Rho GTPases and Their Regulators in Neuronal Functions and Development

Cheng-Gee Koh
School of Biological Sciences, Nanyang Technological University, Singapore, Singapore

Introduction

The development of the neuronal network in an organism is a complex process. During development, neurons receive stimuli and guidance cues that enable their outgrowths to extend and reach their final destinations. It is also during the developmental process that the majority of the neurons become polarized, acquiring the axons and dendrites that define their morphologies. Generally, axons transmit signals from the cell body to other neurons or cells and dendrites receive signals. The cytoskeleton of the axons and dendrites is composed of microtubule and neurofilament bundles in the middle and actin filaments at the periphery. Some dendrites have tiny protrusions called spines which are made of actin filaments. The dendritic spines are usually sites of synaptic contacts. The dynamic interplay between microtubule and actin elements is crucial to the response of neuronal growth cones to guidance cues [1, 2]. The underlying mechanism is unclear, and other molecules such as mDia and stathmin have been found to participate in regulatory pathways that can functionally integrate the actin cytoskeleton and microtubules [3, 4]. The overall shapes and sizes of neurons differ from species to species and vary substantially within a species depending on their activities and locations. The morphologies of these different cell types are determined by cytoskeletal events that are modulated by the activities of Rho GTPases and the downstream pathways they regulate.
The Rho GTPases act as molecular switches to control signaling events. The activities of these GTPases are crucial in regulating functions such as cell movement and motility, transcription, cell growth and proliferation, as well as cell cycle progression. Members of the Rho GTPase family are generally about 20–25 kDa in mass. They alternate between GTP- and GDP-bound states. The Rho GTPases are activated by the guanine nucleotide exchange factors (GEFs) that replace the GDP by GTP (fig. 1). The GTPases are inactivated when the bound GTP is hydrolyzed to GDP. Although Rho GTPases have intrinsic GTPase activity, the rate of hydrolysis is slow. Hence the GTPase-activating proteins (GAPs) act as the negative regulator of the molecular switch by catalyzing the hydrolysis of GTP to GDP. The Rho GTPases are normally sequestered at the cytoplasm by RhoGDI until the cell is stimulated. There are effector proteins which are downstream of the Rho GTPases; they bind to and are activated by the GTP-bound form of the GTPases. Individual members of the Rho GTPases are known to cause specific changes to the actin cytoskeleton of the cells. Three of the most well studied Rho GTPases are RhoA, Cdc42 and Rac1. One of the main functions of these proteins is the regulation of actin cytoskeletal structures in the cell. In fibroblasts and epithelial cells, active RhoA promotes stress fiber formation and enhances focal adhesions, whereas active Cdc42 and Rac1 induce filopodia and lamellipodia, respectively. Actin polymerization at the leading edge of the cell is thought to be responsible for driving the cell membrane forward, involving peripheral cell structures such as lamellipodia and filopodia. Lamellipodia are made up of short branches of actin filaments forming a network. Each actin filament has branches at an angle of 70°, with the barbed end towards the cell membrane [5]. On the other hand, filopodia do not contain a meshwork of actin branches and are made up of long actin bundles. The rearrangement of the cytoskeletal structures is pivotal to the outcome of the signal transduction events downstream of the Rho GTPases.

Many of the downstream effectors of the Rho GTPases and the pathways they regulate are well characterized. In particular, Rho kinase/ROK [6, 7] and mDia [8], which are downstream of RhoA have been shown to promote the formation of stress fibers. ROK can phosphorylate and inactivate the myosin binding subunit of the light chain (MLC) phosphatase [9]. This results in an increase in the phosphorylation of MLC, an enhancement of actin binding/bundling activity and consequently, an increase in the formation of stress fibers. mDia has actin polymerization activities and can promote long thin stress fibers along the cell. The effector proteins downstream of Rac1 in lamellipodia formation are mainly the WAVE subfamily of the WASP proteins [10, 11]. POR1 may also be involved in this process [12]. N-WASP mediates the link between Cdc42 and the Arp2/3 proteins in actin polymerization, and participates in the formation of filopodia [10, 13]. MRCK, a ROK-related target of Cdc42, is involved in the formation of focal complexes and filopodia since a kinase-inactive mutant of MRCK can block these processes downstream of Cdc42 [14].

