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RHO GTPase Signaling for Axon Extension: Is Prenylation Important?

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Abstract Many lines of evidence indicate the importance of the Rho family guanine nucleotide triphosphatases (GTPases) in directing axon extension and guidance. The signaling networks that involve these proteins regulate actin cytoskeletal dynamics in navigating neuronal growth cones. However, the intricate patterns that regulate Rho GTPase activation and signaling are not yet fully defined. Activity and subcellular localization of the Rho GTPases are regulated by post-translational modification. The addition of a geranylgeranyl group to the carboxy (C-) terminus targets Rho GTPases to the plasma membrane and promotes their activation by facilitating interaction with guanine nucleotide exchange factors and allowing sequestering by association with guanine dissociation inhibitors. However, it is unclear how these modifications affect neurite extension or how subcellular localization alters signaling from the classical Rho GTPases (RhoA, Rac1, and Cdc42). Here, we review recent data addressing this issue and propose that Rho GTPase geranylgeranylation regulates outgrowth.

Keywords Cytoskeleton · Geranylgeranylation · Guanine dissociation inhibitors · Guanine nucleotide exchange factors · Guanine nucleotide triphosphatases · Neurite outgrowth · Rac1 · RhoA · Signal transduction

Abbreviations

Arp	Actin-related protein
ATP	Adenosine triphosphate
CAAX	C—cysteine, A—aliphatic amino acid, X—any amino acid
cAMP	Cyclic adenosine monophosphate
C domain	Central domain
CNS	Central nervous system
Cys	Cysteine
F-actin	Filamentous actin
FPP	Farnesyl pyrophosphate
FT	Farnesyl transferase
G-actin	Globular actin
GAP	GTPase activating protein
GDI	Guanine dissociation inhibitor
GDP	Guanosine diphosphate
GEF	Guanine exchange factor
GGOH	Geranylgeraniol
GGPP	Geranylgeranyl pyrophosphate
GGT	Geranylgeranyl transferase
GGTI	Geranylgeranyl transferase I inhibitor
GTP	Guanosine triphosphate
GTPases	Guanine nucleotide triphosphatases
HMG-CoA	3-Hydroxy-3-methylglutaryl coenzyme A
LIMK	LIN-11 Islet-1, MEC-3 domain kinase
mDia	Mammalian diaphanous-related formin
NGF	Nerve growth factor
N-WASP	Neuronal Wiskott Aldrich syndrome protein
PAK	p21-Activated kinase
P domain	Peripheral domain
ROCK	Rho-associated coiled-coil-forming protein kinase
WAVE	WASP family verprolin-homologous protein

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Understanding the regulation of axon growth and guidance is important for deciphering the biochemical mechanisms responsible for correct wiring of the nervous system and to identify therapeutic targets to encourage axon regeneration following damage. There are many nervous system disorders that could benefit from uncovering the mechanisms

regulating axon guidance, including spinal cord and brain injury, cerebrovascular accident, and neurodegenerative disorders like Alzheimer's disease [1–3]. In both traumatic and degenerative diseases, axon regrowth and connection to appropriate targets could improve functional recovery and damage in the adult central nervous system.

Axon growth and regeneration are directed by actin cytoskeletal dynamics in the neuronal growth cone, a sensory motile structure located at the tips of extending axons. Growth cone actin dynamics are regulated, in large part, by the guanine nucleotide triphosphatases (GTPases) of the Rho family [4, 5]. Rho GTPases are activated by guanine triphosphate (GTP) loading in response to interaction with guanine exchange factors (GEFs). In addition, Rho GTPases are post-translationally modified by prenylation [5]. Prenylation directs translocation of the Rho GTPases to the plasma membrane, perhaps facilitating their interaction with GEFs. However, the role of prenylation in regulating Rho GTPase signaling and axon extension is incompletely elucidated. In this review, we attempt to determine how prenylation affects: (1) neurite outgrowth; (2) growth cone actin dynamics; and (3) Rho GTPase signaling. To understand how alteration of prenylation affects Rho GTPase activation and neurite outgrowth, a discussion of the current understanding of the mechanisms of Rho GTPase-mediated axon extension is warranted.

