BioArchitecture

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/kbia20

The cytoskeleton and neurite initiation

Kevin C Flynn[ab]

[a] Axonal Growth and Regeneration; German Center for Neurodegenerative Diseases (DZNE); Bonn, Germany

[b] Department of Molecular Medicine; Max-Planck Institute of Biochemistry; Martinsried, Germany

Published online: 26 Aug 2013.

To cite this article: Kevin C Flynn (2013) The cytoskeleton and neurite initiation, BioArchitecture, 3:4, 86-109, DOI: 10.4161/bioa.26259

To link to this article: http://dx.doi.org/10.4161/bioa.26259

BioArchitecture 3:4, 86–109; July/August 2013; © 2013 Landes Bioscience

The cytoskeleton and neurite initiation

Kevin C Flynn[1,2]

[1]Axonal Growth and Regeneration; German Center for Neurodegenerative Diseases (DZNE); Bonn, Germany; [2]Department of Molecular Medicine; Max-Planck Institute of Biochemistry; Martinsried, Germany

Keywords: neuronal morphogenesis, neurite formation, cytoskeleton, actin, microtubules

Neurons begin their life as simple spheres, but can ultimately assume an elaborate morphology with numerous, highly arborized dendrites, and long axons. This is achieved via an astounding developmental progression which is dependent upon regulated assembly and dynamics of the cellular cytoskeleton. As neurites emerge out of the soma, neurons break their spherical symmetry and begin to acquire the morphological features that define their structure and function. Neurons regulate their cytoskeleton to achieve changes in cell shape, velocity, and direction as they migrate, extend neurites, and polarize. Of particular importance, the organization and dynamics of actin and microtubules directs the migration and morphogenesis of neurons. This review focuses on the regulation of intrinsic properties of the actin and microtubule cytoskeletons and how specific cytoskeletal structures and dynamics are associated with the earliest phase of neuronal morphogenesis—neuritogenesis.

Introduction

Cellular morphogenesis—the ability for cells to change their shape—is a universal and essential phenomenon in biology. The importance of cell morphogenesis is especially evident during development. Whether it is coordinated movement of a multitude of cells participating in convergent extension or the migration of a single neural crest cell, cells must be able to change their shape to achieve specific phases of embryogenesis. In all eukaryotic organisms, the cellular cytoskeleton is largely responsible for these types of morphological changes. The cytoskeleton can be partitioned into three major classes: microtubules, microfilaments, (i.e., actin), and intermediate filaments. Together, these filamentous protein assemblages impart the cell with shape and structure by interacting with each other, a multitude of other proteins, and with cellular membranes. The cytoskeletal polymers are also dynamic, changing their composition and assembly kinetics in response to various cues in order to change cellular shape. The regulation of the cytoskeleton is thus crucial for all cellular migration and morphogenesis that occurs during development.

Correspondence to: Kevin C Flynn; Email: kflynn@biochem.mpg.de
Submitted: 06/24/2013; Revised: 08/22/2013; Accepted: 08/24/2013
http://dx.doi.org/10.4161/bioa.26259

Arguably, neurons are the cells with the most complicated morphogenesis in the developing organism. Arising from terminal mitotic cell divisions in disperse regions of the nervous system, neurons begin their life like many other cells, with a simple spheroid shape. From these morphologically humble beginnings, most neurons undertake a developmental voyage that will completely transform their shape and, in most cases, reposition their cell bodies to become integrated into the functional circuitry of the brain. This is a profound undertaking; multiple intracellular processes must run simultaneously to coordinate biosynthesis, intracellular transport, membrane dynamics, and motility to achieve specific phases of neuronal development. For example, certain actin regulators appear to match up with distinct components of the exocytotic machinery in order to induce neurites under different conditions.[1] Thus, even at the earliest phases of neuronal development, these intracellular processes need to be running in harmony to achieve the initial formation of a neurite.

Neurite initiation (or neuritogenesis) is the foremost event of neuronal morphogenesis. Filopodial and lamellipodial structures form nascent growth cones that protrude away from the cell body and extend the neurite in their wake. These neurites later become the axons and dendrites of mature neurons, forming the intricate circuitry of the entire nervous system. In recent years, significant progress has been made understanding the specific cytoarchitectural mechanisms and molecules required to extend neurites. This body of work, however, has built upon earlier seminal research that simply showed how to induce non-neuronal cells to extend neurite-like processes.[2] This study nicely demonstrated that there are two basic requirements for neurite formation: (1) a malleable or dynamic peripheral actin network and (2) bundling of microtubule arrays. Although these concepts are simple, there are a plethora of molecular players orchestrating the cytoskeleton to achieve efficient neurite formation and growth. There are hundreds of signaling molecules, actin binding proteins (ABPs), and microtubule binding proteins (MBPs) that are expressed in young neurons that can influence neuritogenesis, complicating the matter considerably.[3] In the following, I will begin with an overview of neuronal development, describe the building blocks of the cytoskeleton and how they are regulated, and then illustrate the mechanisms of neurite formation by focusing on the actin- and microtubule- based mechanisms of assembling a growth cone and a neurite. I will focus on key cytoskeletal players shown to be crucial for neurite formation. In order to fill in the gaps, I will also extrapolate on the role of a few actin and microtubule regulators from studies in more mature neurons (i.e., after they

have neurites), non-neuronal systems and fundamental players in in vitro assays. In closing, I will offer perspectives for future work on the cytoarchitectural changes that occur during neuritogenesis.

