CHAPTER 1

Molecular Mechanisms of Axonal Growth

Céline Bouquet and Fatiha Nothias*

Abstract

Outgrowth of axons during neuronal development, as well as their regeneration after injury, of the adult nervous system is controlled by specific extracellular cues which are diffusible, or bound to cell membranes or extracellular matrix. The exact molecular mechanisms through which these extracellular signals are integrated by the growing axon, are not yet well defined. However, it is widely accepted that most, if not all, signaling cascades triggered by guidance cues eventually converge onto the cytoskeleton. The action of extracellular guidance factors is thus modulated not only by specific membrane receptors, but also by cytoskeletal and cytoskeleton-associated molecules within the axon. In fact, the cytoskeleton represents a point of convergence and integration of both neuron-intrinsic and extrinsic factors. Moreover, in recent years, there has been increasing evidence for the involvement of a coordinated cross-talk between actin filaments and microtubules, the two main components of the growth cone cytoskeleton. Their reorganization is complex and involves numerous cytoskeleton-associated proteins whose function is regulated via activation or inhibition of particular signaling pathways.[1-4]

Introduction

The growth cone, highly motile distal tip of the axon, shares many properties with other motile structures, such as the leading edge of migrating cells. This is reflected in a similar cytoskeletal organization of these subcellular compartments, and the use of common signaling pathways, such as the one involving Rho-GTPases (see below). Despite these similarities, the behavior of neurons appears more complex than that of other cell types, in that they extend very long processes, and exhibit quite "sophisticated" responses when confronted to extracellular cues. Expression of cytoskeleton-associated molecules specific for the neuronal growth cone may, at least in part, explain some unique features of this motile structure (for review refs. 5-8).

Here, we will describe in some detail the cytoskeletal network within the neuronal growth cone, and how its organization is regulated in response to extracellular factors by integration of signaling pathways.

The Neuronal Growth Cone and Its Cytoskeletal Organization

Neurites should be thought of as exceptionally differentiated cellular processes. The growth cone tipping an axon (or dendrite) is an extremely motile and dynamic structure that explores the environment. To guide an axon towards the appropriate target, the growth cone fulfills different functions: it acts as sensor of environmental cues, signal transducer, and motility device. Growth cone advance is mediated by the polymerization/depolymerization of cytoskeletal

*Corresponding Author: Fatiha Nothias—UMR7101/IFR83, CNRS-UPMC, Bat. A/3ème Etage, Case-2, Université P&M Curie, 7 Quai Saint Bernard, 75005-Paris, France. Email: fatiha.nothias@snv.jussieu.fr

Axon Growth and Guidance, edited by Dominique Bagnard.

elements, and their specific interactions. The axonal cytoskeleton is composed of three main filamentous polymers: neurofilaments, microtubules and actin microfilaments. Within the growth cone, microtubules and actin filaments are actually the major cytoskeletal components, and have been the focus of most studies. Their spatial organization and relative position in the growth cone define different, functionally specific zones, described in Figure 1.

The Peripheral Domain

The peripheral domain (P-domain) is the most distal part of the growth cone, a highly dynamic, actin-rich structure. This domain bears lamellipodia, membranous flat veil-like protrusions, from which extend many filopodia. These very thin, finger-like structures contain mainly actin filament bundles, and undergo permanent elongation and retraction cycles as they organize their content in response to the environment.[9,10] In the P-domain, equilibrium between actin polymerization and depolymerization (actin "treadmilling") constantly generates protrusion forces, and retrograde flow of actin (see below).

The Transition Zone

The transition zone (T-zone) is situated at the interface between the actin-rich P-domain and the MT-rich central domain. The molecular motor myosin, concentrated in the T-zone, can serve to contract the actin network, thereby inducing the formation of an actin-filament arc.[11] Movements of this arc, in association with retrograde actin flow, limit the penetration of MTs into the P-domain.

The Central Domain

The central domain (C-domain) represents the main site of MT polymerization. Neurofilaments, which transport vesicles and organelles along with the MTs, are also present. The size of the C-domain varies in correlation to the growth mode of the axon: Relatively large when the growth cone is pausing, whereas the C-domain exhibits a thinner shape during fast advance mode.

Actin Filaments and Associated Proteins

Actin filaments (AFs) are helical polymers formed by addition of ATP-actin monomers. AFs are polarized structures characterized by a "pointed" and a "barbed" end. Dissociation of ADP-actin is favored at the pointed end, suggesting that polymerization occurs at the barbed end, and depolymerization at the pointed end.[7,12]

Actin is present in both the P-domain and the T-zone, where it is organized in two different types of networks:[10,13,14] filopodia are composed of thick actin bundles, while in lamellipodia, AFs are organized in a loose meshwork. Similarly, contraction of the actin meshwork in the T-zone by myosin action results in formation of a thick actin arc, oriented perpendicularly to the axon.[11]

In the P-domain, AFs polymerize close to the distal membrane, and polymers are retrogradely transported to the T-zone by a myosin-dependent mechanism.[15] Increased contractile forces in the T-zone then induce severing and depolymerization of AFs. This permanent actin treadmilling accounts for the high dynamics of the P-domain. Moreover, the retrograde actin flow generates a backward force suspected to limit MT invasion into the P-domain.

Dozens of actin-associated proteins have been described in the neuronal growth cone, and were classified according to their function (for review see ref. 16). There are two main groups regulating actin polymerization/ depolymerization:

Actin Nucleation/Polymerization

Actin nucleation/polymerization factors increasing the number of free barbed ends incidentally increase actin polymerization. The Arp2/3 complex, thus, not only favors de novo actin polymerization, but also by binding sideways to preexisting filaments, creating a new branch and hence a new barbed end (for review see ref. 17). Members of the formin protein family