Axon Extension and Growth Cone Actin Dynamics

Neuronal growth cones are composed of a peripheral (P) and central (C) domain. The P domain contains long finger-like projections called filopodia and sheet-like structures called lamellipodia (Fig. 1) [6]. Filopodia act as long-range sensors, continuously sampling the environment for guidance cues, while lamellipodial dynamics are associated with directional growth cone movement. Both filopodia and lamellipodia are actin-based structures. Actin filaments are arranged in longitudinal bundles in filopodia and form a branched network in lamellipodia (Fig. 1) [7]; and rearrangements of actin direct growth cone navigation.

Protrusive forces for growth cone extension include coordinated actin nucleation, polymerization, depolymerization, severing, and capping. Nucleation occurs de novo or as side branches originating from existing filaments, leading to actin branching and lamellipodia formation [6]. For polymerization, actin monomers (G-actin) are added preferentially onto the barbed (plus) ends of filamentous actin (F-actin) located toward the P domain and removed from the pointed (minus) ends oriented toward the C domain (see Fig. 1) [6]. Polymerization is promoted by profilin delivering adenosine triphosphate-bound actin monomers to the barbed ends of either linear or branched filaments. Capping proteins prevent the elongation of actin filaments. Nucleation is mediated by the actin-related protein (Arp) 2/3 complex and mammalian diaphanous-related formins (mDia) in branched and linear filaments, respectively. The actin-severing protein, cofilin, results in depolymerization at pointed ends [6]. The regulated action of these actin-binding proteins directs the formation and extension of axons (see Fig. 1).

Once a neuron has migrated to its proper location, growth cone actin dynamics coordinate extension of processes. First, external cues signal the initiation of neurite outgrowth from a spherical cell body. This process is incompletely elucidated, but it is proposed that decreasing the ratio of F- to G-actin in localized areas increases actin instability to promote neurite initiation [8]. The further extension of axons occurs in three stages: protrusion, engorgement, and consolidation [9]. In protrusion, extension of filopodia and lamellipodia happens with polymerization of bundled actin filaments in filopodia tips [10] and radial spreading of actin meshworks at the base of filopodia for lamellipodial expansion [11]. In engorgement, microtubules and organelles invade lamellipodial protrusions following depolymerization of actin at the P/C domain interface, thickening the C domain. Consolidation happens when actin depolymerizes in the central domain of the growth cone, allowing the membrane to shrink around the bundle of microtubules and forming a new extent of axon shaft [9, 12]. Neurite branching occurs as the growth cone stalls and a new growth cone is formed [13, 14].

Rho GTPase Regulation of Axon Growth and Growth Cone Actin

Several recent reviews eloquently address the role of Rho GTPases on axon growth and actin dynamics [15–18]. Therefore, here, we only briefly address this issue. The Rho GTPases constitute a family of small (21–28 kDa) cell-signaling proteins that cycle between inactive guanosine diphosphate (GDP) and active GTP-bound states (Fig. 2) [19]. GTP loading is facilitated by interacting with GEFs, while GDP association is promoted by GTPase activating proteins (GAPs), which increase GTPase activity and guanine dissociation inhibitors (GDIs), which inhibit dissociation of GDP. The amine (N-) termini of Rho GTPases are well conserved while the carboxy (C-) termini vary and may be associated with different functions for the proteins [20, 21]. The classical and best-studied Rho GTPases are RhoA, Rac1, and Cdc42.

Small GTPases in the GTP-bound form interact with downstream effectors that regulate actin dynamics [22, 23]. Immediate downstream Rho GTPase effectors include the serine threonine kinases, p21-activated kinase (PAK,