In this review, we shall describe the roles of Rho GTPases and their regulators in the regulation of neurite formation and their response to guidance cues. The signaling molecules and events involved will also be discussed.
Roles of Actin Cytoskeleton in Neuronal Cells

The cytoskeleton of a neuron is important in the modulation of its morphology and for its targeted movement towards guidance cues. Branching of the neuron is also crucial for its communication with other cells. Recent studies have shed light on the roles of actin cytoskeleton in the determination of dendrite morphology and in axon guidance. It has been reported that WAVE1, a downstream effector of Rac1, is responsible for the number of dendritic spines in the neurons. Phosphorylation of WAVE1 by the cyclin-dependent kinase 5 (Cdk5) inhibits WAVE1’s activity and thus limits its capacity to regulate Arp2/3-dependent actin polymerization [15]. Cdk5 and its regulator p35 have also been shown to interact with both Rac1 and PAK leading to downregulation of PAK activity [16]. The effect of PAK activity on the actin cytoskeleton is well documented [17]. Active PAK induces stress fiber loss and dissolution of the focal adhesions [18]. However, PAK may stabilize actin structures through its action on LIM kinase. PAK phosphorylates and activates LIM kinase [19] which in turn phosphorylates cofillin and inactivates the actin filament severing activity of cofillin. PAK also phosphorylates both the MLC and MLC kinase that have effects on the bundling of actin and myosin in the cell [20].

The focal adhesion complexes also play a role in neurite extension and axon guidance. Adherence to the extracellular matrices (ECM) is mediated through the integrin receptors. The clustering of these receptors results in the formation of adhesion complexes due to the aggregation of adhesion and signaling proteins. Many of the proteins at the focal adhesion complexes are tyrosine-phosphorylated. Accumulation of phosphotyrosine signals has been observed at the focal complexes and at the tip of filopodia of the growth cones [21]. FAK (focal adhesion kinase) and Src are two tyrosine kinases likely to participate in the phosphorylation events at these two sites. FAK activity is required for the formation of the adhesion contact points for the stabilization of lamellipodial protrusions on the ECM. It has been demonstrated that FAK activity is required for ECM-dependent growth cone turning in vitro [22]. An in vivo study using the Rohon-Beard sensory neurons in Xenopus showed that a reduction in FAK activity resulted in less neurite outgrowth and extension. FAK activity is also required for proper path guidance of the commissural interneuron [22]. However, FAK activity is not always associated with neurite outgrowth and extension. Increased FAK activity can also result in RhoA activation which is mediated by p190RhoGEF. The exchange factor for RhoA is phosphorylated and activated by FAK [23]. Neurons expressing a mutant of p190RhoGEF that cannot bind FAK have a similar phenotype to neurons expressing a FAK mutant with disrupted activity [24], both displaying excessive axonal arborization and branching. The observation indicates that FAK can also control axonal arborization and growth via RhoA.

Another focal adhesion complex component, paxillin, is found to be one of the proteins that are tyrosine phosphorylated at the focal point contacts. Paxillin has also been found to be a possible convergent point of neurite extension. Phosphorylation of paxillin at serine-178 is essential for neurite extension. Mutation of serine-178 to alanine results in a mutant that inhibits neurite extension in neuroblastoma N1E115 cells [25].

RhoA and Cdc42/Rac Have Antagonistic Effects on Neurite Outgrowth

It has long been established that the downstream effects of RhoA and Cdc42/Rac can be antagonistic to one another in cells [26]. This antagonism is also manifest in neurite formation and outgrowth (fig. 2). Studies in neuronal cell lines have found that active Cdc42 and Rac are required for neurite formation while dominant negative Cdc42 and Rac1 have been found to inhibit neurite outgrowth in N1E115 cells [27]. Strong Rac1 and Cdc42 activities have also been localized to the tips of the growing neurites in PC12 cells stimulated with nerve growth factor (NGF) [28]. RhoA activity, on the other hand, is frequently associated with inhibition of neurite outgrowth. Constitutively active RhoA has been shown to induce neurite retraction in PC12 and N1E115 cells [29]. Blocking of RhoA activity using C3 toxin from Clostridium botulinum or using dominant negative RhoA induces actin structures or outgrowth similar to the activation of Cdc42 or Rac1 [30]. The C3 response can be blocked by co-expression of dominant negative Cdc42, implying that RhoA and Cdc42 have antagonistic roles in neurite outgrowth [31]. The RhoA-induced neurite retraction was found to be mediated by the actions of ROK. Active ROK can induce cell rounding and neurite retraction with concomitant increase in MLC phosphorylation in N1E115 cells [32]. A specific inhibitor of ROK, Y-27632, can block the ROK-induced neurite retraction as well as MLC phosphorylation. Studies on primary neurons have also confirmed the findings that Cdc42 and Rac1 generally enhance neurite formation and outgrowth whereas...
RhoA activity inhibits these activities. For example, in primary hippocampal neurons constitutively active Rac1 increases while dominant negative Rac1 decreases neurite outgrowth. In the same system, constitutively active RhoA inhibits neurite extension [33]. However, recent data have indicated that it is the balance of Rho GTPase activities that is important in the regulation of neurite outgrowth. Both Rac1 and RhoA can promote neurite outgrowth through the regulation and stabilization of adhesion contacts to the cell matrix [34]. It was found that inhibition of Rac1 prevented adhesion point contact formation at the growth cone that was required for neurite protrusion. On the other hand, expression of dominant active Rac1 resulted in unstable point contacts. Too much or too little Rac1 activity reduces neurite outgrowth. Interestingly, inhibition of ROK leads to unstable point contacts, an effect similar to that of dominant active Rac1. Hence, coordination of activation of Rac1 and RhoA is required for neurite outgrowth.

