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Actin-Based Growth Cone Motility and Guidance

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Abstract

Nerve growth cones, the dilated tip of developing axons, are equipped with exquisite abilities to sense environmental cues and to move rapidly through complex terrains of developing brain, leading the axons to their specific targets for precise neuronal wiring. The actin cytoskeleton is the major component of the growth cone that powers its directional motility. Past research has provided significant insights into the mechanisms by which growth cones translate extracellular signals into directional migration. In this review, we summarize the actin-based mechanisms underlying directional growth cone motility, examine novel findings, and discuss the outstanding questions concerning the actin-based growth cone behaviors.

The formation of complex brain circuits depends on the guided extension of axonal projections from neurons to their specific targets for synaptic connections. During development, each axon is led by a motile tip, termed the growth cone (Ramón y Cajal, 1909). Nerve growth cones possess both the motility machinery and sensory apparatus that enable them to sample the environment and path-find through the complex and changing terrains of developing brain. Over the past two decades, tremendous progress has been made towards identifying the molecules involved in the guidance of axons to their destination (Kolodkin and Tessier-Lavigne, 2011). A majority of these guidance cues act on the growth cone – some promote and attract whereas others inhibit and repel growth cone extension. It is well established that coordinated actions of attractive and repulsive cues in spatiotemporally expressed patterns guide billions of axons to their designated targets. We have also learned a tremendous amount of details about the signaling pathways that relay various extracellular cues to distinct motile growth cone responses. Whether diffusible or surface-bound, guidance molecules primarily target the motility apparatus of the growth cone, of which the actin cytoskeleton is the major player.

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Like motile cells, nerve growth cones move primarily by actin-based machinery. However, this movement requires coordinated cellular events of dynamic formation and turnover of adhesions, expansion and retrieval of plasma membrane, extension and consolidation of growth by microtubules, and MT-based transport that delivers and removes cargos to and from the growth cones. (Vitriol and Zheng, 2012). Extracellular molecules can target any of these aspects of motility to modulate the movement and, importantly, to elicit specific guidance responses of the growth cone. Directed growth cone movement during axon guidance is achieved by protruding towards the attractive cues and retracting from the repulsive molecules in an actin-based manner (Figure 1a). A number of excellent reviews are available that provide comprehensive coverage on the actin structure and dynamics in both non-neuronal cells and nerve growth cones (Dent et al., 2011; Lowery and Van Vactor, 2009; Pollard and Cooper, 2009; Rodriguez et al., 2003). In this review, we will selectively discuss some of the recent findings that yield novel insights into the actin-based mechanisms underlying growth cone motility. We will also highlight outstanding questions as well as discuss the future direction of actin-based growth cone research. The goal of this review is to promote creative thinking that will provide a foundation for future studies, thereby allowing us to better understand the actin mechanisms underlying brain wiring and regeneration.

Growth cone structure

In vivo, the growth cone appears as a dilated club-shaped axon terminal that exhibits amoeba-like motility. In cell culture, the growth cone is often seen as flat and fan-shaped with two easily identifiable regions: the peripheral (P-) and central (C-) regions. Each of the regions possesses a unique cytoskeletal organization that underlies growth cone shape and function (Figure 1b). The P-region is a broad, flat area characterized by lamellipodia and filopodia, two types of actin-based membrane protrusions that serve different functions in growth cone navigation: lamellipodia mainly function in movement whereas filopodia are involved in sampling the extracellular environment. The lamellipodia consists of a network of short and branched actin filaments, which is punctuated by long, cross-linked F-actin filopodial bundles. The C-region, located behind the P-region and connected to the axonal shaft, is enriched in cellular organelles such as mitochondria and exocytotic vesicles. A predominant feature of the C-region is a dense microtubule array that extends from the axonal shaft to support growth cone movement and to serve as the tracks for transport of membranous organelles. Between the P- and C-regions resides the transitional zone (T-zone) (Lowery and Van Vactor, 2009; Rodriguez et al., 2003). The T-zone is believed to contain the actomyosin contractile structures that play a strong role in the regulation of both actin and microtubules in the growth cone, including regulating the rearward flow of actin in the P-region and maintaining the C-region localization of the microtubule lattice (Burnette et al., 2008; Medeiros et al., 2006; Zhang et al., 2003).