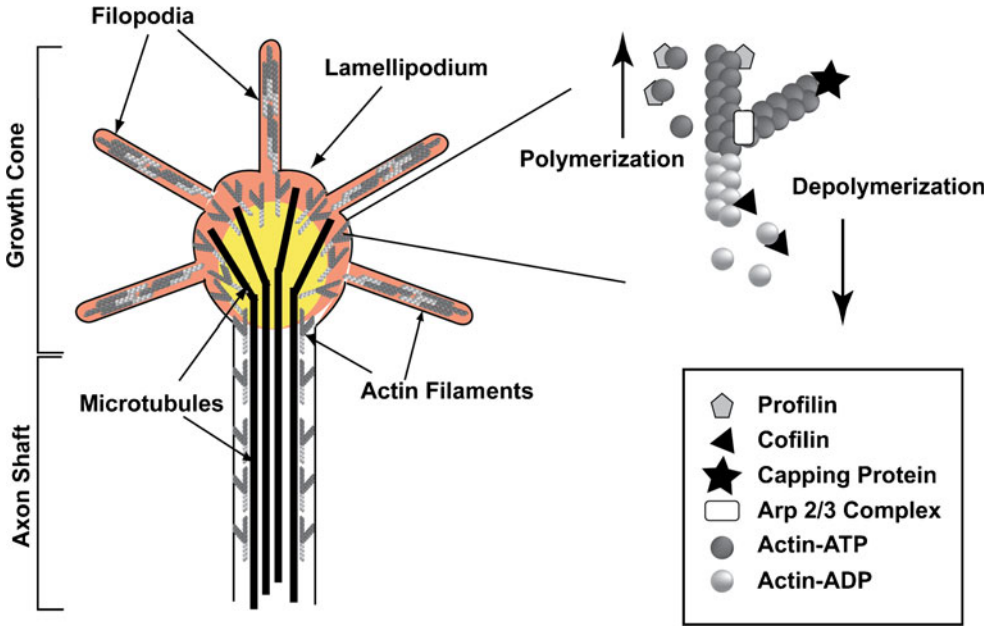

Fig. 1 Growth cone cytoskeletal structure. Growth cones are composed of an actin-based peripheral (P) domain (*in orange*) and a microtubule-rich central (C) domain (*in yellow*). The P domain consists of finger-like filopodia with linearly arranged actin filaments and sheet-like lamellipodia that have branched actin filaments. Actin dynamics including polymerization, depolymerization, severing, and capping are controlled by specific actin-binding proteins including profilin, cofilin, capping protein and the Arp2/3 complex

activated by Rac1 and Cdc42), and Rho-associated coiled-coil-forming protein kinase (ROCK, activated by RhoA). Actin nucleation and polymerization is promoted by complexes of actin-binding proteins. Cdc42 directly activates neuronal Wiskott Aldrich syndrome protein (N-WASP) [24, 25], and all three classical Rho GTPases activate the mammalian diaphanous-related formin mDia [26, 27]. Rac1 leads to activation of WASP family verprolin-homologous (WAVE) protein, by signaling through PAK [28, 29]. To create protrusive force, WAVE and N-WASP work with Arp2/3 to generate branched actin filaments and mDia with profilin which promotes linear actin polymerization [30, 31]. RhoA activation of ROCK leads to increased actin–myosin contractility through direct activation of myosin light chain and inhibition of myosin light chain phosphatase. All three classical Rho GTPases activate LIN-11, Islet-1, MEC-3 domain kinases (LIMKs), which phosphorylate and inactivate the actin depolymerizing factor, cofilin [26].

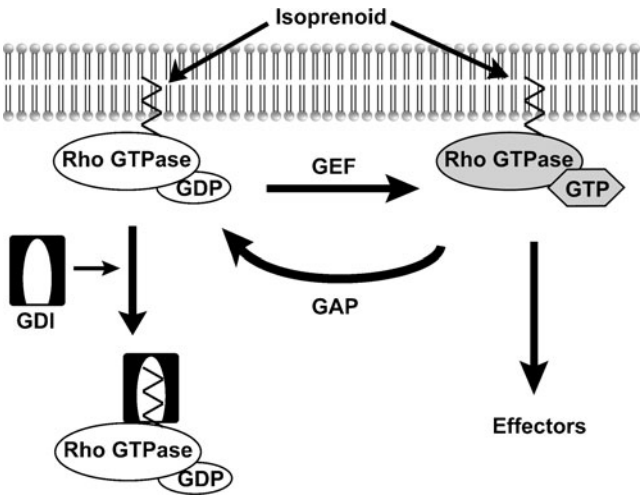

Fig. 2 Rho GTPase cycle. Rho GTPases cycle between active, GTP-bound and inactivate, GDP-bound states. Activation is regulated by interaction with guanine nucleotide exchange factors (*GEFs*), GTPase activating proteins (*GAPs*), and guanine nucleotide dissociation inhibitors (*GDIs*), as well as by membrane localization through geranylgeranylation. Active Rho GTPases regulate the activity of downstream effectors (modified from [97])