Another interesting finding which supports the coordinated activation of Rho GTPases in neuronal development comes from a study of stromal cell-derived factor (SDF)1α, a chemokine involved in the migration of cerebellar granule cells. SDF1α stimulates the activation of RhoA that in turn activates different effectors depending on the concentration of SDF1α present. It was found that low concentrations of SDF1α result in the activation of mDia, a RhoA effector which has actin polymerization activity. mDia can promote axon elongation. High concentrations of SDF1α result in the activation of ROK which leads to inhibition of axon growth [35].

**Rho GTPase Activators in Neuronal Development**

The Rho GTPases are activated by GEFs. The GEFs catalyze the exchange of GDP for GTP on the Rho proteins. They generally contain tandem Dbl homology (DH) and pleckstrin homology (PH) domains. The DH domain is responsible for the exchange activity, whereas the PH domain is reported to have a targeting function [36]. Some of the GEFs involved in neurite formation include Tiam1 (the invasion inducing T-lymphoma and metastasis 1 protein), STEF (SIF and Tiam1 like exchange factor) and FIR (FERM domain including RhoGEF) [37–39]. All three GEFs activate Rac1. Tiam1 is highly expressed in the developing nervous system. It has been shown to cause cell spreading and neurite outgrowth when overexpressed in N1E115 cells [40]. The neurite extension induced by Tiam1 can be blocked by co-expression with active RhoA, whereas dominant negative RhoA
enhances Tiam1-induced neurite outgrowth [40]. Apart from its activities in neurite outgrowth, Tiam1 has been reported to be the link between the NMDA receptor and the development of dendritic arbors and spines [41]. A subunit of NMDA receptor was found to interact with Tiam1. Moreover, knocking down of Tiam1 by RNAi in hippocampal neurons led to reduced dendritic arbor complexity and spine density. The role of Tiam1 in spine formation and development is also coupled to the activity of Par-3 which is a polarity protein. The interaction between Par-3 and Tiam1 is important for the proper localization and thus regulation of Rac activities for spine morphogenesis [42]. STEF is another Rac-specific GEF that is highly expressed in the brain. As with Tiam1, overexpression of STEF in N1E115 cells causes neurite formation. Dominant negative Rac1 can prevent STEF-induced neurites [38]. However, Rac1 activity does not always positively correlate with neurite formation and extension. FIR, a GEF specific for Rac, appears to induce partial neurite retraction or shortened neurites in cortical neurons [39].