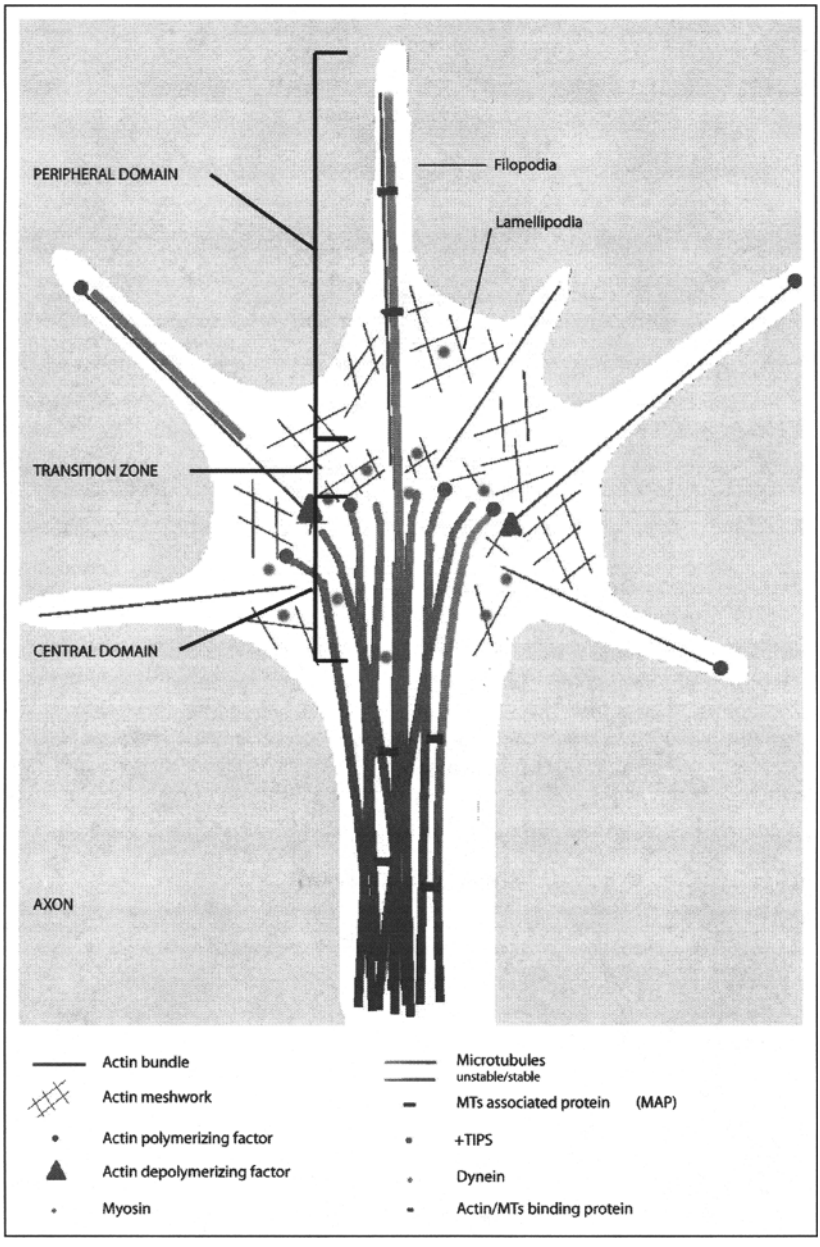

Figure 1. Cytoskeleton growth cone organization. The growth cone, tipping an axon, is divided in three different, functional zones. The C-domain is the polymerization site of MTs, which are thereafter stabilized in the axon shaft. The T-zone limits MTs penetration in the C-domain, and contains a high density actin meshwork associated with myosin. The P-domain is very dynamic and mainly contains actin, organized in bundles in filopodia, and meshwork in lamellipodia. Transient interactions between actin filaments and MTs are observed in the P-domain that are mediated by still unknown factors. MTs- and actin- associated proteins regulate their transport, polymerization and stabilization.

bind to the barbed end of elongating actin polymers, enhance filament elongation, and prevent binding of actin capping molecules (for review see ref. 18).

Actin Depolymerization/Severing

Actin depolymerizing factors (ADFs), as well as cofilins, are important regulators of actin dynamics in the growth cone. Although encoded by different genes, ADFs and cofilin have very similar effects, are regulated by reversible phosphorylation, and colocalize in the cells (for review see ref. 7). When phosphorylated, both bind to the rear end of actin filaments, generating actin fragments. Interestingly, actin severing leads to generation of new free barbed ends, thereby promoting actin polymerization, a mechanism perpetuating the retrograde actin flow (for review see refs. 7,19). Other proteins, such as gelsolin, stop actin polymerization by capping barbed ends, and thereby induce depolymerization (for review see ref. 20).

Thus, modulation of actin polymerization by extracellular guidance cues via actin-associated proteins provides a mechanism to regulate the progress of growth cones.

Microtubules and Associated Proteins

Microtubule protofilaments are formed by spontaneous association of α/β tubulin heterodimers. 13 such protofilaments finally associate to form a hollow microtubule of a diameter of 25 nm (for review see ref. 21). Regulation of expression of different α and β tubulin isoforms during axonal development and regeneration regulates MT stability.

The orientation of tubulin monomers makes MTs intrinsically polarized structures with a "plus" and a "minus" end. In the axon, plus ends are oriented distally toward the growth cone. Depolymerization mainly occurs at the minus end, while a constant cycle of polymerization/depolymerization takes place at the plus end.[22,23]

The frequency of polymerization/depolymerization or "rescue/catastrophe" events, as well as the duration of pauses in between, characterizes the "dynamic instability" of microtubules. The term dynamic instability describes an intrinsic property of MTs that allows them to switch abrubtly between phases of elongation and rapid shortening.[22] MTs are organized in parallel bundles throughout the axon shaft, and splay out when they enter the growth cone C-domain.[13,24] During pauses, MTs extend loops into the C-domain, and breakage of these loops upon regrowth results in highly dynamic, small polymers capable to enter the P-domain and associate with actin bundles. Indeed, while MTs were previously thought to be restricted to the C-domain, recent progress in imaging techniques has provided evidence for MT-actin interactions within the P-domain. This interaction is fundamental for outgrowth, guidance and branching of the axon.[11,25-28] Local stabilization of MTs is also tightly regulated during these events[29,30] by specific post-translational modifications on MTs, and by their interaction with specific associated proteins:

Post-Translational Modifications

Post-translational modifications of MTs include detyrosination/tyrosination, acetylation, phosphorylation, polyglutamylation and polyglycilation (for review see ref. 21). Unmodified tyrosinated tubulin polymers are enriched in the distal part of the axon, while modified detyrosinated or acetylated isoforms are found in the proximal part of the axon, on "older" and stable MTs.[31] Although these modifications do not have a direct effect on MT stabilization,[32] tubulin modifications are frequently used as markers of MT stability. They may, however, facilitate localization and interaction of microtubule binding proteins, such as plus end-tracking proteins (+TIPS[33]) and Microtubule Associated Proteins (MAPs[34,35]).

Proteins Associated to MTs

Proteins associated to MTs include two groups of proteins that interact with MTs and regulate their dynamic instability.

Structural MAPs such as MAP1A, MAP1B, MAP2, and Tau, bind, bundle, and stabilize MTs. Their association with MTs is regulated by post-translational modifications of tubulin, as

well as their own post-translational modification, such as phosphorylation (for review see ref. 36). MAPs are particularly abundant in the nervous system, and their subcellular localization is strictly regulated. Some MAPs are preferentially associated with neuronal processes: MAP2 is concentrated in dendrites, while tau and MAP1B are mainly found in axons (for review see refs. 37,38). As a consequence, the spatial distribution of MAPs defines subcellular zones, in which MTs are more or less stabilized. MAPs are generally important both during development and in the adult nervous system. Maturation of the nervous system is accompanied by a transition from MAPs typically expressed during the phase of axon growth, to other MAPs characteristic of mature neurons.[39,40] Certain MAPs, such as MAP1B and tau, are present in the growth cone, and were shown to play an important role in neurite outgrowth from embryonic neurons in vivo and in vitro.[41-44]

Other MAPs, identified more recently,[46,47] act as potent MT destabilizers. Among these are stathmin and SCG10, members of the same gene family, which are expressed in neurons and promote MT depolymerization by increasing the rate of catastrophes (for review see ref. 48). SCG10 and stathmin are considered as growth-associated proteins, and their expression correlates with neurite outgrowth.

Although the dynamic state of MTs has been shown to be important for neurite elongation and growth cone turning, it is still not clear how MT dynamics are regulated. In fact, MTs are known to be particularly labile within the growth cone,[31,49] despite the rather high concentration of MT-stabilizing MAPs, such as MAP1B and tau. Therefore, it has been proposed that the potent MT destabilizer, such as SCG10, might counteract the activity of stabilizing MAPs, contributing to the regulation of MT dynamics.[46,48]

Recently, a novel type of MT binding proteins called +TIPs has been identified as being specifically associated with the distal ends of growing MTs. These proteins have gained considerable interest with respect to the regulation of MT dynamics and the intracellular transport via MTs (for ref and review, see refs. 50,51), and also due to their anchorage to actin filaments and adhesion sites. A few of them have been detected in neurons, namely, cytoplasmic linker protein-170 (CLIP-170), CLIP-115, end-binding protein 1 (EB1) and EB3. Functions of these proteins in growth cone MTs remains to be determined.

Intermediate Filaments

Neurofilaments (NFs) are the major intermediate filaments in neurons. The NF network is composed of a NF-L (low molecular weight NF, 70 kD) core, associated to NF-M (medium molecular weight NF, 150 kD) and NF-H (high molecular weight NF, 200 kD) chains.[53] The function of NFs in transport of vesicles, membrane material and organelles has been extensively studied (for review see ref. 54). In contrast, even if NFs are found in the C-domain of the growth cone, they do not seem to interact with axonal growth and pathfinding. Thus, transgenic mice lacking axonal NFs are perfectly viable, and do not present any major defect in their neural connections.[55]

Molecular Motors

These molecules present a molecular motor domain capable of generating forces on cytoskeletal polymers by means of ATP hydrolysis. They serve in transporting vesicles back and forth along the axon shaft, and in addition, can generate forces on the cytoskeleton by moving polymers relatively to each other.[56] Kinesin and dynein proteins are microtubule-dependent motors and the polarized structure of MTs induces specificity in the direction of motor molecules. Most kinesins move towards the plus end of MTs, whereas dynein complex moves towards the minus end (for review see ref. 57). Myosin proteins are actin-dependant motors and rather move to the plus end of actin filaments.[58]

The diverse cytoskeletal proteins expressed in the growth cone act in concert to mediate axonal growth and pathfinding. They are regulated by extracellular cues, but also by the axon-intrinsic program. Thus, throughout development and regeneration, the expression of particular components, and their transport and final localization in the axon are tightly regulated.