Overview of Neuronal Morphogenesis

Seminal studies on neuronal morphogenesis extensively characterized mammalian pyramidal neurons in cell culture, which gained popularity in the ensuring years to become one of the most commonly used model systems for neuronal cell biology. Pyramidal neurons undergo a stereotypical development divided into five different consecutive stages[4,5] (**Fig. 1**). These studies utilized dissociated neurons from the hippocampus, but the same stage classification holds true for other pyramidal neurons isolated from the mammalian and avian forebrain.[6] Cerebellar granule neurons are another model system which follow a pattern of development similar to pyramidal neurons, but show some disparities such as the more prominent formation of unipolar and bipolar morphologies during the early stages of growth.[7] Although most of the information discussed below is from studies of pyramidal neurons, the basic mechanisms and molecules are likely applicable to other neuronal subtypes. However, caution must be observed when extrapolating data from one neuronal system to another.

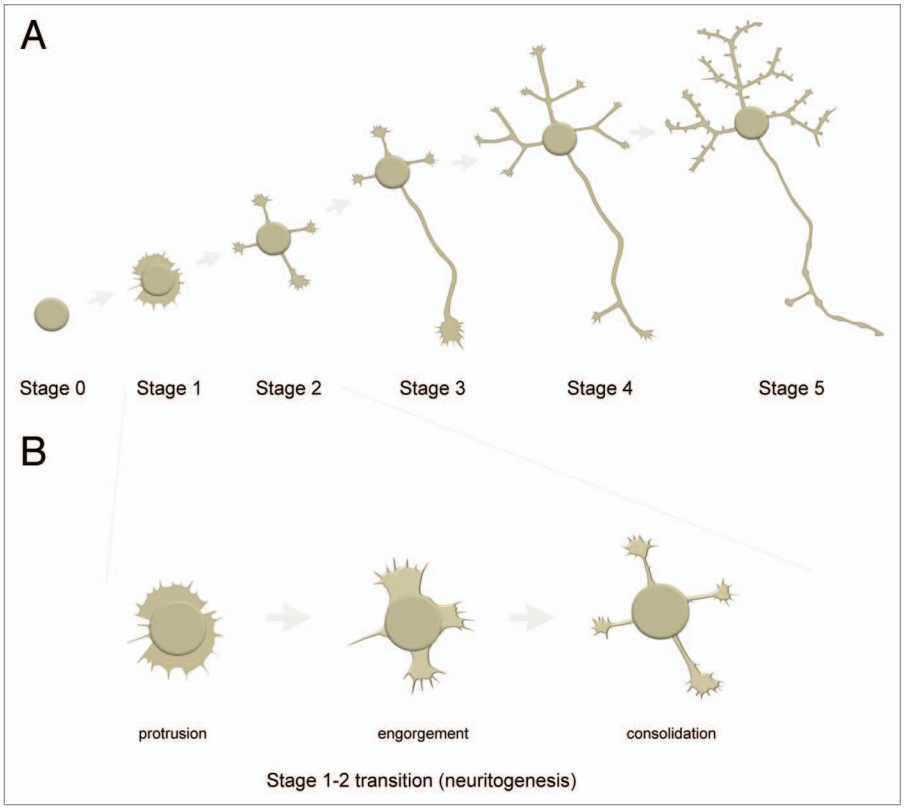

Figure 1. Neuronal development and neurite initiation. (A) Neuronal morphogenesis can be divided into a series of stages which were initially characterized in culture (Dotti et al., 1988), but also seem to occur in vivo. The times provided here are broad generalizations and specific culture conditions determine the exact timing of the developmental milestones. Shortly after their birth (or after plating in culture), initially spherical neurons begin extending circumferential lamellipodia and filopodia (Stage 1, 0–6 h). During the Stage 1–2 transition, the lamellipodia protrude forward and stable filopodia become engorged forming into neurites (neurite initiation or neuritogenesis). A Stage 2 (6–24hrs) neuron typically exhibits multiple minor neurites, all of which have the potential to become an axon. In the absence of graded external signals a stochastic process occurs by which one neurite with a large and dynamic growth cone begins elongating at a rapid rate and becomes the axon (Stage 3, 24–72 h). In vivo, this typically occurs in a directed manner, as the presence of extrinsic cues guide axonal development. The axon continues to grow and differentiate while remaining processes then grow and arborize acquiring dendritic identities (Stage 4, 3–7 d). Neurons then begin to make synapses, develop dendritic spines, and form neuronal circuits (Stage 5, 7–28 d). (B) Neurite initiation or neuritogenesis occurs during the stage 1–2 transition and can be subdivided into 3 phases. F-actin assembly drives membrane protrusion as either broad lamellipodia or finger-like filopodia. Then microtubules and other components move out into the lamellipodia and filopodia during the engorgement phase. Finally neurite formation at the base of the growth cone is consolidated as the membrane and underlying cytoskeleton reorganizes to form the cylindrical shaft of the neurite.