Each of the best-studied Rho GTPases (RhoA, Rac1, Cdc42) is implicated in neurite outgrowth. RhoA is associated with inhibition of neurite outgrowth and Rac1 and Cdc42 are associated with increased axon extension [32]. Activation of RhoA correlates with inhibition of neurite initiation [9, 33], axon retraction [34], and growth cone collapse [35, 36]. Moreover, inhibiting RhoA or its signaling increases axon growth and branching in cultured neurons [32, 35, 37] and regeneration of axons following nervous system injury [38, 39]. Rac1 promotes neurite outgrowth across inhibitory substrata [40] and in response to activation by outgrowth promoting cues (e.g. netrin-1) [41, 42]. For instance, Rac1 expression is necessary for branching in *Drosophila* neurons [43]. Activation of Cdc42 is also associated with promotion of neurite outgrowth [44]. In mammalian neurons, downregulation of Cdc42 expression allows elaboration of several neurites from a cell but limits the growth of axons [45].

However, several works contradict the studies mentioned above. For instance, in some reports, RhoA activity is necessary for efficient axon outgrowth [35, 46]. The reasons behind these disparate results are as yet unclear but may indicate that spatial/temporal regulation of threshold levels of RhoA activation are important for orchestrating

its biological effects. In support of this, RhoA activation is associated with netrin-induced neurite outgrowth [47, 48]; and RhoA activation in the growth cone P domain is seen in extending neurites [49] where it may be involved in regulating point contacts and adhesion [46]. Similar contradictory scenarios are also observed for Rac1 and Cdc42. Rac1 activation is involved in mediating growth cone collapse [50, 51]; and dominant negative Cdc42 inhibits induction of growth cone collapse [50]. It may be that these apparently contradictory reports arise from complexity in regulating and coordinating Rho GTPase activity and/or signaling to Rho GTPase effectors. For example, constitutively active RhoA leads to long, unbranched neurites in primed cells, but a ROCK inhibitor induces formation of highly branched neurites, an effect potentially explained by RhoA signaling to Rac1 [35, 52]. Equally plausible is that a heretofore unappreciated complexity in regulating Rho GTPase activation, perhaps via post-translational modification by prenylation.

Prenylation

Prenylation is an irreversible lipid modification where the isoprenoid intermediates farnesyl (15-carbon) or geranylgeranyl (20-carbon) pyrophosphates are added to the C-terminus of proteins [53]. Isoprenoid intermediates, farnesyl pyrophosphate (FPP), and geranylgeranyl pyrophosphate (GGPP) are produced from mevalonate in the same biosynthetic pathway that produces cholesterol. These intermediates are linked to cellular proteins [54] by thioether linkages at a C-terminal cysteine (Cys) residue [55] in reactions catalyzed by the protein prenyl transferases farnesyl transferase (FT) and geranylgeranyl transferase (GGT) [53]. The sequence CAAX (C—cysteine, A—aliphatic amino acid, X—any amino acid) at the C-terminus determines whether a protein is farnesylated or geranylgeranylated. GGT recognizes leucine or phenylalanine in the X position, while FT utilizes mostly serine or methionine, as well as other amino acids in the X position [56]. Prenylation is followed by truncation and methylation [57] and the lipid moiety makes the C-terminus hydrophobic and helps anchor it to membranes. Membrane translocation allows the proteins to interact with regulatory and effector proteins [58].