Building a network: actin dynamics and actin binding proteins

The actin cytoskeleton is concentrated in the nerve growth cone and its dynamics play a central role in growth cone motility. The forward advancement of the growth cone requires actin polymerization-driven membrane protrusion followed by selective adhesion to the extracellular matrix. In vertebrate cells, a large array of actin regulatory proteins control the

actin network and its dynamics through a diverse set of actions, including filament nucleation, severing, crosslinking, end-capping, and monomer-sequestering. The complex regulation exerted by actin binding proteins accounts for the structural and functional heterogeneity of higher-order actin networks required for growth cone motility. In vertebrate cells, over a hundred actin-binding proteins may exist, but how many of them are expressed and function in nerve growth cones remain to be studied (Dent et al., 2011). We will briefly describe several key actin regulatory proteins –namely, those involved in filament nucleation, severing, end capping, and monomer sequestering – that are believed to be crucial for the formation, organization and remodeling of actin filaments underlying growth cone motility.

Actin filaments (F-actin) display intrinsic polarity, preferentially polymerizing from the addition of ATP-bound G-actin at the barbed end (plus end), and depolymerizing through loss of ADP- bound G-actin subunits at the pointed end (minus end). The polarized nature of actin assembly and disassembly can lead to actin treadmilling, a state of equilibrium in which the filament maintains a constant length while individual actin subunits are being added and removed at an equal rate. Like motile cells, actin polymerization in the growth cone occurs largely in a band at the leading edge of lamellipodia, where the barbed ends of actin filaments concentrate. Such actin polymerization subjacent to the leading edge membrane is believed to push the membrane forward, leading to membrane protrusion. Two classic families of actin nucleating factors are critical for the assembly and dynamics of distinct F-actin networks in the growth cone: the actin-related protein 2/3 (Arp2/3) complex and Formin molecules (Campellone and Welch, 2010; Chhabra and Higgs, 2007; Pollard, 2007). The Arp2/3 complex, which is enriched in the P domain, binds to the side of existing actin filaments to nucleate branched actin filaments that predominate in the lamellipodia. Formins represent a large family of actin regulatory proteins that nucleate and elongate unbranched actin filaments that form the structural basis of filopodia, though some formins can also act upon Arp 2/3-derived filaments through association with growing barbed ends (Block et al., 2012). The activities of both sets of nucleating proteins are under tight control from nucleation-promoting factors (NPFs) (Campellone and Welch, 2010; Takenawa and Suetsugu, 2007), which in turn are often subjected to regulation by the Rho family of GTPases (Hall, 1998).

Because filament ends are the site of actin assembly and disassembly, capping of filament ends represents an important mechanism to regulate filament elongation and stability. Families of molecules that cap the barbed and pointed ends of actin filaments are present in neurons but their functions in growth cones have not been thoroughly studied. In motile lamellipodia, barbed end capping by capping protein (CP) is believed to promote Arp2/3-dependent actin polymerization (Akin and Mullins, 2008) but whether it functions the same in nerve growth cones has not been determined. The pointed ends of actin filaments generated by Arp2/3-mediated nucleation are often capped by the Arp2/3 complex, while the actin filaments not associated with Arp2/3 complex would require other proteins to regulate their pointed end dynamics. The tropomodulin (Tmod) family of proteins are well known for their pointed end capping of actin thin filaments in many actin-based structures, most notably, the thin filaments of skeletal muscle cells and the actin filaments in the spectrin-actin membrane skeleton of erythrocytes (Yamashiro et al., 2012). Several members of

Tmods are expressed in brain and present in the growth cone (Fath et al., 2011), but their functions in growth cone remain unknown. In principle, capping the pointed ends of actin filaments in growth cones can impair their disassembly and turnover, which, if spatiotemporally regulated, can play an important role in growth cone extension and guidance. Further discussion on pointed end capping of actin filaments in growth cone is provided later in this review.