Pyramidal neurons follow a developmental program that is reproducible under equivalent cell culture conditions. After initial attachment, neurons are in stage 0 with a spherical shape and typically begin extending lamellipodia and filopodia protrusions within minutes and lasting for a few hours (stage 1). During the stage 1–2 transition, neurites first emerge from the cell body and begin extending away from the cell body (**Fig. 1**). As discussed below, neuritogenesis may occur in multiple ways. Neurons that are in stage 2 display proper neurites (normally regarded as protrusions equal to or greater than the diameter of the cell body). These neurites are essentially indistinguishable, as any of them has the potential to become the axon.[8] During the stage 2–3 transition one of these neurites begins elongating at a faster rate developing into the axon and the neuron becomes polarized (stage 3). The remaining neurites later grow and arborize into morphologically distinct dendrites (stage 4). Continued development can proceed up to and exceeding a month in culture, whereby neurons form dendritic spines and functional synaptic connections (stage 5). Each of these stages requires specific changes in the dynamics and cytoarchitechture of the cytoskeleton. Recent reviews have outlined some of the cytoskeletal changes associated with axon specification,[9] dendrite arborization,[10] and dendritic spine formation.[11] Historically, the focus of research has been aimed at understanding the breaking of symmetry initiated by axon formation. However, this is not the first symmetry breaking event in neuronal morphogenesis. The breaking of spherical symmetry of newborn neurons occurs with the initiation of neurite protrusions (i.e., neuritogenesis) and

this is the first morphogenetic event upon which all other development builds.

Recent advances in tissue culture and imaging techniques have shown that some of the basic features of neuronal development that were described in culture are analogous in vivo and ex vivo.[12-14] However, a truly definitive in vivo description of neuritogenesis has not been published. The vast majority of the molecular mechanisms described below have been elucidated in cell culture. Many of these mechanisms are likely the same in vivo, however, diligence should always be employed when extrapolating events observed in cell culture to the in vivo situation. Retinal ganglion neurons, for example, undergo a multi-polar stage in culture but only exhibit bipolar and unipolar neurite growth in vivo.[15] There are in fact a few notable differences in cortical neurons as well. For example, as neurons begin extending neurites (stage 1–2), their cell bodies are not fixed in space, as normally occurs in culture. Rather, neurite extension occurs coincidentally with neuronal migration.[14] In the cortex, neurons are derived from asymmetric cell divisions from radial glia at the ventricular zone (VZ) and symmetric cell divisions in the subventricular zone (SVZ).[12] Regardless of the origin, most neurons undergo a multipolar stage with 3–5 processes, similar to neuritogenesis in culture.[16] There is some controversy if the initial processes are bona fide neurites or some sort of migratory extensions with different properties than neurites.[12,17] More recently it was observed that neurite formation and axon growth can occur before the formation of migratory processes.[18] Interestingly, there are numerous examples of molecule disruption (dominant negative, genetic ablation or siRNA) in which neurons display perturbed migration and neurite growth.[19] But in all of these cases the signaling molecules that were disrupted are part of central cell motility pathways which would unhinge any motility-dependent process. Whether neurite initiation and migration in vivo are inextricably linked or autonomous processes thus remains unclear. Interestingly, neurons can migrate without axon extension,[17,20] indicating that at least polarization and migration can be independently regulated. If care is taken to not over interpret the findings, it should be useful to apply the basic principles learned from neuronal culture studies to the in vivo setting.

Neuritogenesis: The Synopsis

The morphological events of the stage 1–2 transition can be sub-divided in an analogous manner as described for axon extension in classical studies by Goldberg and Burmeister,[21] but with some nuances. Neuritogenesis occurs in three stages: protrusion, engorgement, and consolidation (**Fig. 1B**). Protrusion occurs as F-actin-based rearrangements and polymerization "pushes" the leading edge of membrane forward. There are at least two mechanisms that give rise to the neurite. In many stage 1 cells, there is a circumferential lamellipodium which collapses in discrete regions while extending in others, thereby establishing nascent growth cones of what will become newly formed neurites.[22,23] Microtubules (and neurofilaments) then follow the lead of the advancing leading edge with the transport of vesicles and organelles into the periphery during the engorgement phase. Finally, the contiguous, proximal regions of membrane and underlying cortical cytoskeleton collapse and undergo a consolidation to form the cylindrical neurite. During consolidation, the microtubules become progressively more bundled and construct the core of the nascent neurite cylinder. Concomitantly, in bordering regions of the soma, lamellipodial, and filopodial protrusions largely regress and transform into more stable cortical structures. In a second mechanism, a single filopodium can give rise to a neurite in the absence of discernable lamellipodia protrusions[23-25]. These neuritogenic filopodia first protrude, become stable, then engorge with cytoplasm and microtubules, and later develop growth cones. A similar process has been observed to enhance branching of neurites when growth-cone like waves cause engorgement of neurite filopodia.[26] Other groups have described a "bud" directly emerging from the spherical cell body, which with phase contrast imaging may be the distension of a stable filopodium and/or represent the accumulation of microtubules into the forming neurite.[4,27]

Although analogous, the mechanisms of neuritogenesis and axon growth are not completely transposable. Neuritogenesis and axonogenesis use the same basic machinery, but in a number of distinct ways. For example, low levels of the microtubule stabilizing drug taxol induces axon differentiation and growth[28] but does not enhance neuritogenesis in primary neurons.[23] Moreover, inhibition of signaling pathways crucial for axon specification has no impact on neuritogenesis.[1] Thus, casually assuming that any factor that plays a certain role in during axon specification is doing the same during neuritogenesis (or axon growth for that matter) is an over-simplification of neuronal morphogenesis. That being said, one must borrow from studies in axon specification, neurite growth, and even other motile cells out of necessity, since direct studies of neuritogenesis are few compared with the multitude of data available regarding neuronal polarization and axon growth. Some useful information regarding the essential players regulating the dynamics actin and microtubules in growth cones and motile cells can shed light on how neurites protrude, engorge and consolidate from out of the cell soma.