The subsequent assembly of components and their interactions in the growth cone eventually modulates axonal outgrowth and pathfinding.

Mechanisms of Axonal Elongation

Synthesis of Cytoskeletal Proteins

The bulk of new cytoskeletal proteins is produced in the cell body. Recently, however, there is increasing evidence for a local synthesis in growing and regenerating axons, at least in small amounts (estimated at 5%[59]), which can play a crucial functional role. Thus, it has been demonstrated that ribosomal proteins, translational initiation factors, and ribosome-bound mRNA are present in axons. Moreover, protein synthesis occurs even when processes are separated from their cell bodies.[60,61] The rapidly growing list of identified intra-axonally synthesized proteins includes cytoskeletal proteins (intermediate filaments as well as actin and tubulin), heat shock proteins, endoplasmic reticulum proteins, metabolic proteins, anti-oxidant proteins, and proteins associated with neurodegenerative diseases (see ref. 62). When communication between processes and the cell body is interrupted by axotomy or colchicine treatment, blocking local protein synthesis in regenerating axons results in rapid retraction of growth cones, indicating a physiological importance for local synthesis during axonal regeneration[60] as well as during development[63] to respond to guidance factors.

Cytoskeletal Protein Transport

After their synthesis, cytoskeletal proteins have then to be transported to the site of axonal growth. The first studies on axonal transport were performed in the adult during axonal regeneration, and used radio labeled-methionine for tracing of newly synthesized proteins. They showed a correlation between the rate of axonal regeneration, and the rate of the slow axonal component (SC[64]). Tubulin and actin are transported in two peaks, differing in their velocity and content. In mammals, the slower peak, SCb, is mostly composed of tubulin, while actin moves faster in association with the SCa peak (reviewed in ref. 65).

The polymerization status of actin and tubulin during their transport, as well as the exact mechanism of their transport are still unclear and have been much debated in recent years. Novel methods using fluorescent proteins and time lapse imaging may now yield new insight into this problem.[66] Two models have been proposed: The classical "cargo" model assumes that tubulin and neurofilament polymer transport uses the classical motor molecules. The "sliding filament" model[67,68] suggests that short tubulin polymers can be moved anterogradely on longer MTs by dynein. NF transport was suspected to be linked to this MT transport with the NF "piggy backed" on MTs, but recent evidences suggest that it may rather rely on the classical cargo model.[67,68]

Less is known about anterograde transport of actin. Myosin seems to be the motor for at least a subpopulation of para-axially aligned actin filaments.[68]

Axonal Elongation

During axonal elongation, 3 phases can be distinguished (reviewed in refs. 16,69): In the initial protrusion phase, lamellipodia and filopodia extend from the tip of the axon, forming the growth cone. This phase is mainly governed by actin dynamics, which in turn, are regulated by Rho family GTPases, but is also modulated by MTs dynamics.[70] The engorgement phase that follows protrusion, consists in the invasion of MTs and organelles into the growth cone. It depends on the dynamic instability of MTs, since inhibition of these dynamics leads to a reduction in axonal growth.[27,71,72] During the final consolidation phase, the formation of actin protrusion stops, and MTs become bundled. This phase probably relies on the activity and interaction of microtubule- and actin-associated proteins, although it is still not well elucidated.

Axonal elongation is modulated by extracellular factors that the growth cone senses in the environment. Extracellular guidance cues elicit diverse intracellular signaling cascades. Here

we will particularly focus on the signaling mediated by Rho-GTPases, as the consequences of their activation on reorganization of the cytoskeleton has been well characterized.

Regulation of the Cytoskeleton by Extracellular Cues, Role of Rho-GTPases

Regulation of Rho-GTPases

Rho-GTPases act as "molecular switches" by oscillating between an active, GTP-bound and an inactive, GDP-bound state. The three best-characterized members of this family are Rho, Rac and Cdc42. Their regulatory function on the actin cytoskeleton during axon outgrowth and guidance has been extensively demonstrated (for review see refs. 73-75). Rho, Rac and Cdc42 are generally considered to regulate formation of stress fibers (actin- and myosin-rich structures), lamellipodia, and filopodia, respectively.

The activity of Rho-GTPases is itself modulated by three families of factors. GAPs (GTPases activating proteins) facilitate hydrolysis of GTP by GTPases, and hence favor the inactive, GDP-binding state of Rho-GTPases.[76] GEFs (guanine nucleotide exchange factors) activate GTPases by facilitating GDP/GTP exchange.[77] Finally, GDIs (guanine nucleotide dissociation inhibitors) inhibit GDP dissociation and maintain GTPases in an inactive state. GEFs/GDIs/GAPs can be either specific for a given GTPase, or act simultaneously on several molecules.

In addition, Rho-GTPase activity can also be modulated by second messenger cyclic nucleotides. Indeed, cAMP-dependent protein kinase A (PKA) reduces Rho-GEF activity,[78] while activating Rho-GDIs.[79] RhoA is also directly inhibited upon phosphorylation by PKA.[80,81] In addition to its action on Rho-GTPases, PKA can directly act on their downstream targets, including cytoskeletal components (see chapter by Piper et al.).

Binding of permissive or inhibitory factors to their neuronal receptors induces different signaling cascades, which in turn leads to activation/inactivation of Rho-GTPases. Since Rho, Rac and Cdc42 may also interact and thereby modulate themselves, it seems that the balance between the activities of different GTPases, rather than activation of a single group, will control the axonal response.

After binding to their membrane receptors, repulsive guidance cues activate Rho, while inhibiting Cdc42 and Rac activity by acting on GTPase modulators (see Fig. 2). For example ephrines, via Src kinase and RasGAP, inhibit p190RhoGAP.[82,83] In parallel, the RhoGEF ephexin is activated,[84] which leads to RhoA activation.

Semaphorins activate Rho and inhibit Rac via a slightly different mechanism. The semaphorin receptors Plexins are able to directly bind Rac and Rho. This binding then activates Rho, while it sequesters Rac and inhibits its interaction with its downstream effector Pak.[85]

In contrast, outgrowth- and regeneration-permissive factors activate Cdc42 and Rac, while inhibiting Rho. Neurotrophins for example, besides their trophic effect mediating gene transcription in the cell body, activate GEFs via a PI3-K signaling pathway,[86,87] and thereby Cdc42. Binding of the neurotrophin receptor p75 to RhoA inactivates this Rho-GTPase, and further contributes to the attractive effect of neurotrophins.[88] Moreover, it has recently been demonstrated that RhoA-kinase and myosin-II are required for the maintenance of growth cone polarity and guidance mediated by nerve growth factor,[89] suggesting that localized activation of different RhoGTPases is necessary for axonal pathfinding.

Effect of Rho-GTPases on the Cytoskeleton

Activation of Rho-GTPases leads to an important cytoskeletal remodeling. Their effects converge on three main systems: actin polymerization/depolymerization, actin/myosin contractility, and microtubule reorganization, as represented in Figure 2.