Effect of Statins on Protein Prenylation

Statins have emerged as important pharmacological tools for studying protein prenylation. These cholesterol-lowering drugs inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme for the mevalonate pathway. In this pathway, HMG-CoA is reduced to mevalonate which is converted through a series of enzymatic reactions, to first FPP and then to GGPP (Fig. 3). The addition of a prenylation precursor (e.g., geranylgeraniol) in statin-treated cells restores lost

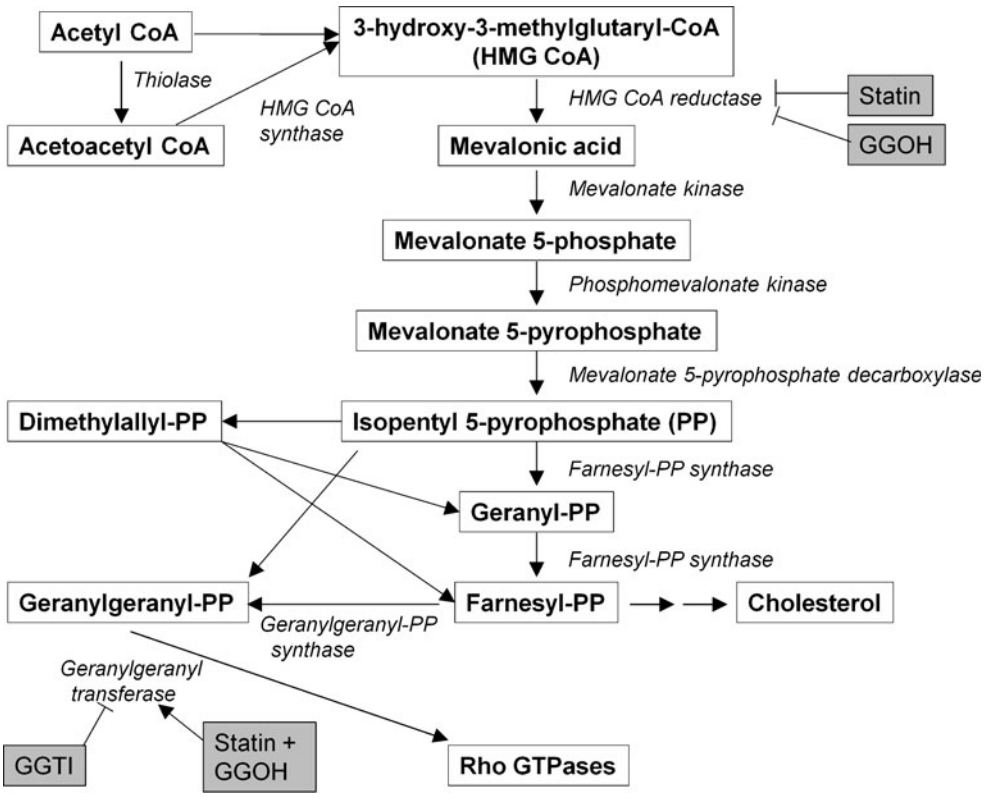

Fig. 3 The mevalonate pathway produces prenylation precursors. This pathway produces protein prenylation precursors, farnesyl pyrophosphate, and geranylgeranyl pyrophosphate, as well as cholesterol. Treatments commonly used to manipulate the mevalonate pathway are shown in *shaded boxes*. Statins act on 3-hydroxy-3-methylglutaryl coenzyme A (*HMG-CoA*) reductase, geranylgeraniol (*GGOH*) promotes geranylgeranylation, and geranylgeranyl transferase inhibitor (*GGTI*) prevents transfers of geranylgeraniol to proteins

protein prenylation [59, 60]. Since the mevalonate pathway also produces cholesterol, data obtained from studies using statin treatments could result from decreasing cholesterol content. Cholesterol levels clearly influence neurite outgrowth in some studies. Depleting cholesterol increases outgrowth in hippocampal neurons but decreases outgrowth in cortical neurons [61]. Interestingly, cholesterol appears to regulate dendrite, but not axon, extension in sympathetic neurons [62]. Fortunately, supplying a source of cholesterol allows attribution of observed effects to statin action on protein prenylation, providing a nice tool for studying axon growth in response to decreased protein prenylation.

Prenylation and Neurite Outgrowth

Decreasing protein prenylation levels with statins promotes neurite outgrowth in some systems [34, 63–71] but decreases it in others [62, 72, 73]. The reasons behind the different results reported in these studies have yet to be fully uncovered but may include (1) the use of different measures of outgrowth, (2) employment of different model systems, (3) assessment of dendrite versus axon outgrowth, or (4) testing of different types of statins.