The Building Blocks of Neuronal Structure

Actin filaments, microtubules, and intermediate filaments compose the cytoskeletal framework of the neuron. These fibrous polymers impart different biophysical character to the neuron; microtubules resist compressive loads while F-actin and intermediate filaments bear tensile forces.[29] This is crucial for neuritogenesis, as the external application of tension induces neurite formation,[30,31] suggesting that tensile forces generated at interface of the cortical cytoskeleton and substrate are necessary for neuritogenesis. Although neurofilaments, a class of intermediate filaments of the neuron, are involved in enlarging and maintaining axon caliber, regulating axonal conduction velocity of electric transmission and facilitating axon growth,[32] the evidence thus far suggests they are not essential for neurite initiation or growth. Therefore, neurofilaments will not be discussed further in this review, which will focus on the roles of actin and microtubules, both of which are indispensible for neuritogenesis. The basic

principles of actin and microtubule assembly and dynamics as they pertain to neuritogenesis will be discussed below.

Building a Growth Cone—Actin

Actin dynamics—its assembly from monomers into filaments, motor driven movements, interactions with adhesions, fragmentation, and reorganization of fragments, and disassembly back into monomers—provides a major force for the cell movement. It is clear that actin polymerization at the submembranous oriented barbed ends of actin filaments drives the protrusion of lamellipodia and filopodia at the leading edge of migrating cells and in the growth cones of extending neurites.[33] In concert, F-actin disassembly occurs at the proximally oriented pointed ends of actin filaments, balancing the actin assembly at the leading edge in a process termed actin treadmilling.[34] This treadmilling is an essential component of the actin retrograde flow "engine" upon which neurite outgrowth depends. Seminal studies in cell-free paradigms highlighted how relatively simple collections of proteins recapitulate actin-based motility.[35,36] These proteins are certainly major players in neuronal development. In motile cells and growing neurons, however, the situation is much more complex, with the actin motor, myosin, and other actin binding proteins modulating actin filament dynamics, and actin organization into higher-order superstructures.[37] Actin isoforms, treadmilling kinetics, and important modulators of actin organization and dynamics that influence neuritogenesis and the formation of growth cones will be reviewed in this section.

In mammals, there are six actin isoforms including three α (α) isoforms, a β (β), and two gamma (γ) isoforms which, in spite of showing overall similar biochemical properties, have distinct expression patterns and functions. α-Actin isoforms are notable for their muscle-specific expression and constituting the thin filaments of the sarcomere, the functional unit of muscle cells. The cytoplasmic β- and γ- actin are highly expressed in brain at roughly equal levels[38-40]and therefore of more interest for neuritogenesis. Although over 98% homologous and having nearly similar biochemical activities, β-, and γ- actin show distinct mRNA and protein localizations and have distinct transcriptional and post-translational modifications that may be of importance to actin dynamics in the neuron.[41,42] In neurons, there is a higher distribution of γ- actin in the cell body and lower levels in the growth cone while β-actin is most highly localized to the growth cones.[43] In spite of partial distribution differences, there is definite overlap of protein localization in dynamic actin of the growth cone[40]and γ-, and β-actin can co-polymerize in the same actin filaments[44] suggesting possible redundancy. Indeed, recent genetic knockout models show that actin isoforms can compensate for each other during neuronal development. Complete genetic ablation of γ-actin in mouse does not cause any phenotype, including in the nervous system.[45] Similarly, the brain-specific ablation of β-actin did not result in any gross changes in neuronal development, including neuritogenesis.[46] In both knockout models, the upregulation of other actin isoforms likely minimizes phenotypic manifestations. Intriguingly, in the β-actin knockout mice, while only a modest 20% upregulation in γ-actin is observed, a massive 35 fold upregulation of smooth muscle α-actin occurs in brain tissue. This is the first report of α-actin expression in brain. It will be interesting to see if the upregulated smooth muscle α-actin is sufficient for neuronal development in the absence of β-actin and γ-actin since various actin binding proteins important for cell motility have been shown to bind with higher affinity to β-actin-rich compared with α-actin-rich preparations.[47,48]

The tips of developing neurites are characterized by a dynamic, fan-like structure, coined "growth cone" by Ramon y Cajal.[49] Growth cones are classically sub-divided into 3 regions based primarily on their underlying cytoskeletal organization and organelle composition. The peripheral (P) domain is defined by an extensive actin filament network and is devoid of organelles. The central (C) domain contains organelles (i.e., mitochondria) and a core of microtubules that splay out as they penetrate from the neurite shaft. The interface between the P and C domains is the transitional zone (T) where contractile actin networks are compressed and deconstructed while impeding the growth of microtubules farther into the periphery. Actin filaments are assembled into different higher-order networks or superstructures in developing growth cones.[37] Of these superstructures, filopodia, lamellipodia, and arcs have been the most widely studied in growing neurons. These require different ensembles of the actin binding proteins, which collectively affect actin assembly/disassembly kinetics, retrograde flow, and remodeling. Even before neurites are fully formed in young neurons, these actin superstructures are discernable (**Fig. 2**). Other higher order structures such as stable cortical actin lattices and dynamic comet-like intrapodia are also present in neurons but have received less attention and will not be discussed at length in this review.[50] Although heterogenous, all growth cones have some combination of these actin superstructures.