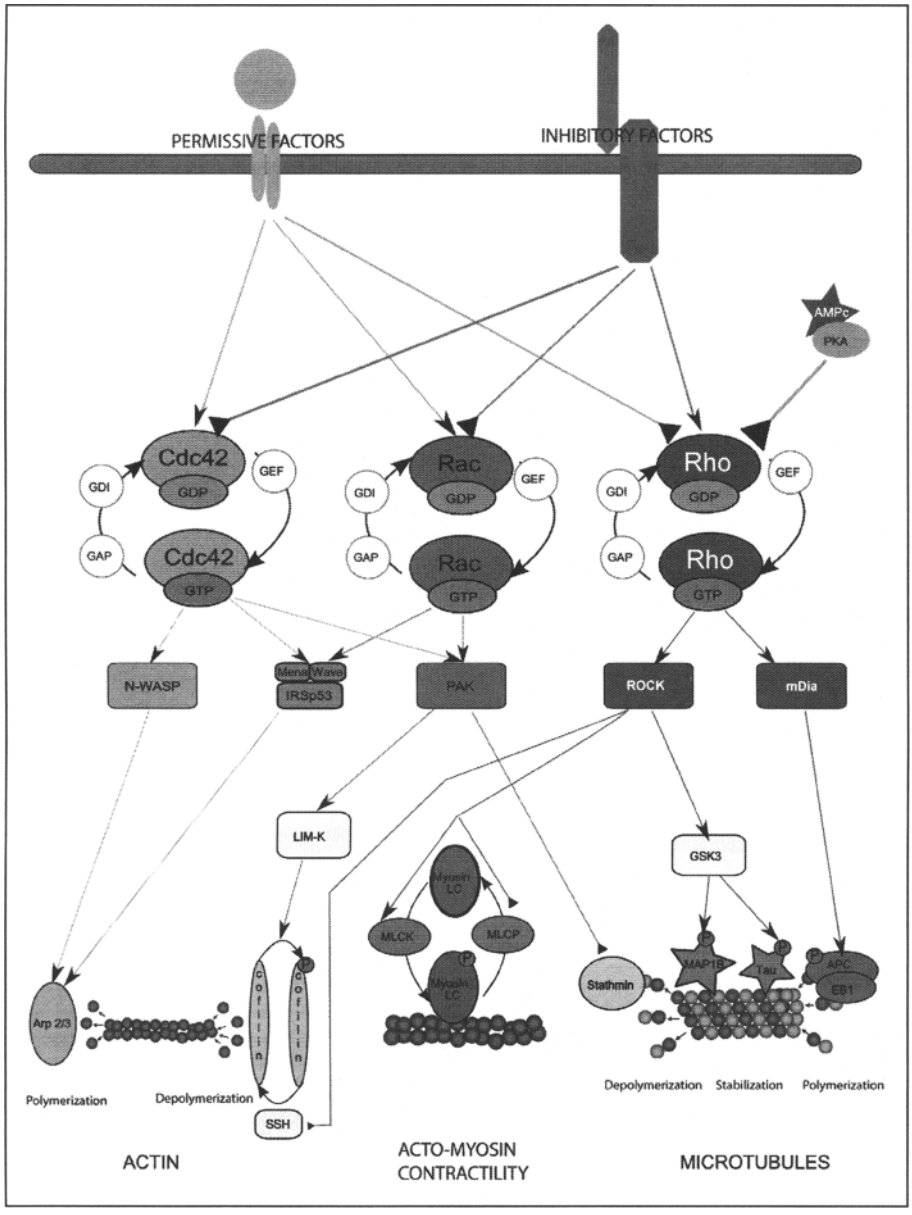

Figure 2. Rho GTPases signalling to the cytoskeleton. Rho-GTPases are controlled via their associated partners by extracellular cues. Specific effectors mediate actions of Rho-GTPases on the cytoskeleton. They focus on three main effects: actin polymerization/depolymerization, regulation of acto/myosin contractility, and microtubules polymerization/depolymerization/stabilization.

Regulation of Actin Polymerization

Upon Cdc42 activation, N-WASP (N-Wiskott-Aldrich related protein), activates the Arp2/3 factor.[90] Arp2/3 stimulates de novo actin polymerization (for review see ref. 91). This factor is also activated by Rac effectors IRSp53 and WASP related protein SCAR/WAVE.[92]

Conversely, actin depolymerization is induced by cofilin/ADF (Actin Depolymerizing Factor). Cofilin is activated by phosphatase SSH (slingshot), and inhibited by LIM kinase.[93] Rac and Cdc42 activate LIM-K,[94] while Rho and its downstream effector ROCK (Rho-associated kinase) activate SSH, and as a consequence, promote actin depolymerization.[95]

Regulation of Acto-Myosin Contractility

Rho activation of ROCK increases myosin contractility by two converging pathways. ROCK activation induces phosphorylation of myosin light chain 2 (MLC2) by activating myosin light chain kinase, while at the same time inhibiting myosin light chain phosphatase.[96,97] An increase in MLC phosphorylation and myosin activity then leads to contraction of the actin network.

Regulation of Microtubules

Rho-GTPases have been extensively described as actin modulators, but a growing number of studies also suggest a function in regulation of MT dynamics. In particular, it has been described that after Rho activation, the formin mDia not only favors actin nucleation,[98] but also stabilizes MTs via TIPS proteins EB1 and APC.[99] Rac1 activity has a MT stabilizing effect, since the Rac effector PAK inhibits the MT-severing protein Stathmin/Op18.[100,101] These pathways have mainly been characterized in nonneuronal cells, but we assume that the same or similar mechanisms should also regulate neuronal cell motility. In neuronal cell lines, the Rho/ROCK pathway has been shown to induce hyperphosphorylation of two major MAPs, tau and MAP1B, by GSK3β,[102] thereby destabilizing MTs. Interestingly, Rho-GTPase activity is inversely regulated by MT polymerization/depolymerization in nonneuronal cells: MT depolymerization leads to release of a RhoA activating GEF,[103] whereas their polymerization activates Rac1 by a still unknown mechanism.[104]

This "retrograde signaling" from MTs to Rho-GTPases could be one way of coregulating MTs and actin dynamics during motile events. Indeed, a strict coordination of actin and MT systems is required for proper growth cone advance, turning and branching.[13,25,29,105,106] Besides those based on regulatory interactions involving Rho-GTPases, several hypotheses have been put forward for a model of coupling between actin and MTs (for review see refs. 2,8,107). Structural interactions mediated by MT- and actin-binding proteins or protein complexes might physically couple actin and MT movements (for review and see refs. 8,45). For example, in addition to their interaction with MTs, some MAPs directly or indirectly interact with actin filaments. Another hypothesis proposes that actin and MT movements are controlled by the equilibrium between forces generated by molecular motors on the 2 types of filaments. According to this hypothesis, a balance between backward forces generated by myosin and forward forces generated by dynein or kinesins should control advance or retraction of the axon.[56]

In summary, during axonal outgrowth, extracellular signals converge on the reorganization of cytoskeletal proteins, particularly actin filaments and microtubules, and thereby control the advance of the growth cone. On the other hand, specific expression and intrinsic modification of cytoskeletal proteins also modulates the neuronal response to extrinsic factors, allowing for diversity in the response to a specific guidance cue, and underlying the role of the cytoskeleton as a convergence point during axonal outgrowth.

During the past two decades, a huge amount of data has been acquired detailing the molecular mechanisms of axon outgrowth and guidance. Today, it seems possible to exploit these data also in view of a better understanding of phenomena related to axonal plasticity in adult nervous system. Thus, especially our growing knowledge of how exactly extracellular cues and intracellular pathways ultimately converge on the axonal cytoskeleton, is of particular interest for studies of axonal regeneration in the adult following a traumatic lesion.

Growth Cone of Regenerating Axons

In the adult nervous system, particularly of mammals, understanding why certain types of neurons regenerate their axons while others do not, may provide clues to establish a therapy for each type of lesion, be it traumatic, degenerative, or linked to developmental (genetic) anomalies.

In order to regenerate, adult neurons should have the intrinsic capacity to survive a traumatic or degenerative lesion, and to activate a cell-autonomous program that will end in plastic changes in their network. At the same time, this program is influenced by interactions between neurons and neighboring cells (glia in particular), mediated by cell- and substratum- adhesion molecules and their receptors, and by a variety of secreted factors into the extra-cellular space. A number of key players in these regenerative processes have already been identified. However, the relationship between individual molecular events, especially the triggering of gene expression and the corresponding cascade of signaling pathways, are still poorly understood. Here we summarize some relevant findings from studies that were undertaken to understand how the axonal cytoskeleton is reorganized in response to a lesion, particularly in response to axotomy.