The most common outgrowth measurement to be employed is the percent of neurite-bearing cells, a measure of neurite initiation [63, 66, 67, 72]. Of these works, only the study conducted by Schulz and colleagues [72] reports a decrease in the percent of neurite-bearing cells with statin treatment. Interestingly, predifferention of PC12 cells before treatment with atorvastatin leads to a transient increase and then a subsequent decrease in the proportion of neurite-bearing cells [72]. Other assessments of statin effects on neurite outgrowth include measurements of elongation or arborization. Here, reported results continue to be disparate. Statin treatment is reported to not affect axon elongation in cortical [62] or sympathetic neurons [73], increase neurite length in hippocampal neurons [65], and decrease neurite length in PC12 [72] and sympathetic secondary and tertiary dendrites [73]. Similarly, statin treatment decreases axonal or dendritic branching in some systems [62, 73] and increases it in others [65]. Thus, it is difficult to identify any pattern in statin-based neurite outgrowth responses based on the measurement used to assess outgrowth.

It is likely that the cellular identity is important in determining the response to manipulating protein prenylation. The majority of investigations assess statin effects in neuronal cell line models including PC12 [63, 69, 70, 72], Neuro2A [66, 67, 74], and N1E-115 [34]. Here, studies in the same model system (PC12) show that statin treatment could variably increase [63, 69, 70] or decrease outgrowth [72], whereas the other two models (Neuro2A and N1E-115) both indicate that statins increase outgrowth [34, 66, 67, 74]. Studies using primary neuronal cultures or investigating effects of manipulating protein prenylation in vivo do not report more consistent results. In cortical or sympathetic neurons, treatment with statins decreases dendrite or axon branching without affecting elongation [62, 73], but in hippocampal neurons, statins increase outgrowth [65]. However, this latter result is inconsistent with a report showing that overexpression of geranylgeranyl transferase increases outgrowth from hippocampal neurons [71]. Additionally, neurite elongation is decreased in statin-treated cortical neurons [72]. Futhermore, statin treatment decreases cellular responses to outgrowth inhibitors [64], but this may also lead to detrimental changes following statin treatment in vivo [75]. Although neuronal identity may play a role in determining the outgrowth response to statins, these results may also arise from investigating different neuronal extensions or from administration of different types of statins.

There does appear to be a differential effect in statin actions on dendritic versus axonal outgrowth. In dendrites, statin treatment is consistently reported to decrease initiation and arborization [62, 73], an effect attributable to RhoA activation by geranylgeranylation [73]. Additionally, Purkinje and hippocampal neuronal dendrite development requires GGT [71, 76]. Similarly, statins are consistently reported to not affect axon elongation, but decrease axon branching [62, 73]. Thus, some of the variable effects attributed to statin treatment may be explained by the type of extension. More studies in primary cultured neurons and assessment of statin-induced effects on process development or regeneration in vivo will help to better define the differential statin actions.

Finally, the use of different statins might explain the different results achieved in studies measuring statin effects on outgrowth. In commonly used PC12 cells, simvastatin and lovastatin increase [63, 69, 70], and atorvastatin decreases [72] neurite outgrowth, while pravastatin has no effect [70]. In primary cortical cells, atorvastatin decreases outgrowth [72], while simvastatin results in increased outgrowth [64]. Studies employing pravastatin reported either enhanced or unaffected outgrowth [65, 66, 70, 73], while studies with mevastatin show it increases outgrowth [66, 67]. Simvastatin [64, 69, 70, 73] and lovastatin [34, 63, 73] are reported to both enhance and inhibit outgrowth. Taken together, these studies consistently indicate that atorvastatin decreases outgrowth [72, 73], and mevastatin increases outgrowth [66, 67], but results obtained with other statins (pravastatin, lovastatin, simvastatin) are more variable [34, 63, 64, 69, 70, 73]. The different responses do not correlate with hydropho-

bicity but may indicate a difference between the actions of naturally derived type 1 (lovastatin, pravastatin, simvastatin, mevastatin) and synthesized type 2 (atorvastatin) statins on specific isoprenylated targets.