Filopodia consist of long unipolar, bundled actin filaments that extend from the peripheral domain into the transitional domain of the growth cone. The peripheral domain also exhibits lamellipodia, which are made up of short branched actin filaments that compose a mesh-like gel. Whether filopodia or lamellipodia are initialized at certain locales within the peripheral domain depends on the repertoire of actin binding proteins engaged at those sites. It begins at the membrane, where actin nucleators are locally activated (or dis-inhibited). Certain actin nucleators, such as formins initiate bundled F-actin in filopodia,[51] while others such as Arp2/3 complex extend branched F-actin arrays in lamellipodia. In the competing tip nucleation model, filopodia and lamellipodia are considered separate compartments and specific ensembles of ABPs protein accumulate at distinct locales at the membrane and compete for actin nucleation thereby driving filopodia or lamellipodia formation.[52] Some proteins in these ensembles are overlapping, such as Ena/Vasp and IRSp53, but the main distinction is the type of actin nucleator in these ensembles; formins drive filopodia formation and Arp2/3 initiates lamellipodia.Thus, according to this model,, the identities of actin filament superstructures are determined at their birth[53] and other ABPs assist with maintaining, disassembling or interconverting these actin superstructures. Other

observations support the convergent elongation model, whereby filopodia formation occurs out of the dendritic network in lamellipodia by the convergence of barbed ends of growing filaments which gradually coalesce, accumulate Ena/Vasp which out-competes capping protein and subsequently recruits fascin to generate nacent filopodia.[54] In vitro, the interconversion does not need to be so complex;decreasing the concentration of fascin in favor of filamin is enough to remodel actin filaments from bundled arrays to a branched filament network.[55] It is unclear which of these mechanisms are at play in neurons; however, overexpression of fascin alone increases filopodia numbers in hippocampal neurons, apparently at the expense of lamellipodia[23] (Flynn et al., unpublished observations). These models will hopefully be tested as data from complete genetic knockout of the important actin nucleators becomes available.

Actin acrs are transversely oriented actin bundles located behind the lamellipodia and filopodia of the leading edge in the transition zone of growth cones. In non-neuronal systems, actin arcs have been shown to form from the shedding of actin filaments from the lamellipodia and actin microspikes (filopodia).[56,57] Since the actin in arcs is derived from shedding in both directions, the resulting actin filaments have mixed polarity. Observations in keratocytes showed that myosin II formed clusters in the proximal lamellipodia and coincided with the change of orientation of actin filaments from diagonal to perpendicular.[58] Moreover, ultrastructural analysis clearly showed these actin filaments had mixed polarity in contrast to the leading edge which is composed almost exclusively of actin bundles with barbed ends oriented toward the membrane. Live cell imaging studies have also demonstrated that as lamellipodial actin flows away from the cell edge, α actinin-decorated filaments seed the formation of actin arcs in a process dependent on Arp2/3 complex.[59] Although some of the details are lacking, these studies have largely been comfirmed in the large growth cone Aplysia bag cells, where actin arcs are formed in the transition zone from peripheral actin structures as the result of compressive forces of peripheral retrograde F-actin flow and myosin II activity.[60,61]

The shape and motile properties of growth cones are governed primarily by the dynamic rearrangements of these actin superstructures. Actin exists in a balance of unassembled, globular actin (G-actin) and filamentous actin (F-actin), which is composed of actin monomers assembled in a helical head to tail (polar) fashion (**Fig. 3**). Actin filaments can be extremely dynamic in growth cones, both in terms of their assembly/disassembly kinetics and in how they move relative to one another. As in other motile cells, the vector of actin filament subunit flow always occurs from the leading edge membrane inward toward the central domain of the growth cone. The important consequence of this is a dynamic organizational plane in which actin subunit addition and filament elongation occurs at the membrane while disassembly occurs proximally within the growth cone. This process is essential to neuritogenesis.

Actin filaments are "born" at or near the cell membrane by one of three different classes of actin nucleation factors including the Arp2/3 complex, formins, and tandem-monomer-binding nucleators.[62] It is still controversial whether cofilin represents a fourth class of actin nucleator in vivo[37,63], but cofilin may provide the pieces of F-actin that serve as mother filaments necessary for Arp2/3 complex binding and nucleation of branched filaments.[64] All of these types of actin nucleators have been found in brain.[65,66] Spontaneous nucleation of actin is unfavorable due to the instability of the actin dimer nucleus.[67] Only after a stable oligomer of three or four subunits is formed can more rapid elongation of actin filaments occur (**Fig. 3**). Actin nucleators circumvent this problem via different mechanisms. The Arp2/3 complex requires the binding to the sides of an existing mother actin filament, preferentially to ADP-Pi-actin and nucleation promoting factors such as WASP and WAVE which funnel actin monomers to Arp2/3 complex, which mimics a lateral actin dimer. Filaments generated by Arp2/3 complex occur at 70° angles to the mother filament resulting in an overall branched filament network. Formins function as hinged dimers binding 2 actin monomers to promote the formation of linearly aligned actin filaments.[68] Formins not only nucleate new actin filaments, but also promote the continued polymerization of actin filaments due to their proccessivity (i.e., their ability to stay associated with growing barbed ends) and their ability to work cooperatively with profilin-actin complexes to accelerate monomer addition (**Fig. 3**). The tandem-monomer binding nucleator, Spire has been proposed to collect four actin monomers into long-pitch helix nuclei with Wasp Homology 2 (WH2) domains which can initiate further polymerization.[69] Another tandem-monomer nucleator, cordon bleu (Cobl), uses two WH domains to first form a linear dimer and then a third WH2 domain to complete an actin trimer nucleus.[70]