Initiation of Axonal Regeneration after Axotomy

The regeneration of an amputated axon involves the transformation of a stable axonal segment, i.e., a stable structure specialized in propagating action potentials, into a highly motile and complex tip, a new growth cone, that will sense the surrounding environment and guide regenerating neurites to their targets. This is a critical step in the process of recovery from neural injury. Most of the studies on the initiation of the regeneration of damaged axon were done on Aplysia neurons[108,109] where it has been shown that cytoskeleton reorganization promotes a growth cone formation, allowing elongation of a new axon.

Gene Expression Recapitulates Developmental Program during Axonal Regeneration

In response to injury, such as axotomy, adult neurons shutdown their specific differentiated functions and activate growth program through local intracellular signaling cascade. Coordinated sequence of gene expression is induced for synthesis and transport of proteins that maintain axonal plasticity, growth cones are formed and ultimately functional synaptic contacts are restored. In vertebrates, these events occur only in the peripheral nervous system (PNS). In contrast, most lesions in the central nervous system (CNS) result in abortive regeneration associated with decrease in protein synthesis and may ultimately induce atrophy or death. The coordination of gene expression pattern after axonal injury is complex and is determined by both intrinsic factors to neurons as well as environment factors.

Cytoskeleton Synthesis in Injury-Induced Axonal Plasticity

The contribution of cytoskeleton proteins to the axonal regeneration process is crucial. Although several studies on neuronal cytoskeleton were undertaken during the development, its regulation during axonal regeneration remains poorly understood. Nevertheless, cytoskeleton proteins in regenerating axon undergo quantitative and qualitative changes in synthesis, organization and protein transport, similar to that of growing axon during development.[110]

In vertebrates, the recapitulation of the developmental cytoskeleton-protein expression has been mainly demonstrated in the peripheral sensory neurons of the dorsal root ganglia (DRG) and motor neurons (MN, in the CNS). These neurons are able to regenerate after peripheral injury.

MN or DRG axotomy is followed by an increase in levels of specific tubulin isoforms, as well as beta actin and peripherin, while levels of neurofilaments (NF), known to regulate the axon caliber[111] decrease. The down regulation of NF gene expression was suggested to facilitate supplying structural elements toward the distal end of the regenerating axon, resulting in a selective acceleration in the transport rate of tubulin and actin (for review see ref. 112).

It is important to note that although the capacity of axonal regeneration is attributed to PNS neurons, it is now well accepted that, in response to CNS injury, there are some neuronal populations able to initiate an axonal growth program to regenerate. This has been observed during the first days after axotomy of rubrospinal neurons, where the amounts of GAP43 and cytoskeleton proteins such actin and tubulin increase. Sustained only in few neurons, a decrease in these proteins occurs thereafter, associated with neuronal atrophy.[113] This study demonstrated that for some CNS neurons, the failure to regenerate after axotomy is not due to the failure to initiate gene-expression changes, but mainly to due to the environment. Depending on extracellular cues, the signals converge in growth cone on cytoskeleton protein reorganization to promote axonal regeneration (PNS) or to impede regeneration (CNS).

Following a traumatic lesion, several inhibitory guidance cues are expressed in the CNS and are partly responsible for the poor regenerative response of axotomized neurons. Besides the inhibitory effect of these molecules, loss of regenerative capacities in the adult nervous system is thought to coincide with myelination. Indeed, several inhibitors of regeneration have been described on the myelinating cells surface, and, in the adult, contribute to the failure of regeneration in the CNS (for review see ref. 114). Furthermore, the effect of these molecules, as well as other extracellular factors, on axonal regeneration is modulated by the intrinsic neuronal state.

How Intrinsic Neuronal Properties Control the Success of Regeneration?

cAMP

The best characterized example of intrinsic neuronal state controlling axonal regeneration comes from demonstration that elevating intracellular cAMP concentration of adult neurons to reach that of young neurons allow them to regenerate on a central myelin substrate.[115-117]

Binding of myelin inhibitors to their receptors induces, repulsive guidance cues during development, an elevation of Rho activity via a RhoGDI.[118,119] The exact mechanisms by which myelin inhibitors inhibit axonal regeneration are still unclear, but probably involve, as during the development, an actin depolymerization/contraction and a MTs destabilization. The precise effect of cAMP in overcoming myelin inhibition is not known. However, for a short phase, PKA action on Rho-GTPases may explain a part of the mechanism (see above). A second, transcription-dependant phase is induced by CREB activation. The multiple targets of this transcription factor are unknown, but one can reasonably consider that it may include cytoskeleton proteins. Moreover, it has been demonstrated that CREB activation leads to polyamines synthesis, which are known to modulate the cytoskeleton.[121,122]

Cytoskeleton Associated Protein, GAP43/CAP32

One of the first and best studied example of a cytoskeletal regeneration-associated protein is GAP43, a phosphoprotein associated with growth cones, whose expression is also induced in adult regenerating axons (for review see ref. 123). GAP43 is one of the final targets of calcium signals. GAP43 is an actin capping protein that blocks microfilament elongation and appears to be an important regulator of growth cone motility during development. Phosphorylation of GAP43 by the protein kinase C (PKC) affects its interaction with actin filament and might therefore trigger actin polymerization and hence regulating axonal outgrowth (for review see ref. 135). Furthermore, it plays a significant role in regeneration, together with CAP23, a functionally related protein that is also upregulated by injury (ref. 124 and references therein).

Cytoskeleton Associated Protein, MAP1B

Several studies strongly suggest an axon growth-related function of MAP1B that is regulated by phosphorylation (for review see ref. 38). Although generally down-regulated in the adult, MAP1B is constitutively highly expressed in adult DRG and MN. After sciatic nerve lesion, the phosphorylated forms of MAP1B (MAP1B-P) is enriched in the more distal portion of the axon and is associated with peripheral regeneration of these neurons.[125,126] In adult CNS, axonal MAP1B-P remains detectable in areas that retain axonal plasticity,[127,128] and can also be reinduced

in injury-induced axonal reorganization.[129,130] Adult DRG from MAP1B null allele mutant mice[131] are able to regenerate their axons but exhibit two main abnormalities: (1) the number of terminal and collateral branching is significantly increased and (2) the turning capacity of growth cones, i.e., "choice" of a proper orientation, is impaired.[45] In developing neurons, both growth cone turning[106] and axonal branch formation[25] are known to involve local cross-talk between actin and MTs. MAP1B capacity to bind both actin filaments and microtubules[132-134] suggests that MAP1B is involved in the locally coordinated assembly of cytoskeleton components required for branching and straight directional axon growth.[45] The developmental role of cytoskeleton-associated proteins in the organization of the cross-talk between MTs and actin-filaments[8] appears thus to be maintained during axonal regeneration in the adult.

In conclusion, it seems evident that most, if not all signaling cascades triggered by extracellular stimuli converge onto the cytoskeleton. The subsequent reorganization of actin-filaments and microtubules is a complex phenomenon, and involves numerous cytoskeleton-associated proteins, whose function is fine-tuned via activation or inhibition of particular signaling pathways. Specific expression of some of these cytoskeleton-associated proteins in the neuronal growth cone may, at least in part, explain some unique features of this motile structure. Further studies, such as the one examining the coordinated cross-talk between actin filaments and microtubules during axonal branching and growth cone guidance to the appropriate target, will help determine the precise molecular mechanisms of axonal growth.

In contrast to neural development, the pathways inolved in triggering cytoskeletal reorganization during regeneration are less well known, and this field of research is attracting great interest. Indeed, the ability of regenerating axons to respond to extracellular signals present in their environment depends on both the intrinsic neuronal state, and the presence (or absence) of specific cytoskeleton-associated proteins. It may be particularly interesting to determine a potential central convergence point of inhibitory extrinsic signaling. Modulating the intrinsic state of the neuron, and the response of the cytoskeleton to environmental factors, may provide clues for search of therapeutic targets to promote axonal regeneration after injury.