The dynamics of actin filaments is crucial for growth cone motility and involves rapid turnover of existing filaments, which is in part mediated by filament severing through the actin depolymerizing factor (ADF)/cofilin family of proteins (hereafter referred to as AC). AC was initially identified for its ability to increase the rate of dissociation of ADP-actin from the pointed end of actin filaments to promote depolymerization (Carlier et al., 1997) and to sever actin filaments into small fragments for disassembly (Maciver, 1998). These small fragments generated by AC and other mechanisms can then be recycled into the G-actin pool. Once G-actin is made available, several families of G-actin binding proteins may contribute to G-actin localization, nucleotide state, and assembly. For example, profilin binds G-actin, promotes its nucleotide exchange for polymerization-competent ATP-G-actin, and delivers ATP-G-actin to the growing barbed end for further actin assembly at the leading edge. In addition to filament disassembly, the severing of actin filaments by AC paradoxically creates new barbed ends, which can in turn promote filament assembly and membrane protrusion (Kuhn et al., 2000; Pollard et al., 2000). In the growth cone, AC has been shown to promote both growth cone motility and collapse, likely due to the complex and opposing functions caused by filament severing. The spatiotemporal localization and activity of the aforementioned actin regulatory proteins are essential for organizing the actin networks required for advance of the leading edge during growth cone motility.

Growth cone movement: the molecular clutch

Like motile cells, the F-actin in growth cone lamellipodia undergo “retrograde flow”, which is generated by a combination of actin polymerization at the leading edge, actomyosin-based contraction at the T-zone, and AC-mediated actin depolymerization preferentially at the rear region of the lamellipodia. At rest, the retrograde flow contributes to little or no net advancement of the growth cone. Forward lamellipodial protrusion and growth cone movement are only generated when the actin cytoskeleton is “connected” to the extracellular matrix, thus generating the traction needed for the forward movement. This so-called “molecular clutch” was first hypothesized by Mitchinson and Kirschner (Mitchison and Kirschner, 1988; Suter and Forscher, 2000) over twenty years ago and has been the leading model used to conceptualize the coupling of the intracellular cytoskeleton to the adhesion machinery, an association which is required for cell migration and growth cone movement (Case and Waterman, 2015; Lin et al., 1996; Lin and Forscher, 1995; Mitchison and Kirschner, 1988). The “molecular clutch” is essentially a physical link composed of macromolecular complexes between the actin cytoskeleton and the adhesion receptors bound to extracellular matrix molecules (Case and Waterman, 2015). The engagement of the “molecular clutch” is apparently required to translate actin polymerization into forward movement of the growth cone, whereas clutch disengagement can stall the growth cone (Figure 1c). The strength of coupling between the actin cytoskeleton and adhesion

complexes i.e. the level of ‘clutch engagement’ is determined by a complex interplay of parameters such F-actin flow, the amount of F-actin coupled to adhesive sites, substrate stiffness, adhesion strength, and complex cell signaling (Giannone et al., 2009).