Role of Prenylation in Rho GTPase Function

The Rho GTPases are geranylgeranylated [77], targeting them to the plasma membrane [78] and regulating their function [79, 80]. For example, prenylated Rho GTPases localize to the plasma membrane [21, 81]. Impairing geranylgeranylation also leads to accumulation of RhoA in the nucleus [82]. In fibroblasts and other non-neuronal cells, prenylation is important for Rho GTPase function [83], where membrane attachment generally promotes activation [84]. In neuronal cells, functional activity of Rho family proteins is also regulated by the cellular location of the protein [5]. Interestingly, some functions associated with activation of Rho GTPases may be separable from their membrane association. For instance, translocation of RhoA to the membrane is required for neurite retraction, but not for stress fiber formation [34]. Thus, the role of prenylation in regulating Rho GTPase activity in neurons is still unclear. In non-neuronal cells, statins increase Rac1 GTP loading [85] and decrease RhoA GTP loading [63, 86]. The state of activation (defined by the loading with GTP) of both Rac1 and RhoA in response to manipulating prenylation has not been simultaneously studied in neuronal cells. With the help of tools to manipulate Rho GTPase function and signaling, the ability to elucidate the mechanisms through which prenylation modulates Rho GTPase activation is now possible.

Prenylation and Effects on Rho GTPase Interaction with Regulators

Rho GTPase prenylation and targeting to the plasma membrane regulates their interaction with activating GEFs and inactivating GAPs and GDIs. GEFs are more active on prenylated GDP-bound Rac1 associated with membranes than towards non-prenylated GDP-bound Rac1 [84]. Membrane-bound GAPs aid in GTP hydrolysis of prenylated Rho GTPases, but not unprenylated forms [58]. Most work has, however, focused on the role of prenylation in inhibiting the exchange of GTP for GDP, an action facilitated by GDIs [87, 88]. Binding to RhoGDI masks the isoprenoid modification of Rho GTPases, preventing membrane localization and sequestering the Rho GTPases in the cytosol to control their activity [89, 90]. However, this may not always be the case. In one study, only geranylgeranylated RhoA is reported to interact with RhoGDI [83], but a non-prenylatable mutant RhoA is able to interact with RhoGDI in another work [91]. In addition, RhoGDI can interact with Rho GTPases in both their GTP and GDP-bound states [92]. Interestingly, when RhoGDI is bound with GTP-loaded Rac1, the action of GAP on Rac1 is inhibited and causes sustained activation of Rac1 [92]. Other studies report that the activity of GAP can be distinguishable for RhoA and Rac1 according to the phospholipid environment [58, 93]. Prenylation, therefore, likely plays a role in Rho GTPase activation, but the specifics regarding this regulation are not yet completely defined.