References

1. Zhou FQ, Cohan CS. How actin filaments and microtubules steer growth cones to their targets. J Neurobiol 2004; 58:84-91.
2. Rodriguez OC, Schaefer AW, Mandato CA et al. Conserved microtubule-actin interactions in cell movement and morphogenesis. Nat Cell Biol 2003; 5:599-609.
3. Kodama A, Lechler T, Fuchs E. Coordinating cytoskeletal tracks to polarize cellular movements. J Cell Biol 2004; 167:203-7.
4. Baas PW, Luo L. Signaling at the growth cone: The scientific progeny of Cajal meet in Madrid. Neuron 2001; 32:981-4.
5. Suter DM, Forscher P. Substrate-cytoskeletal coupling as a mechanism for the regulation of growth cone motility and guidance. J Neurobiol 2000; 44:97-113.
6. Kornack DR, Giger RJ. Probing microtubule +TIPs: Regulation of axon branching. Curr Opin Neurobiol 2005; 15:58-66.
7. Gungabissoon RA, Bamburg JR. Regulation of growth cone actin dynamics by ADF/cofilin. J Histochem Cytochem 2003; 51:411-20.
8. Dehmelt L, Halpain S. Actin and microtubules in neurite initiation: Are MAPs the missing link? J Neurobiol 2004; 58:18-33.
9. Letourneau PC. Differences in the organization of actin in the growth cones compared with the neurites of cultured neurons from chick embryos. J Cell Biol 1983; 97:963-73.
10. Bridgman PC, Dailey ME. The organization of myosin and actin in rapid frozen nerve growth cones. J Cell Biol 1989; 108:95-109.
11. Schaefer AW, Kabir N, Forscher P. Filopodia and actin arcs guide the assembly and transport of two populations of microtubules with unique dynamic parameters in neuronal growth cones. J Cell Biol 2002; 158:139-52.
12. Pollard TD, Borisy GG. Cellular motility driven by assembly and disassembly of actin filaments. Cell 2003; 112:453-65.
13. Forscher P, Smith SJ. Actions of cytochalasins on the organization of actin filaments and microtubules in a neuronal growth cone. J Cell Biol 1988; 107:1505-16.

14. Lewis AK, Bridgman PC. Nerve growth cone lamellipodia contain two populations of actin filaments that differ in organization and polarity. J Cell Biol 1992; 119:1219-43.
15. Brown J, Bridgman PC. Role of myosin II in axon outgrowth. J Histochem Cytochem 2003; 51:421-8.
16. Dent EW, Gertler FB. Cytoskeletal dynamics and transport in growth cone motility and axon guidance. Neuron 2003; 40:209-27.
17. Millard TH, Sharp SJ, Machesky LM. Signalling to actin assembly via the WASP (Wiskott-Aldrich syndrome protein)-family proteins and the Arp2/3 complex. Biochem J 2004; 380:1-17.
18. Kovar DR. Molecular details of formin-mediated actin assembly. Curr Opin Cell Biol 2006; 18:11-7.
19. Huang TY, DerMardirossian C, Bokoch GM. Cofilin phosphatases and regulation of actin dynamics. Curr Opin Cell Biol 2006; 18:26-31.
20. McGough AM, Staiger CJ, Min JK et al. The gelsolin family of actin regulatory proteins: Modular structures, versatile functions. FEBS Lett 2003; 552:75-81.
21. Luduena RF. Multiple forms of tubulin: Different gene products and covalent modifications. Int Rev Cytol 1998; 178:207-75.
22. Mitchison T, Kirschner M. Dynamic instability of microtubule growth. Nature 1984; 312:237-42.
23. Baas PW. Microtubules and axonal growth. Curr Opin Cell Biol 1997; 9:29-36.
24. Tanaka EM, Kirschner MW. Microtubule behavior in the growth cones of living neurons during axon elongation. J Cell Biol 1991; 115:345-63.
25. Dent EW, Kalil K. Axon branching requires interactions between dynamic microtubules and actin filaments. J Neurosci 2001; 21:9757-69.
26. Gordon-Weeks PR. Growth cones: The mechanism of neurite advance. Bioessays 1991; 13:235-9.
27. Tanaka E, Ho T, Kirschner MW. The role of microtubule dynamics in growth cone motility and axonal growth. J Cell Biol 1995; 128:139-55.
28. Tanaka E, Kirschner MW. The role of microtubules in growth cone turning at substrate boundaries. J Cell Biol 1995; 128:127-37.
29. Buck KB, Zheng JQ. Growth cone turning induced by direct local modification of microtubule dynamics. J Neurosci 2002; 22:9358-67.
30. Mack TG, Koester MP, Pollerberg GE. The microtubule-associated protein MAP1B is involved in local stabilization of turning growth cones. Mol Cell Neurosci 2000; 15:51-65.
31. Baas PW, Ahmad FJ, Pienkowski TP et al. Sites of microtubule stabilization for the axon. J Neurosci 1993; 13:2177-85.
32. Khawaja S, Gundersen GG, Bulinski JC. Enhanced stability of microtubules enriched in detyrosinated tubulin is not a direct function of detyrosination level. J Cell Biol 1988; 106:141-9.
33. Erck C, Peris L, Andrieux A et al. A vital role of tubulin-tyrosine-ligase for neuronal organization. Proc Natl Acad Sci USA 2005; 102:7853-8.
34. Bonnet C, Boucher D, Lazereg S et al. Differential binding regulation of microtubule-associated proteins MAP1A, MAP1B, and MAP2 by tubulin polyglutamylation. J Biol Chem 2001; 276:12839-48.
35. Goold RG, Owen R, Gordon-Weeks PR. Glycogen synthase kinase 3beta phosphorylation of microtubule-associated protein 1B regulates the stability of microtubules in growth cones. J Cell Sci 1999; 112(Pt 19):3373-84.
36. Gelfand VI, Bershadsky AD. Microtubule dynamics: Mechanism, regulation, and function. Annu Rev Cell Biol 1991; 7:93-116.
37. Matus A. Microtubule-associated proteins: Their potential role in determining neuronal morphology. Annu Rev Neurosci 1988; 11:29-44.
38. Gordon-Weeks PR, Fischer I. MAP1B expression and microtubule stability in growing and regenerating axons. Microsc Res Tech 2000; 48:63-74.
39. Matus A. Microtubule-associated proteins and neuronal morphogenesis. J Cell Sci Suppl 1991; 15:61-7.
40. Hirokawa N. Microtubule organization and dynamics dependent on microtubule-associated proteins. Curr Opin Cell Biol 1994; 6:74-81.
41. Takei Y, Teng J, Harada A et al. Defects in axonal elongation and neuronal migration in mice with disrupted tau and map1b genes. J Cell Biol 2000; 150:989-1000.
42. Takei Y, Kondo S, Harada A et al. Delayed development of nervous system in mice homozygous for disrupted microtubule-associated protein 1B (MAP1B) gene. J Cell Biol 1997; 137:1615-26.
43. Gonzalez-Billault C, Owen R, Gordon-Weeks PR et al. Microtubule-associated protein 1B is involved in the initial stages of axonogenesis in peripheral nervous system cultured neurons. Brain Res 2002; 943:56-67.
44. DiTella MC, Feiguin F, Carri N et al. MAP-1B/TAU functional redundancy during laminin-enhanced axonal growth. J Cell Sci 1996; 109:467-77.
45. Bouquet C, Soares S, von Boxberg Y et al. Microtubule-associated protein 1B controls directionality of growth cone migration and axonal branching in regeneration of adult dorsal root ganglia neurons. J Neurosci 2004; 24:7204-13.