In the classic molecular clutch model, strong mechanical coupling between the F-actin cytoskeleton and the substrate, mediated by cell adhesion complexes, transmit the forces generated by the cytoskeleton into rearward traction forces, enabling forward advance of the growth cone. However, ‘clutch engagement’ is subject to various states defined by the strength of coupling between the cytoskeleton and the extracellular substrate. For example, a ‘slipping clutch’ is characterized by transient and weak interactions between the F-actin cytoskeleton, clutch proteins, and ligand/receptor complexes. In this state, the rate of retrograde flow has not reached maximum attenuation and traction force exerted on the substrate is low. It has been shown that the clutch formation has a latency period underlying the transition from the weak and slippery state to the strong and mature state. A recent study by Buck and colleagues (Buck et al., 2016) proposes that the forces caused by local actin filament assembly may be required for the transition from the initial ‘latency period’ of clutch formation, when retrograde flow is unaffected, to the ‘advanced period’, where strong clutch engagement and significant retrograde flow decrease allows forward advancement. Using physically restrained apCAM coated beads to mimic a noncompliant substrate, the authors found that Arp2/3 and Rac GTPase activity are required for generating branched actin assembly at the site of nascent adhesions in the latency period, producing inductopodia-like actin polymerization. Propulsive forces generated by intrapodia motility can then be converted to traction at the site of nascent adhesions, thereby ‘buffering nascent adhesions’ from the force of retrograde flow. The local actin assembly that occurs in the latent phase may counteract retrograde forces in order to facilitate the assembly of actin structures and clutch components robust enough to handle the traction forces required for growth cone protrusion. The forces generated by intrapodia activity could thereby represent a crucial step in adhesion site maturation. Intrapodia (or inductopodia), comet-like dynamic actin structures, have frequently been observed in nerve growth cones and motile cells (Forscher et al., 1992; Rochlin et al., 1999; Schafer et al., 1998). Therefore, they may function in growth cone forward movement and the “clutch” maturation. Buck et al lays the groundwork for future studies that can provide answers to critical questions regarding how forces generated by the dynamic actin cytoskeleton function together with the clutch machinery to generate and maintain the traction need for growth cone advance. For example, what are the mechanisms that regulate the formation and elimination of local actin assembly near nascent adhesions? What signaling mechanisms are involved in the transition from nascent adhesions to adhesions characterized by strong coupling between the cytoskeleton and ligand/receptor complexes? How does environmental stiffness modify adhesion strength and actin organization? The answer to this and other open questions will add depth to the traditional ‘molecular clutch’ model, thereby enhancing our understanding of actin-based growth cone advance.

Growth cone motility: monomers and caps

Cell based motility, including that of nerve growth cones, depends on actin-based lamellipodial protrusion. Such rapid polymerization can only be sustained by continuously

available polymerization-competent actin monomers (G-actin). Traditionally, G-actin is considered to exist as a single diffusible pool in the cell that passively supports actin assembly. However, recent work has shown that G-actin appears to be more concentrated in the leading edge of the lamellipodia, creating a G-actin gradient that may function in actin-based growth cone motility (Lee et al., 2013; Van Goor et al., 2012). Further investigation shows that G-actin enrichment at the leading edge of lamellipodia is dynamically regulated and plays a role in actin-based lamellipodial protrusion, cell migration, and growth cone guidance (Lee et al., 2013). Given that the local concentration of polymerization-competent G-actin directly impacts the filament assembly rate (Pollard and Borisy, 2003), these findings provide the evidence that spatiotemporal regulation of the G-actin concentration in specific cellular compartments represents a mechanism to regulate the actin dynamics underlying cell motility.

In cells, G-actin is often bound by monomer-binding proteins to regulate their nucleotide state and polymerization competence (Paavilainen et al., 2004). While both profilin and thymosin β 4 (T β 4) have been implicated in G-actin enrichment at the leading edge of lamellipodia, how they function in G-actin localization has yet to be determined. Furthermore, G-actin may come from different pools, including the cytosolic pool, recycling pool (from F-actin depolymerization), and newly synthesized pool. Whether G-actin from different pools behaves and functions the same remains unclear. For example, newly synthesized β -actin has been shown to play a role in cell motility and growth cone guidance (Leung et al., 2006; Yao et al., 2006). It remains unclear if and how newly synthesized β -actin is different from the existing pools of G-actin. Interestingly, a recent study shows that cytosolic and recycling pools of G-actin are also different and contribute to distinct aspects of actin dynamics in lamellipodia (Vitriol et al., 2015). Specifically, the cytosolic pool of G-actin appears to be T β 4-bound and destined for formin-based actin polymerization in lamellipodia underlying stimulated protrusion. On the other hand, G-actin recycled from lamellipodial F-actin appears to be function in maintaining the steady state of the lamellipodial F-actin network. This work raises the exciting possibility that discrete mechanisms exist in the growth cone to selectively generate or maintain various pools of G actin required for the formation of distinct actin substructures. Because filopodia and lamellipodia are differentially involved in growth cone navigation and advance, it will be exciting to uncover if and how the dynamic G actin localization is required to generate the dynamic properties inherent to both structures.