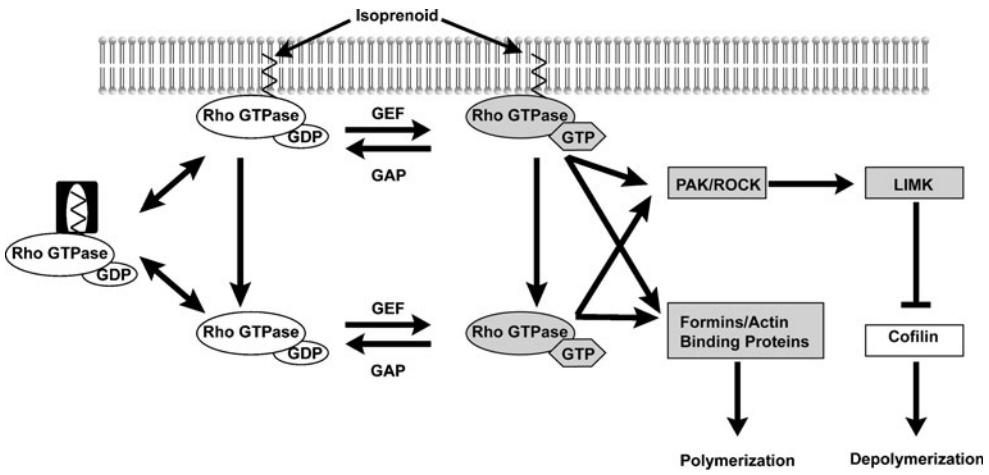

Fig. 4 Simplified diagram of potential signaling mechanisms. Prenylation of Rho GTPases promotes their membrane localization and GTP loading. However, GTP-loaded Rho GTPases may also be located in the cytosol. Likewise, GDP-loaded, inactive Rho GTPases may be prenylated or non-prenylated and localized to the cytosol or plasma membrane. In either case, GTP-loaded Rho family members increase actin filament content by activating: (1) downstream kinases (ROCK and PAK) leading to cofilin inactivation and decreased actin depolymerization; and (2) formins or actin-binding proteins (e.g., WAVE, N-WASP) to promote actin polymerization

Interestingly, preliminary data in our studies suggest an appreciable pool of GTP-loaded Rho GTPases in the cytosol (Samuel et al., in preparation), a result unexpected based on the hypothesis that localization to the membrane is necessary for activation. As indicated briefly above, GDI-bound to GTP-loaded Rho GTPases causes sustained activation of the protein when this protein might still be in the cytosol [92]. In non-neuronal cells, inhibiting the mevalonate pathway with mevastatin results in activation of Rac1, Cdc42, and RhoA [85], indicating that membrane localization is not necessary. We hypothesize that geranylgeranylation of RhoA and Rac1 may not always correlate with promoting GTP loading. A potential mechanism supported by our data and the studies published thus far is depicted in Fig. 4. According to this scheme, pools of GDP- and GTP-loaded Rho GTPases are both associated with the plasma membrane and in the cytosol. This pattern may lead to the intricate regulation of spatial/temporal patterns of Rho GTPase activation necessary to support axon growth and guidance.

Interaction with Downstream Effectors of Rho GTPases

It is likely that membrane localization of Rho GTPases is important for regulating their interactions with downstream effectors. In PC12 cells, cytosolic localization of RhoA prevents its association with ROCK [33]. However, in a study in non-neuronal cells, ROCK is able to bind RhoA in cytosol [94]. Rac1 activation of PAK may also be regulated by membrane localization. In fibroblasts, cytosolic Rac1 does not interact with PAK [28]. This regulation of effectors has been assessed by investigating how altering prenylation affects cofilin activity, since cofilin is regulated by LIMK downstream of ROCK and PAK and can be affected by activity in each of the classical Rho GTPases. This approach is logical since all the classical Rho GTPases can inactivate cofilin. However, interpretation of the main effects may be clouded by the potential crosstalk between the actions of individual Rho GTPases on cofilin activity. For example, cofilin inactivation by phosphorylation is associated with increased growth cone motility [95] and increased neurite outgrowth [63]. However, cofilin co-localizes with actin in growth cones and is required for neurite outgrowth [96], and Rho-mediated neurite retraction occurs through phosphorylation of cofilin [36]. Our own data suggest that treatment with statins decreases actin filament content without significantly affecting cofilin activity (Samuel et al., submitted for publication). Future studies that comprehensively address the consequences of geranylgeranylation on Rho GTPase subcellular localization, activation and signaling should help illuminate the intricate mechanisms involved in regulating these proteins and axon guidance.

Summary

The investigations detailed above present some intriguing results associated with statins and neurite outgrowth. The responses, rather than being attributable to decreases in cholesterol, might be due to promotion of neural plasticity through synapse formation and axon and dendrite extension. If this is true, then prenylation may have an important role in regulating process outgrowth during development and regeneration following nervous system damage. However, recent studies reporting the action of statins on neurite outgrowth yield contradictory results with statins both inhibiting and promoting outgrowth. Since Rho GTPases are regulated by prenylation and play important roles in directing neurite outgrowth, future work will likely focus on these proteins. Thus far, statins appear to be important regulators of Rho GTPases and subsequent outgrowth. However, experiments conducted also leave several questions unanswered, including: “How does prenylation of Rho GTPases modulate neurite outgrowth?”; “Does prenylation affect activation of Rho GTPase effectors?”; and “What effect does prenylation have on GTP loading of Rho GTPases generally thought to have opposite effects on neurite outgrowth (e.g., RhoA and Rac1)?”

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