Another well documented Rho GEF in the promotion of neurite outgrowth is Trio. Trio has two GEF domains. The first GEF domain is active towards RhoG and Rac1 and the second domain is for RhoA. The first domain is responsible for the induction of neurite outgrowth. It has been demonstrated that Trio protein starts to accumulate under NGF treatment and that the activation of RhoG by Trio is essential for the NGF-induced neuronal differentiation in PC12 cells [43]. It has also been reported that Trio activity is required for axon pathfinding. The Trio mutants of *Drosophila* have misguided axons and axons which lose the ability to reach their target [44–46]. A genetic study of the photoreceptor system in *Drosophila* has implicated PAK, a downstream effector of Rac1 and Cdc42, in the Trio-Rac signaling pathways. PAK is recruited to the membrane by the adaptor protein Dock. Loss of PAK function causes the photoreceptor axons to be misguided and results in over extension and projection of the axon into deeper regions of the brain beyond the medulla. This medulla bypass phenotype is also observed in Trio and Dock loss of function mutants [46]. PAK and Rac1 mutation also cause axon guidance defects in *Drosophila* mushroom body neurons [47]. Similar to Trio, Kalirin is another GEF that contains two GEF domains, the first specific for RhoG and Rac1 and the second for RhoA [48]. Interestingly, the two GEF domains promote different phenotypes when overexpressed in cortical neurons. Overexpression of the first GEF domain alone resulted in shorter neurites and axons, whereas overexpression of the second GEF domain alone induced extension of the axon [49]. Overexpression of full length Kalirin-9 induced longer processes. Clearly the coordination of activities of the two GEF domains is important for the regulation of neurite extension and retraction. Kalirin interacts with the neurotrophin receptor TrkA and this interaction provides a possible mechanism to explain activation of tyrosine kinase receptor as well as of Rho GTPases by extracellular stimuli in the neuronal cells [50]. An isoform of Kalirin which lacks the second GEF domain, Kalirin-7, was found to interact with PSD-95, a PDZ domain containing protein enriched at the postsynaptic density [48]. It is believed that the interaction with PSD-95 targets Kalirin-7 to the PSD where it can regulate Rac1 activity required for the modulation of dendritic morphologies. Kalirin-7 is localized to the dendritic spines. Transfection of Kalirin-7 into primary cortical neurons increases spine like structures. In contrast, transfection of Kalirin-7 mutant with an inactivated GEF domain results in a reduction of dendritic spines.

Another interesting GEF which participates in neurite morphogenesis is PIX. PIX is a GEF for Rac and Cdc42 [51]. The various PIX isoforms contain different domains for interaction with other proteins but all contain the SH3 domain for interaction with PAK, the conserved DH and PH domain found in all RhoGEFs as well as the GIT-interacting domain. Certain patients with mental retardation have abnormal dendritic spine morphology, and mutations in PAK and αPIX have been found in these patients implying the involvement of the Rho signaling pathway [52, 53]. GIT is another component of the PAK-PIX complex. GIT targets the PIX complex to the focal adhesion [54] and dendritic spines [55]. PIX regulates the localized activation of Rac1 at the dendritic spines with PAK acting as the downstream effector [56]. In cultured hippocampal neurons, expression of dominant negative GIT resulted in the mislocalization of GIT that led to numerous dendritic protrusions and a reduction in the number of synapses. Constitutively active Rac1 produces a similar phenotype as the GIT mutant, possibly due to mislocalized activation of Rac1. Dominant negative Rac1 can block formation of the dendritic protrusions caused by the mislocalized GIT [55]. Interaction between PIX and Shank/Pro SAP has also been reported [57]. Shank is a multi-domain scaffold protein involved in the organization of the PSD. It may function to recruit the PAK-PIX-GIT complex to post-synaptic structures.
Table 1. Summary of the regulators of Rho GTPases and their functions in neurons

<table>
<thead>
<tr>
<th>Regulators</th>
<th>GTPases</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ephexin</td>
<td>RhoA, Cdc42, Rac</td>
<td>growth cone turning</td>
</tr>
<tr>
<td>FIR</td>
<td>Rac</td>
<td>neurite retraction</td>
</tr>
<tr>
<td>Intersectin</td>
<td>Cdc42</td>
<td>branching, spine formation</td>
</tr>
<tr>
<td>Kalirin</td>
<td>RhoG, Rac (GEF1)</td>
<td>spine formation; neurite and axon extension</td>
</tr>
<tr>
<td>RhoA (GEF2)</td>
<td>extension and retraction</td>
<td></td>
</tr>
<tr>
<td>PIX</td>
<td>Rac, Cdc42</td>
<td>regulation of spine protrusion</td>
</tr>
<tr>
<td>P190GEF</td>
<td>RhoA</td>
<td>dendrite branching</td>
</tr>
<tr>
<td>Vav</td>
<td>Rac</td>
<td>axon guidance</td>
</tr>
<tr>
<td>GEF KIAA03880</td>
<td>RhoA</td>
<td>neurite retraction</td>
</tr>
<tr>
<td>STEF</td>
<td>Rac</td>
<td>neurite formation</td>
</tr>
<tr>
<td>Tiam1</td>
<td>Rac</td>
<td>neurite outgrowth and extension; spine morphogenesis</td>
</tr>
<tr>
<td>Trio</td>
<td>RhoG, Rac (GEF1)</td>
<td>neurite outgrowth, axon pathfinding</td>
</tr>
<tr>
<td>RhoA (GEF2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grit</td>
<td>RhoA, Cdc42</td>
<td>neurite elongation</td>
</tr>
<tr>
<td>p190RHoGAP</td>
<td>RhoA</td>
<td>neurite outgrowth</td>
</tr>
<tr>
<td>RICS</td>
<td>Cdc42, Rac</td>
<td>shorter neurites</td>
</tr>
<tr>
<td>srGAP</td>
<td>Cdc42</td>
<td>axon guidance</td>
</tr>
<tr>
<td>Vilse</td>
<td>Rac</td>
<td>axon guidance</td>
</tr>
</tbody>
</table>