46. Riederer BM, Pellier V, Antonsson B et al. Regulation of microtubule dynamics by the neuronal growth-associated protein SCG10. Proc Natl Acad Sci USA 1997; 94:741-5.
47. Belmont LD, Mitchison TJ. Identification of a protein that interacts with tubulin dimers and increases the catastrophe rate of microtubules. Cell 1996; 84:623-31.
48. Grenningloh G, Soehrman S, Bondallaz P et al. Role of the microtubule destabilizing proteins SCG10 and stathmin in neuronal growth. J Neurobiol 2004; 58:60-9.
49. Tanaka E, Sabry J. Making the connection: Cytoskeletal rearrangements during growth cone guidance. Cell 1995; 83:171-6.
50. Stepanova T, Slemmer J, Hoogenraad CC et al. Visualization of microtubule growth in cultured neurons via the use of EB3-GFP (end-binding protein 3-green fluorescent protein). J Neurosci 2003; 23:2655-64.
51. Galjart N. CLIPs and CLASPs and cellular dynamics. Nat Rev Mol Cell Biol 2005; 6:487-98.
52. Jimenez-Mateos EM, Paglini G, Gonzalez-Billault C et al. End binding protein-1 (EB1) complements microtubule-associated protein-1B during axonogenesis. J Neurosci Res 2005; 80:350-9.
53. Lee MK, Cleveland DW. Neuronal intermediate filaments. Annu Rev Neurosci 1996; 19:187-217.
54. Gallant PE. Axonal protein synthesis and transport. J Neurocytol 2000; 29:779-82.
55. Eyer J, Peterson A. Neurofilament-deficient axons and perikaryal aggregates in viable transgenic mice expressing a neurofilament-beta-galactosidase fusion protein. Neuron 1994; 12:389-405.
56. Baas PW, Ahmad FJ. Force generation by cytoskeletal motor proteins as a regulator of axonal elongation and retraction. Trends Cell Biol 2001; 11:244-9.
57. Hirokawa N, Takemura R. Molecular motors in neuronal development, intracellular transport and diseases. Curr Opin Neurobiol 2004; 14:564-73.
58. Medeiros NA, Burnette DT, Forscher P. Myosin II functions in actin-bundle turnover in neuronal growth cones. Nat Cell Biol 2006; 8:215-26.
59. Lee SK, Hollenbeck PJ. Organization and translation of mRNA in sympathetic axons. J Cell Sci 2003; 116:4467-78.
60. Zheng JQ, Kelly TK, Chang B et al. A functional role for intra-axonal protein synthesis during axonal regeneration from adult sensory neurons. J Neurosci 2001; 21:9291-303.
61. Hanz S, Perlson E, Willis D et al. Axoplasmic importins enable retrograde injury signaling in lesioned nerve. Neuron 2003; 40:1095-104.
62. Willis D, Li KW, Zheng JQ et al. Differential transport and local translation of cytoskeletal, injury-response, and neurodegeneration protein mRNAs in axons. J Neurosci 2005; 25:778-91.
63. Ming GL, Wong ST, Henley J et al. Adaptation in the chemotactic guidance of nerve growth cones. Nature 2002; 417:411-8.
64. Wujek JR, Lasek RJ. Correlation of axonal regeneration and slow component B in two branches of a single axon. J Neurosci 1983; 3:243-51.
65. Galbraith JA, Gallant PE. Axonal transport of tubulin and actin. J Neurocytol 2000; 29:889-911.
66. Wang L, Brown A. Rapid movement of microtubules in axons. Curr Biol 2002; 12:1496-1501.
67. He Y, Francis F, Myers KA et al. Role of cytoplasmic dynein in the axonal transport of microtubules and neurofilaments. J Cell Biol 2005; 168:697-703.
68. Baas PW, Buster DW. Slow axonal transport and the genesis of neuronal morphology. J Neurobiol 2004; 58:3-17.
69. Gallo G, Letourneau PC. Neurotrophins and the dynamic regulation of the neuronal cytoskeleton. J Neurobiol 2000; 44:159-73.
70. Gallo G, Letourneau PC. Axon guidance: GTPases help axons reach their targets. Curr Biol 8:R80-2.
71. Challacombe JF, Snow DM, Letourneau PC. Dynamic microtubule ends are required for growth cone turning to avoid an inhibitory guidance cue. J Neurosci 1997; 17:3085-95.
72. Gallo G, Letourneau PC. Different contributions of microtubule dynamics and transport to the growth of axons and collateral sprouts. J Neurosci 1999; 19:3860-73.
73. Hall A. Rho GTPases and the actin cytoskeleton. Science 1998; 279:509-14.
74. Luo L. Rho GTPases in neuronal morphogenesis. Nat Rev Neurosci 2000; 1:173-80.
75. Luo L. Actin cytoskeleton regulation in neuronal morphogenesis and structural plasticity. Annu Rev Cell Dev Biol 2002; 18:601-35.
76. Moon SY, Zheng Y. Rho GTPase-activating proteins in cell regulation. Trends Cell Biol 2003; 13:13-22.
77. Schmidt A, Hall A. Guanine nucleotide exchange factors for Rho GTPases: Turning on the switch. Genes Dev 2002; 16:1587-609.
78. Manganello JM, Huang JS, Kozasa T et al. Protein kinase A-mediated phosphorylation of the Galpha13 switch I region alters the Galphabetagamma13-G protein-coupled receptor complex and inhibits Rho activation. J Biol Chem 2003; 278:124-30.
79. Ellerbroek SM, Wennerberg K, Burridge K. Serine phosphorylation negatively regulates RhoA in vivo. J Biol Chem 2003; 278:19023-31.

80. Feoktistov I, Goldstein AE, Biaggioni I. Cyclic AMP and protein kinase A stimulate Cdc42: Role of A(2) adenosine receptors in human mast cells. Mol Pharmacol 2000; 58:903-10.
81. O'Connor KL, Mercurio AM. Protein kinase A regulates Rac and is required for the growth factor-stimulated migration of carcinoma cells. J Biol Chem 2001; 276:47895-900.
82. Brambilla R, Schnapp A, Casagranda F et al. Membrane-bound LERK2 ligand can signal through three different Eph-related receptor tyrosine kinases. EMBO J 1995; 14:3116-26.
83. Bruckner K, Klein R. Signaling by Eph receptors and their ephrin ligands. Curr Opin Neurobiol 1998; 8:375-82.
84. Wahl S, Barth H, Ciossek T et al. Ephrin-A5 induces collapse of growth cones by activating Rho and Rho kinase. J Cell Biol 2000; 149:263-70.
85. Hu H, Marton TF, Goodman CS. Plexin B mediates axon guidance in Drosophila by simultaneously inhibiting active Rac and enhancing RhoA signaling. Neuron 2001; 32:39-51.
86. Ozdinler PH, Erzurumlu RS. Regulation of neurotrophin-induced axonal responses via Rho GTPases. J Comp Neurol 2001; 438:377-87.
87. Yamauchi J, Chan JR, Miyamoto Y et al. The neurotrophin-3 receptor TrkC directly phosphorylates and activates the nucleotide exchange factor Dbs to enhance Schwann cell migration. Proc Natl Acad Sci USA 2005; 102:5198-203.
88. Yamashita T, Tucker KL, Barde YA. Neurotrophin binding to the p75 receptor modulates Rho activity and axonal outgrowth. Neuron 24:585-93
89. Loudon R, Silver LD, Yee Jr HF et al. RhoA-kinase and myosin II are required for the maintenance of growth cone polarity and guidance by nerve growth factor. J Neurobiol 2006; 66:847-67.
90. Rohatgi R, Ma L, Miki H et al. The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. Cell 1999; 97:221-31.
91. Welch MD, Mullins RD. Cellular control of actin nucleation. Annu Rev Cell Dev Biol 2002; 18:247-88.
92. Miki H, Yamaguchi H, Suetsugu S et al. IRSp53 is an essential intermediate between Rac and WAVE in the regulation of membrane ruffling. Nature 2000; 408:732-5.
93. Arber S, Barbayannis FA, Hanser H et al. Regulation of actin dynamics through phosphorylation of cofilin by LIM-kinase. Nature 1998; 393:805-9.
94. Edwards DC, Sanders LC, Bokoch GM et al. Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. Nat Cell Biol 1999; 1:253-9.
95. Hsieh SH, Ferraro GB, Fournier AE. Myelin-associated inhibitors regulate cofilin phosphorylation and neuronal inhibition through LIM kinase and Slingshot phosphatase. J Neurosci 2006; 26:1006-15.
96. Hirose M, Ishizaki T, Watanabe N et al. Molecular dissection of the Rho-associated protein kinase (p160ROCK)-regulated neurite remodeling in neuroblastoma N1E-115 cells. J Cell Biol 1998; 141:1625-36.
97. Amano M, Chihara K, Nakamura N et al. Myosin II activation promotes neurite retraction during the action of Rho and Rho-kinase. Genes Cells 1998; 3:177-88.
98. Pruyne D, Evangelista M, Yang C et al. Role of formins in actin assembly: Nucleation and barbed-end association. Science 2002; 297:612-5.
99. Palazzo AF, Cook TA, Alberts AS et al. mDia mediates Rho-regulated formation and orientation of stable microtubules. Nat Cell Biol 2001; 3:723-9.
100. Daub H, Gevaert K, Vandekerckhove J et al. Rac/Cdc42 and p65PAK regulate the microtubule-destabilizing protein stathmin through phosphorylation at serine 16. J Biol Chem 2001; 276:1677-80.
101. Wittmann T, Waterman-Storer CM. Cell motility: Can Rho GTPases and microtubules point the way? J Cell Sci 2001; 114:3795-803.
102. Sayas CL, Avila J, Wandosell F. Glycogen synthase kinase-3 is activated in neuronal cells by Galpha12 and Galpha13 by Rho-independent and Rho-dependent mechanisms. J Neurosci 2002; 22:6863-75.
103. Krendel M, Zenke FT, Bokoch GM. Nucleotide exchange factor GEF-H1 mediates cross-talk between microtubules and the actin cytoskeleton. Nat Cell Biol 2002; 4:294-301.
104. Waterman-Storer CM, Worthylake RA, Liu BP et al. Microtubule growth activates Rac1 to promote lamellipodial protrusion in fibroblasts. Nat Cell Biol 1999; 1:45-50.
105. Zhou FQ, Waterman-Storer CM, Cohan CS. Focal loss of actin bundles causes microtubule redistribution and growth cone turning. J Cell Biol 2002; 157:839-49.
106. Challacombe JF, Snow DM, Letourneau PC. Actin filament bundles are required for microtubule reorientation during growth cone turning to avoid an inhibitory guidance cue. J Cell Sci 1996; 109:2031-40.
107. Owen R, Gordon-Weeks PR. Inhibition of glycogen synthase kinase 3beta in sensory neurons in culture alters filopodia dynamics and microtubule distribution in growth cones. Mol Cell Neurosci 2003; 23:626-37.