The above discussed G-actin regulation in cell and growth cone motility highlights one of the recent advances in our understanding of the actin mechanisms in growth cone motility. The complexity of actin regulation, however, presents a challenge to fully elucidate how actin dynamics are spatiotemporally regulated by extracellular cues to generate distinct behaviors of the growth cones during axon pathfinding. Many actin-binding proteins have not been well studied in neuronal growth cones, and whether and how they function in growth cone migration and guidance remains to be seen (Dent et al., 2011). For example, it remains to be determined if the G-actin enrichment in the leading edge of the lamellipodia described above is regulated by barbed end capping, as has been demonstrated in the lamellipodia of migrating cells. Potentially, spatiotemporally regulated capping of barbed ends of F-actin, G-actin enrichment, and Arp2/3-mediated polymerization could work in

concert to generate the motile responses of the growth cone during axon extension and guidance.

Finally, the pointed end of an actin filament mainly consists of ADP-actin and tends to depolymerize if not stabilized. The pointed end can be capped by the nucleation complex (e.g. Arp2/3) as long as it stays bound to the complex (Mullins et al., 1998). While Arp2/3-based branched actin networks are predominant in the lamellipodia, linear actin filaments are also present. It is unclear if pointed ends of both branched and linear actin filaments in growth cones are stabilized by other minus end binding proteins. In striated muscle cells, actin filaments are long-lived, which is achieved in part by capping of the both barbed and pointed ends by CP and tropomodulin (Tmod), respectively. Tropomodulins are a family of four proteins (Tmods1-4) that cap the pointed ends of actin filaments (Yamashiro et al., 2012). Both Tmod1 and Tmod2 are highly expressed in brain and are detected in nerve growth cones, but they appear to negatively regulate neurite extension (Fath et al., 2011). It is unknown, however, if this negative effect of Tmods on growth cone extension is mediated by the minus end capping of F-actin. Tmods can cap the pointed ends with and without the presence of tropomyosins (Tms), but binding of Tmods to tropomyosins greatly enhances its minus end capping activity. Several brain-specific Tms have been identified, of which Tms from TPM3 and TPM4 genes have shown to localize to postsynaptic spines (Guen et al., 2011). Whether these Tms are present and function in nerve growth cones during development remains unknown. Binding of Tms to actin filaments could regulate the actin stability and dynamics, e.g. protecting filaments from the severing action of ADF/cofilin (Bernstein and Bamburg, 1982) and gelsolin (Ishikawa et al., 1989). Together, Tms and Tmods could potentially play an important role in local regulation of the actin dynamics underlying growth cone motility.

The dynamic actin cytoskeleton in axon elongation and guidance

Guidance of developing axons involves directional steering of the growth cone towards or away from the attractive or repulsive cues (Figure 1a). This is largely achieved by rapid remodeling of the actin cytoskeleton and its dynamics. Among many actin regulatory molecules, ADF/cofilin (AC) plays an essential role in regulating actin dynamics during growth cone elongation and guidance. The activity of AC is primarily regulated by phosphorylation on a highly conserved serine residue, Ser3 (Agnew et al., 1995). Ser3 phosphorylation inactivates AC's ability to bind F-actin to depolymerize and sever. The LIM (Lin-11/Isl-1/Mec-3) kinases and the TES (testicular protein) kinases are the two kinase families that phosphorylate and inactivate ACs. Two distinct families of phosphatases, named Slingshot (SSH) and chronophin (CIN), dephosphorylate AC for re-activation (Meng et al., 2004; Niwa et al., 2002; Ohta et al., 2003). Both LIM kinases and SSH phosphatases are regulated by a wide range of signaling pathways including Rho GTPases and Ca^{2+} , thus placing AC at the converging point of intricate signaling pathways to regulate actin dynamics (Bernstein and Bamburg, 2010).