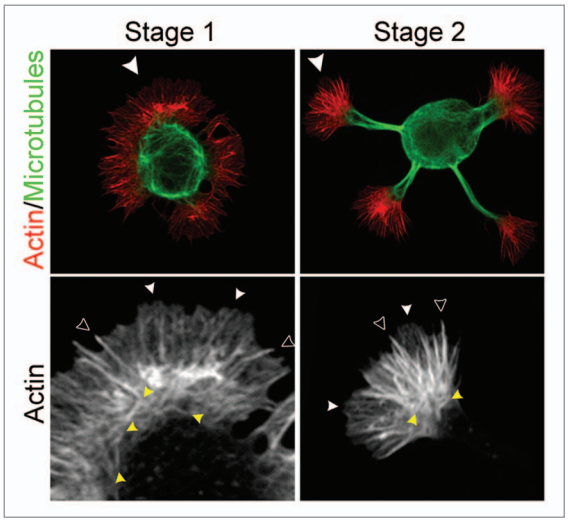

Figure 2. Actin organization in Stage 1 and Stage 2 neurons. A comparison of Stage 1 and Stage 2 neurons illustrates similarities in the general architecture of actin filaments in the circumferential membrane extensions and growth cones, respectively. Filamentous-actin (Red, stained with phalloidin) and microtubules (Green, stained with β-tubulin) are depicted in upper panels. The lower panels show a magnified view of F-actin staining of regions indicated by arrows in upper panels. Note that in Stage 1 neurons and Stage 2 growth cones, different F-actin superstructures are discernible including F-actin bundles in filopodia (open arrowheads), F-actin network in lamellipodia (white arrowheads) and F-actin arcs (yellow arrowheads).

