell lines or primary cortical neurons, we find a significant shift in the localization of activated Rho GTPases as measured by their ability to interact with effectors. For instance, expressing non-prenylatable Rac1 in neuroblastoma cells leads to a significant increase in the proportion of activated Rac1 that is localized to the cytosol (Reddy et al., 2015). Pull-down assays demonstrate that non-prenylatable Rho GTPases can be activated, although they are not translocated to the plasma membrane. Furthermore, we have identified endogenous Rho GTPases that are found in their activated forms in the cytosol or nucleus of untreated cells (Samuel et al., 2014; Reddy et al., 2015). Results from these studies and studies in other laboratories suggest that there are some mechanisms that allow Rho GTPases to be activated in the cytosol, necessitating a re-assessment of the current model of Rho GTPase activation (**Figure 1A**).

Affecting Rho GTPase activity at specific cellular locations may well activate different signaling networks at those location and lead to differential signaling events. If this is true, it may explain some of the pathological conditions that involve Rho GTPases. For instance, aberrant localization of RhoA is implicated in the pathology of Alzheimer's and Parkinson's diseases (Huesa et al., 2010). Furthermore, we observe a redistribution of activated Rac1 to the cytosol under conditions where we express a non-prenylatable form of the protein (Reddy et al., 2015). Interestingly, studies on ageing mouse brains suggest that a decrease in function in GGTase I correlates with decreases in geranylgeranylation of its target proteins even though brain concentrations of the substrate GGPP increases in ageing (Hooff et al., 2012). This could well indicate that redistribution of Rho GTPases occurs in natural ageing, perhaps contributing to loss of cognition. Furthermore, it has been demonstrated that the classical view of Rho GTPase activity, with Rac1 and Cdc42 supporting process extension from neurons and RhoA inhibiting it, may be more complicated than originally thought. For example, it is now known that activation of RhoA is required for growth cone lamellipodial consolidation at its interface with the process shaft, allowing efficient process extension (Loudon et al., 2006). Together, these studies suggest that differential distribution of Rho GTPases may be important both for normal functioning and in cases of neurological dysfunction.

The mechanisms that may regulate the spatial and temporal

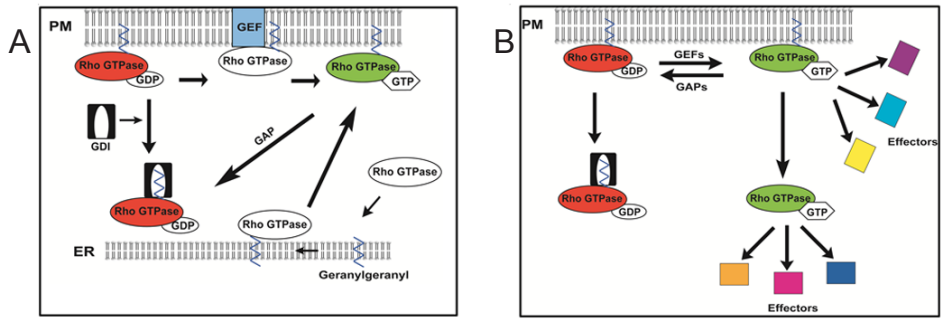

Figure 1 The current model of Rho GTPase activation and signaling.
(A) states that Rho GTPases are geranylgeranylated for transport to the plasma membrane, where their interaction with GEFs increases their GTP loading and activation. In this model, GTPases can be inactivated through GTP hydrolysis facilitated by the action of GAPs or through sequestration in the cytosol *via* complexing with GDIs. Since recent evidence has suggested that some Rho GTPases can be found in their activated form in the cytosol, additional aspects of the current model should be considered, as depicted in panel B, where activated forms of Rho GTPases may be associated with the plasma membrane or be localized to the cytosol, where they have the potential to activate a different set of effectors to mediate their biological consequences. GEFs: Guanine exchange factors; GTP: Guanosine triphosphate; GDIs: guanosine dissociation inhibitors; PM: plasma membrane; GAP: GTPase activating protein; ER: endoplasmic reticulum.

localization of Rho GTPase activation are far from being completely elucidated. Many Rho GTPases have additional post-translational modifications that may influence their activity. Phosphorylation of different residues in some Rho GTPases affects their activity. For example, phosphorylation of RhoA at serine 188 decreases RhoA activity by increasing binding to GDI (Liu et al., 2012). How this works in the presence or absence of geranylgeranylation is unknown. Similarly, the interactions between prenylation and other Rho GTPase post-translational modifications (*e.g.*, AMPylation, SUMOylation, transglutamination), some of which also affect membrane targeting (*e.g.*, palmitoylation), are incompletely defined. In addition, spatial and temporal localization of Rho GTPase signaling may be influence by intra-axonal translation of the GTPases (Walker et al., 2010) or may occur differently in specific types of neurons (Joshi et al., 2015). Furthermore, the spatial and temporal patterns of Rho GTPase activation may well control the localization of specific cellular signaling modules. Thus, it is possible that activation of a particular Rho GTPase will lead to activation of different sets of effectors, depending on the subcellular location where it is being activated (**Figure 1B**). It is not hard to see that having a more complete understanding of the spatial/temporal patterns of Rho GTPase activation will aid in the development of therapies designed to target and manipulate Rho GTPase activity at the cellular and molecular levels, potentially leading to development of individualized treatments to encourage the regeneration of neuronal processes after central nervous system damage.

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