Rho Regulators in Neuronal Development

The major proteins involved in the downregulation of the GTPases are the GAPs. They accelerate the hydrolysis of GTP to GDP thereby inactivating the Rho protein. Some of the RhoA GEFs have been shown to induce neurite retraction, while RhoGAPs have been reported to support neurite outgrowth and extension. A Rho-specific GEF KIAA03880 has been identified to induce neurite retraction in the neuroblastoma Neuro2 cells [58]. On the other hand, overexpression of p190 RhoGAP results in extensive neurite outgrowth presumably through the inactivation of RhoA [59]. Another GAP found mainly in neuronal cells is Grit, which is active towards RhoA and Cdc42 but not Rac1. Grit also interacts with TrkA. Overexpression of Grit promotes neurite elongation in NGF-stimulated PC12 cells [60].

RICS, found mainly in the brain, is a GAP for Cdc42 and Rac1. RICS knockout mice are found to develop normally, but their neurons exhibit higher Cdc42 activity when compared with wild-type neurons [61]. Hippocampal and cerebellar granule neurons from RICS−/− mice have longer neurites than wild-type neurons. These observations indicate that RICS play a role in modulating neurite extension by controlling the activity of Cdc42. A summary of Rho GEFs and GAPs and their function in neuronal cells is given in table 1.

Guidance Cues and the Rho GTPases

Some axons travel long distances to reach their target sites of action and to form connections to the cells they communicate with. The paths taken by the axons are dependent on the guidance cues they are exposed to. These guidance cues can be attractive or repulsive. Cytoskeletal changes are required to make the turns or movements needed. Usually, an attractive cue will increase actin polymerization and induce morphological changes to allow the axons to extend. A repulsive cue will usually cause the axon to turn away from the signal by the collapse of its growth cones. There are four major families of guidance molecules that provide the signals for axon guidance. They are the semaphorins, ephrins, slit and netrins. The semaphorins are secreted or membrane-associated proteins that direct axons away from cells which express them. All semaphorins contain a conserved ‘sema’ domain of 400 amino acids and they are divided into 8 classes dependent on their sequence homologies. The receptors for semaphorins are the Plexins. They are required to mediate growth cone collapse in response to semaphorins. It has been reported that Plexin-B1 contains a GAP domain for R-Ras in vitro and in cultured hippocampal neurons [62]. The Plexin GAP activity is stimulated by the binding of semaphorin as well as of another small GTPase, Rnd1, to the linker region on Plexin-B1. The Ras
GAP activity has been implicated in neurite remodeling through R-Ras regulation of integrin and ECM interaction [63]. When R-Ras activity is reduced, the resultant reduction in integrin activity will lead to the suppression of attachment towards the ECM and thus repulsion from the guidance signals [64, 65]. Plexin has also been shown to interact with Rac1. This interaction prevents Rac1 from binding and activating Pak and also enhances RhoA activity [66]. Interestingly, it is also reported that Rac1 can be activated by semaphorin 3A stimulation that in turn activates Plexin-A1 [67].

The ligands of the Ephrin family are membrane-bound proteins which are either GPI-linked (ephrinA) or containing transmembrane domains (ephrinB). The receptors for the ephrins are the Eph receptor tyrosine kinases. Interaction between Ephrin and Eph receptors can induce either attraction or repulsion of growth cone during development. Eph signaling has been shown to affect RhoA activities. Ephrin 5A causes growth cone collapse by activating RhoA and Rho kinase [68]. However, the Eph receptor EphA4 inhibits RhoA in Xenopus [69]. Recently Ephrin-Eph signaling has been linked to two GEFs and their activities resulted in the repulsive behavior in axon guidance. Ephexin1 and Vav2 are two GEFs identified as Eph4A-interacting proteins in a yeast two hybrid screen for signaling molecules downstream of Eph. Ephexin1 can activate RhoA, Cdc42 and Rac1. When there is no ephrin stimulation, there is a balance of GEF activities towards all three Rho GTPases. In the presence of ephrin, EphA receptor activation leads to the phosphorylation of ephexin1 that enhances its exchange activity towards RhoA while the activities toward Rac1 and Cdc42 remain unchanged [70].