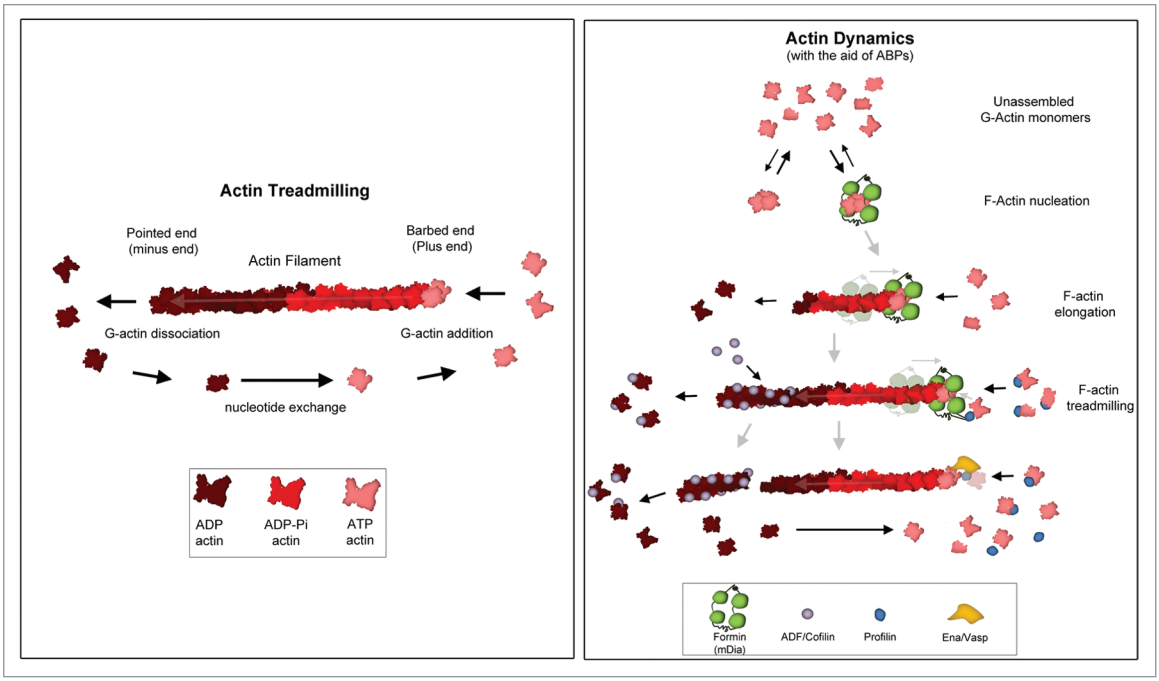

Figure 3. Actin subunits (Globular or G-actin) can spontaneously assemble into elongated, polar, bi-helical filamentous polymers (F-actin) in vitro217. Actin filaments are often called microfilaments because they are the slimmest cytoskeletal polymer with an average thickness of 6–9 nm. The polarity of actin filaments is determined by the orientation of the monomers in the filament and by the non-covalent attachment of adenine nucleotides. This polarity is essential for the intrinsic dynamics of actin filaments – treadmilling. Simply stated, actin treadmilling is the process by which actin subunits are added preferentially to one end of an actin filament (A.K.A. plus or barbed end) while disassembly occurs at the other end of the filament (A.K.A. minus or pointed end). Treadmilling occurs because of the two ends of an actin filament maintain different "critical concentrations" of G-actin needed for assembly. When the concentration of G-actin lies between these two critical concentrations, net actin assembly occurs at the barbed end and disassembly at the pointed end. An intrinsic ATPase activity of actin underlies treadmilling, and this is critical for actin-based motility217. ATP bound actin monomers dominate the unassembled pool of G-actin and are preferentially added to the barbed end of actin filaments. The ATP is rapidly hydrolyzed into ADP-Pi, with the subsequent release of the phosphate taking 300–600X longer, resulting in ADP-actin subunits. The resultant ADP-actin thus dominates the older portion of the filament and is released at the pointed end of the filament. Barbed end growth and treadmilling speed is determined by the availability of ATP-actin monomers and increased depolymerization at the pointed end. An important consequence of the actin ATPase cycle and treadmilling is that actin monomers age as they progress from their incorporation at the barbed end through their removal at the pointed end. This results in a molecular stratification in which the barbed end consists of a small number of freshly added ATP-actin monomers, followed by a "middle-aged" portion consisting of ADP-Pi-actin with ADP-actin making up the old segment toward the pointed end. Since many actin binding proteins show preferential binding based on the status of the bound nucleotide, this stratification is essential to actin organization and turnover in cells. For example the preferentially binding to ADP-actin sorts ADF/cofilin to the older, cytoplasmic, portions of actin filaments37. The fact that actin treadmilling is 100 times faster in cells than in isolated preparations indicated the modification of actin dynamics by additional mechanisms218. There are multiple factors influencing actin turnover in cells including the catalysis of F-actin assembly by actin nucleators and polymerization enhancing proteins, the increase in pointed end subunit dissociation by actin severing and depolymerizing factors, and the regulation of the pool of unassembled, G-actin. Out of hundreds of ABPs expressed in cells, only an actin nucleator, such as formin or Arp2/3 complex, capping protein, and ADF/cofilin proteins are required in cell-free systems to emulate actin turnover rates observed in cells 35, 36. No other motor proteins or actin crosslinking proteins are needed. However, other actin binding proteins can modulate the turnover of actin in vitro and may be important modulators of actin in neuronal cells (e.g., Ena/Vasp enhances actin polymerization in vitro). The left panel here shows how nucleation and treadmilling is enhanced by ABPs. In cells nucleation mediated by formins occurs at the plasma membrane. Formins remain associated with the barbed ends of actin filaments and aid in the polymerization of F-actin. Profilin can aid actin polymerization by enhancing the exchange of ATP for ADP on G-actin and funneling actin to the barbed ends by interacting with formins and Ena/Vasp. ADF/cofilin bind to the ADP-actin portion of the actin filament, enhance the ATPase activity of actin, and increase severing which increases the number of ends from which disassembly occurs. This increases the overall disassembly of actin filaments and recycles actin monomers for further actin polymerization.

Other factors can increase actin turnover by accelerating the on-rate of actin assembly in existing filaments. In addition to the formin, mDia, the multifunctional proteins of the Ena/Mena/Vasp family (hereafter called Ena/Vasp proteins) are potent actin polymerizing agents found in neurons.[71,72] Ena/Vasp proteins promote actin polymerization by binding the barbed ends of filaments, interacting with profilin-G-actin complexes, and promoting the transfer of the G-actin subunit from profilin to the barbed end. Moreover, Ena/Vasp proteins exhibit "anti-capping" activity, by which they protect actin filaments from capping protein, which inhibits actin polymerization.[73] Other Ena/Vasp activities such as anti-branching and filament bundling also contribute to organizing actin filaments in linear bundles as observed in filopodia.[3,51]

At the other end of the filament, the off-rate of actin subunits from the pointed end can be enhanced by increased

depolymerization and severing. There are multiple actin binding proteins that can enhance F-actin severing and actin subunit disassociation at the minus end including ADF/Cofilin, gelsolin, and mical proteins.[74,75] The motor protein, myosin II has also been proposed to directly deconstruct actin filaments.[76] The widely studied ADF/Cofilin proteins (AC) bind cooperatively to ADP-bound portion of the actin filament and binding to or induce a more twisted form of the F-actin helix.[77] This is thought to introduce mechanical discontinuities and destabilized interfaces between actin subunits in the filament to induce severing at the boundary between the AC bound portion of F-actin and the unbound portion.[78] AC proteins also increase the ATPase activity of actin, the rate of phosphate release, and promote the acceleration of monomer loss from the pointed ends of actin filaments.[79] The overall effects of AC protein activity, thus is to increase the overall rate of actin treadmilling by enhancing the ATPase cycle in actin filaments, accelerating the disassembly of F-actin and, thereby, increasing the pool of actin monomers able to re-associate with the barbed ends (**Fig. 3**). Gelsolin is one member of the gelsolin/villin superfamily of ABPs that typically bind the barbed ends of actin filaments.[80] After binding along actin filaments, gelsolin mediates calcium (Ca^{2+})-dependent actin severing activity and remains attached to newly formed barbed ends. Other work contends that the principal activity of gelsolin is, in fact, as a capping protein and that observed F-actin depolymerization proceeds due to diminished actin polymerization.[81] Recent studies have also suggested that under certain conditions, gelsolin cooperates with ADF/Cofilin to enhance actin depolymerization.[82] Mical proteins use redox enzymatic activity and the coenzyme NADPH to decrease actin polymerization, increase actin depolymerization and lead to a disassembly of actin bundles.[75] As discussed below, actin turnover driven by ADF/Cofilin F-actin severing is crucial for neurite initiation.