While AC primarily functions to sever and depolymerize F-actin, the effects of AC activity on the growth cone motility are complex. On one hand, AC activity is essential for the actin dynamics underlying growth cone motility, and overexpression of AC in neurons leads to

increased neurite outgrowth (Meberg et al., 1998). This is consistent with the observation that high actin turnover is associated with increased growth cone motility (Bradke and Dotti, 1999). In this case, AC is likely responsible for turning over the F-actin at the rear of the lamellipodia to support the rapid forward protrusion in part by supplying G-actin to the leading edge for polymerization. On the other hand, AC activation has been associated with growth cone collapse in response to repulsive cues (Hsieh et al., 2006; Piper et al., 2006). In this case, overall depolymerization of F-actin in growth cones was observed, suggesting that AC was activated globally throughout the growth cone to cause the destruction of F-actin structures. Therefore, different spatiotemporal patterns of AC activities may be responsible for generating distinct effects on the growth cone motility. We reported previously that attractive and repulsive turning of nerve growth cones is mediated by asymmetric AC inhibition and activation, respectively (Wen et al., 2007). Our findings are consistent with the depolymerizing/severing functions of AC on the actin cytoskeleton, but appear to differ from that observed in fibroblasts and carcinoma cells in which local AC activation promotes actin-based protrusion (DesMarais et al., 2005; Ghosh et al., 2004). Moreover, activation of AC in growth cone attraction was also observed in cultured dorsal root ganglion neurons (Marsick et al., 2010). It is plausible that different types of cells and their unique cytosolic environments, together with the spatiotemporal patterns of AC activation, may determine the final outcome on the actin structure and dynamics in the growth cones. As discussed previously, the final effects of AC on the actin structure and dynamics likely depend on a number of factors such as the spatiotemporal pattern, degree of the activation, the local G- and F-actin ratio, among other factors.

Adding to the complexity of AC regulation and effects on the actin cytoskeleton, recent studies show that AC-mediated F-actin disassembly is enhanced by actin oxidation through the redox enzyme Mical. Specifically, the synergistic actions of AC and Mical mediate growth cone collapse in response to the repulsive cue Semaphorin in invertebrates (Grintsevich et al., 2016; Hung et al., 2010). Mical associates with PlexA, a receptor for Semaphorins in *Drosophila* (Hung and Terman, 2011). Activated Mical can cause F-actin disassembly via oxidation of the bound actin subunits at their M55 residues and the oxidized G-actin also exhibits disrupted polymerization (Hung et al., 2011). Interestingly, Mical-mediated oxidation of F-actin improves cofilin binding to F-actin to augment cofilin's filament severing and disassembly properties (Grintsevich et al., 2016). The coordinated efforts between the oxidation of actin by Mical and the severing of actin filaments by AC lead to growth cone collapse, which is an example of how AC recruitment can contribute to repulsive signaling. While these findings are interesting, whether Mical functions in vertebrate semaphorin signaling remains to be determined.

The Rho family of small GTPases is consisted of three members, RhoA, Rac, Cdc42, and they play a crucial role in mediating complex signaling to the remodeling of the actin cytoskeleton. In nerve cells, RhoA is associated with inhibitory signaling that impairs growth cone motility, whereas Rac1 and Cdc42 are primarily downstream effectors of growth promoting and attractive cues (Hall and Lalli, 2010). Activation of the RhoA GTPase has been associated with growth cone inhibition by a number of inhibitory/repulsive cues such as myelin associated glycoprotein, NOGO, and Semaphorins (Fujita and Yamashita, 2014). RhoA acts through Rho kinase ROCK, which can in turn regulate several distinct

downstream targets to regulate the actin cytoskeleton in growth cones. ROCK can phosphorylate and activate LIMK1, leading to the inactivation of cofilin. Such a pathway has been indicated in growth cone inhibition by several repulsive cues (Aizawa et al., 2001; Marsick et al., 2012). In this case, however, inhibition of growth cone motility is likely achieved through the activation of the actomyosin contractility through ROCK activation of myosin light chain kinase (Amano et al., 1998; Kubo et al., 2008). Indeed, inhibition of myosin II has been shown to promote growth cone extension over inhibitory molecules (Hur et al., 2011). The finding that pharmacological disruption of F-actin abolished actomyosin-mediated growth cone retraction (Wang and Zheng, 1998) suggests its requirement for intact F-actin structure. Therefore, ROCK inhibition of cofilin may be important for actomyosin contractility and growth cone inhibition. Given that different guidance receptors could be coupled to distinct downstream targets, the above described multiple pathways involving AC exemplifies the complexity, flexibility, and adaptability of the guidance mechanisms underlying precise wiring of the nervous system.