108. Ziv NE, Spira ME. Induction of growth cone formation by transient and localized increases of intracellular proteolytic activity. J Cell Biol 1998; 140:223-32.
109. Spira ME, Oren R, Dormann A et al. Critical calpain-dependent ultrastructural alterations underlie the transformation of an axonal segment into a growth cone after axotomy of cultured Aplysia neurons. J Comp Neurol 2003; 457:293-312.
110. Hoffman PN, Cleveland DW. Neurofilament and tubulin expression recapitulates the developmental program during axonal regeneration: Induction of a specific beta-tubulin isotype. Proc Natl Acad Sci USA 1988; 85:4530-3.
111. Hoffman PN, Thompson GW, Griffin JW. Changes in neurofilament transport coincide temporally with alterations in the caliber of axons in regenerating motor fibers. J Cell Biol 1985; 101:1332-40.
112. Tetzlaff W, Leonard C, Krekoski CA et al. Reductions in motoneuronal neurofilament synthesis by successive axotomies: A possible explanation for the conditioning lesion effect on axon regeneration. Exp Neurol 1996; 139:95-106.
113. Tetzlaff W, Alexander SW, Miller FD et al. Response of facial and rubrospinal neurons to axotomy: Changes in mRNA expression for cytoskeletal proteins and GAP-43. J Neurosci 1991; 11:2528-44.
114. Domeniconi M, Filbin MT. Overcoming inhibitors in myelin to promote axonal regeneration. J Neurol Sci 2005; 233:43-7.
115. Qiu J, Cai D, Dai H et al. Spinal axon regeneration induced by elevation of cyclic AMP. Neuron 2002; 34:895-903.
116. Neumann S, Bradke F, Tessier-Lavigne M et al. Regeneration of sensory axons within the injured spinal cord induced by intraganglionic cAMP elevation. Neuron 2002; 34:885-93.
117. Cai D, Qiu J, Cao Z et al. Neuronal cyclic AMP controls the developmental loss in ability of axons to regenerate. J Neurosci 2001; 21:4731-9.
118. Fournier AE, Takizawa BT, Strittmatter SM. Rho kinase inhibition enhances axonal regeneration in the injured CNS. J Neurosci 2003; 23:1416-23.
119. Niederost B, Oertle T, Fritsche J et al. Nogo-A and myelin-associated glycoprotein mediate neurite growth inhibition by antagonistic regulation of RhoA and Rac1. J Neurosci 2002; 22:10368-76.
120. Mimura F, Yamagishi S, Arimura N et al. Myelin-associated glycoprotein inhibits microtubule assembly by a Rho-kinase-dependent mechanism. J Biol Chem 2006; 281:15970-9.
121. Gao Y, Deng K, Hou J et al. Activated CREB is sufficient to overcome inhibitors in myelin and promote spinal axon regeneration in vivo. Neuron 2004; 44:609-21.
122. Cai D, Deng K, Mellado W et al. Arginase I and polyamines act downstream from cyclic AMP in overcoming inhibition of axonal growth MAG and myelin in vitro. Neuron 2002; 35:711-9.
123. Skene JH. Axonal growth-associated proteins. Annu Rev Neurosci 1989; 12:127-56.
124. Bomze HM, Bulsara KR, Iskandar BJ et al. Spinal axon regeneration evoked by replacing two growth cone proteins in adult neurons. Nat Neurosci 2001; 4:38-43.
125. Ma D, Nothias, F, Boyne LJ et al. Differential regulation of microtubule-associated protein 1B (MAP1B) in rat CNS and PNS during development. J Neurosci Res 1997; 49:319-32.
126. Ma D, Connors T, Nothias F. et al. Regulation of the expression and phosphorylation of microtubule-associated protein 1B during regeneration of adult dorsal root ganglion neurons. Neuroscience 2000; 99:157-70.
127. Nothias F, Vernier P, von Boxberg Y et al. Modulation of NCAM polysialylation is associated with morphofunctional modifications in the hypothalamo-neurohypophysial system during lactation. Eur J Neurosci 1997; 9:1553-65.
128. Nothias F, Fischer I, Murray M et al. Expression of a phosphorylated isoform of MAP1B is maintained in adult central nervous system areas that retain capacity for structural plasticity. J Comp Neurol 1996; 368:317-34.
129. Soares S, von Boxberg Y, Lombard MC et al. Phosphorylated MAP1B is induced in central sprouting of primary afferents in response to peripheral injury but not in response to rhizotomy. Eur J Neurosci 2002; 16:593-606.
130. Soares S, Fischer I, Ravaille-Veron M. Induction of MAP1B phosphorylation in target-deprived afferent fibers after kainic acid lesion in the adult rat. J Comp Neurol 1998; 396:193-210.
131. Meixner A, Haverkamp S, Wassle H et al. MAP1B is required for axon guidance and Is involved in the development of the central and peripheral nervous system. J Cell Biol 2000; 151:1169-78.
132. Togel M, Wiche G, Propst F. Novel features of the light chain of microtubule-associated protein MAP1B: Microtubule stabilization, self interaction, actin filament binding, and regulation by the heavy chain. J Cell Biol 1998; 143:695-707.
133. Pedrotti B, Islam K. Dephosphorylated but not phosphorylated microtubule associated protein MAP1B binds to microfilaments. FEBS Lett 1996; 388:131-3.
134. Noiges R, Eichinger R, Kutschera W et al. Microtubule-associated protein 1A (MAP1A) and MAP1B: Light chains determine distinct functional properties. J Neurosci 2002; 22:2106-14.
135. Bolsover, SR. Calcium signalling in growth cone migration. Cell Calcium 2005; 37:395-402.