The mechanism of Vav2-induced axon guidance is different from that of ephexin1. Upon stimulation by ephrin to Ephs, Vav-dependent endocytosis of the ligand-receptor complex is triggered. This causes growth cone collapse, turning the original adhesive behavior to a repulsive one. Vav2-Vav3 double knockout mice showed projection defects of their retinogeniculate axons [71]. Other GEFs such as Tiam1, Kalirin-7 and Intersectin have also been implicated in axonal guidance or neuronal development downstream of the Eph receptors. It has been reported that Tiam1 which is GEF for Rac1 interacts with the cytoplasmic regions of ephrin-B1 and EphA2 [72]. Dominant negative Tiam1 or mutants of ephrin-B1 or EphA2 which do not contain the Tiam1-interacting regions prevented neurite extensions in cortical neurons and neuroblastoma cells. Studies on hippocampal neurons showed that Kalirin translocated to the synapses upon the activation of the EphB receptor and led to the activation of Rac1 and Pak, which in turn induced the formation of dendritic spine projections [73]. The EphB receptor also associates with Intersectin, a GEF for Cdc42 [74]. The activity of Intersectin is enhanced through its binding to EphB and N-WASP. This complex can drive Cdc42 signaling to modulate actin cytoskeletal changes during neuronal branching and spine formation.

Similarly, the Slit family of guidance molecules and its receptor Robo have been found to influence neuronal migration through the interference of Rho signaling. Slit was first identified in Drosophila but have homologues in mammals, frogs and chickens. Besides having a repulsive property in neuronal guidance, Slit also inhibits chemotaxis in leukocytes [75]. The intracellular region of Robo was found to interact with the Slit-Robo GAP (srGAP) which is specific to Cdc42 [76]. Slit binding to Robo increases the srGAP activity towards Cdc42. The downregulation of Cdc42 results in the repulsion of the neurons. Constitutively active Cdc42 can block the repulsive effect of Slit. More recently, another GAP found in the Drosophila, Vilse, has also been reported to mediate Slit-Robo repulsion in axons [77]. Vilse shows GAP activity towards Rac1 and to a lesser extent Cdc42. It is suggested that Vilse participates in the modulation of Cdc42 through its downregulation of Rac1 and its effectors and thus their regulation of the actin cytoskeleton.

The netrins are another family of secreted guidance molecules. They can act as both attractive and repulsive signals. The major receptors for netrins are DCC (deleted in colorectal cancer) and UNC-5. The many proteins reported to be targets downstream of DCC include the small Rho GTPases, MAP kinases, second messenger system involving cAMP and the microtubules [reviewed in 78]. It has been reported that Rac1 and Cdc42 activities are required for the induction of neurites downstream of DCC and that downregulation of RhoA and ROK induced by DCC lead to neurite outgrowth in neuroblastoma cells [79]. Rac1 activities can be increased up to fourfold through netrin-1 stimulation of DCC. DCC also signals through FAK after netrin-1 stimulation. FAK is found to interact with DCC and is activated downstream of DCC. Interfering with the FAK activity results in the inhibition of netrin-1-induced neurite outgrowth and growth cone turning [80–82].
Conclusion

The activities of RhoA and Cdc42/Rac are essential for neurite outgrowth and extension as well as axon guidance. Generally, Rac and Cdc42 induce the formation of lamellipodia and filopodia and hence promote neurite outgrowth and extension. The GEFs and GAPs for these GTPases would therefore contribute positively and negatively towards neurite outgrowth respectively. RhoA, on the other hand, is generally associated with neurite retraction. However, there are exceptions. FIR, a GEF for Rac, induces partial neurite retraction in cortical neurons and RhoA effector mDia promotes axon elongation. Similarly, recent findings have suggested that the balance of Rho GTPase activities is required for the control and regulation of neurite outgrowth and extension; too much or too little Rac activity reduces neurite outgrowth. Different Rho GTPases and the signaling pathways associated with them are activated in response to the interaction between neuronal cells and the ECM, and to the presence of different guidance signals. GEF such as Ephexin can vary its activities for RhoA but not Cdc42 and Rac dependent on the guidance cues. How the GTPases and their effector proteins are regulated will eventually determine the changes in the actin cytoskeleton that are so critical to the final morphologies and functions of neurons.

References