The Rho GTPases are activated by the guanine exchange factors (GEFs) and inactivated the GTPase-activating proteins (GAPs)(Hall, 1998). In neurons, it has been shown that both RhoA and Rac1 can be activated by a GEF named TRIO, or Triple Functional Domain Protein. TRIO has been the subject of significant interest recently due to its unusual structure and key role in axon guidance. First discovered in 1996, TRIO is named for its three functional domains: a RhoG/Rac1 GEF domain, a RhoA GEF domain, and a serine/threonine kinase domain (Debant et al., 1996; van Rijssel and van Buul, 2012). Through these domains, TRIO can lead to LIM kinase activation to inhibit AC activity (Ng and Luo, 2004). The presence of GEF domains for two Rho GTPases in opposing guidance responses makes TRIO unique and suggests its involvement in multiple signaling pathways. For example, the Rac1 GEF domain of TRIO was found to function in non-canonical Notch signaling in *Drosophila* motor neurons, in which Notch activates TRIO and Rac1 to reduce fasciculation of the motor axons (Song and Giniger, 2011). TRIO is phosphorylated by Fyn Src Kinase at Y2622 in response to Netrin signaling in rat cortical neurons to activate Rac1 and produce axon outgrowth (DeGeer et al., 2013). Phosphorylated TRIO in turn increases surface expression of the Netrin receptor DCC. Thus, the growth cone behaviors produced by TRIO-based activation of Rac1 seem to depend on the cellular and signaling contexts.

Rac1 can also be activated by a second GEF found in the growth cone, known as Tiam-1. Similar to TRIO, Tiam-1 is a common GEF for both ced-10 (Rac1 in *C. elegans*) and mig2 (RhoG in *C. elegans*). Tiam-1 activation of ced-10/Rac1 was found to activate lamellipodial and filopodial protrusion downstream of Netrin/DCC signaling, in a mechanism distinct from TRIO activity (Demarco et al., 2012). This indicates that though TRIO activates the same Rho GTPases as Tiam-1, it acts downstream of different receptor pathways independently of Tiam-1, and may therefore elicit different growth cone behaviors from Rac1 activation. Further research is required to elucidate how these differing contexts of Rac1 activation can produce such distinct effects on the actin cytoskeleton and overall growth cone behavior.

Final thoughts

It is well established that a cell's ability to sense the environment and to determine the direction and proximity of an extracellular stimulus, followed by correct movement, is fundamental not only for neural development but also for immunity, angiogenesis, wound healing, and embryogenesis, as well as underlies many pathological events such as cancer-cell metastasis. Directional motility is known to be largely powered by the actin cytoskeleton and we have accumulated a wealth of information on the actin cytoskeleton and its regulation. However, there are still many outstanding questions regarding the actin cytoskeleton and its regulation in brain development and function. An increasing number of studies have shown that the actin cytoskeleton and its regulation represent an important cluster of alterations in many neurological diseases (Almuqbil et al., 2013; Fromer et al., 2014; Gilman et al., 2011; Levy et al., 2011; Sanders et al., 2011). Future studies on the actin cytoskeleton in neurons will help us better understand its disease connection and underlying mechanisms, thus providing the foundation for developing clinic interventions.

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Highlights

1. Nerve growth cones are the actin-based motile structure of developing axons.
2. Actin-based growth cone motility drives the extension of axons
3. The actin cytoskeleton of the growth cone is the major target of complex signaling elicited by extracellular molecules.
4. Guided growth cone extension is achieved by concerted modifications of the actin cytoskeleton, membrane recycling, adhesions dynamics, and microtubule growth.

Figure 1. Schematic illustration summarizes the actin-based growth cone motility
 (a) Nerve growth cones protrude towards or retract from attractive or repulsive cues during guidance. (b) A representative fluorescent image of a rat hippocampal growth cone showing the actin-rich P-region (blue) and the microtubule-concentrated C-region (red). (c) The clutch model and the local enrichment of G-actin enable the forward movement of the growth cone. The right panel highlights that the propulsive force generated by Arp2/3-mediated intrapodia contributes to the generation of traction force for growth cone movement.