A final mechanism to accelerate actin turnover is having a surplus of free monomeric actin that is available for F-actin polymerization on demand. In developing neurons, this is no problem with up to 80% of total actin in the unassembled form,[23] where it is specifically enriched at the leading edge.[83] This suggests that the monomeric actin is available in ample supply and at the right location to support rapid F-actin growth. Cells regulate the production and availability of the monomer pool in different ways. At the transcriptional level, cells use a complex of myocardin related transcription factors (MRTFs) such as megakaryocytic acute leukemia (MAL) with Serum Response Factor (SRF) which act as a G-actin sensor to regulate increased actin transcription.[84] Rho activation can shift F:G actin ratios to increasing F-actin levels at the expense of G-actin, leading to a release of MRTF from G-actin, which forms a complex with SRF to increase the synthesis of actin (both β- and γ- actin).[84,85] Knockout of SRF or its coactivator MAL severely inhibits neurite outgrowth in hippocampal neurons.[86] Although SRF-MAL can control the expression of many other genes, overexpression of active SRF, which increases actin synthesis, reverses neurite growth defects induced by an actin mutant that impedes actin turnover, suggesting that SRF-mediated actin synthesis contributes to the G-actin pool thereby moderating neurite growth

G-actin concentration in cells greatly exceeds the critical concentration for actin assembly. However, cells resist the spontaneous nucleation of assembly with G-actin binding proteins such as β-thymosin (Tβ) or profilin that sequester G-actin thereby regulating the controlled polymerization of actin.[80,83] The binding of profilin and β-thymosin to G-actin are mutually exclusive and generally have opposing effects on actin polymerization; activated profilin promotes while β-thymosin attenuates actin polymerization.[72] Profilin increases actin polymerization by facilitating the exchange of ADP for ATP on G-actin and interacting either directly with or via intermediaries to actin nucleators which directs ATP-actin to the barbed end.[87] β-thymosin is highly expressed in neurons suggesting that the Tβ-actin pool is a major source of actin monomer in neurite growth. RNA knockdown of Tβ in Lymnaea snail neurons increased outgrowth rates suggesting that local translation of thymosin and sequestering of G-actin may slow outgrowth rates and growth cone motility,[88] perhaps allowing more time to process guidance cue signaling for pathfinding.

Profilin has been shown to directly regulate neuritogenesis and control F-actin levels in developing neurons.[89] The downregulation of profilin II either with antisense RNA or via genetic ablation leads to an increase in neuritogenesis, with increased neurite numbers and branching while the overexpression of profilin II has the opposite effect. Interestingly, in neurons with decreased profilin II expression, there was a decrease in F- and increase in G-actin (overexpression of profilin II had the opposite effect) suggesting that increasing profilin II activity alone increases F-actin assembly. In other studies, knockdown of profilin I decreased G-actin levels, protrusion velocity, distance, and persistence in neuroblastoma cells, suggesting that profilin-mediated actin assembly helps mediate the protrusions of neurites.[83] The discrepancies between these studies may be due to inherent differences in the cell type examined whereby G- and F-actin levels can lead to differential effects of the same ABP family[40] or by differences in the activities of profilin I and II. Nevertheless, taken together these results suggest that during neuritogenesis, too much actin assembly is refractory to neuritogenesis, while too little leads to excessive neurite formation. The key for normal neuritogenesis thus seems to be a balance of actin assembly and disassembly.

Monomer binding to the barbed ends of filaments is also regulated by barbed end capping proteins (e.g., Capping protein (CP) and EPS8) which block further polymerization. In this regard, capping protein can lead to the reduction of actin filament length in vitro.[90] However, in cells, capping protein cooperation with actin nucleators and antagonism with F-actin polymerization factors may lead to enhanced filament elongation by funneling actin monomers to uncapped filaments[91] or by increasing actin nucleation rates.[92] It remains unknown if barbed end capping proteins regulate neuritogenesis, but given their importance in generating actin motility in vitro, it is likely an important player in neuronal morphogenesis.

The first phase of forming a growth cone from the uniform lamellipodium of a stage 1 neuron is the protrusion of the leading edge membrane. Thus, understanding the actin superstructures

that compose the most peripheral region of emerging neurites is essential to understand neuritogenesis. Indeed, evidence suggests that actin-based processes initiate neurites, while other cytoskeletal components (i.e., microtubules) follow the lead of the advancing actin superstructures[23,25]. The unipolar, linearly-arranged F-actin bundles underlying filopodia have been suggested to play a particularly important role during neuritogenesis. Careful time-lapse microscopy of sympathetic neurons plated on laminin showed that the first dynamic morphological change of round neurons is the formation of long, dynamic filopodia.[24] Some of these filopodia become engorged with microtubules, and then develop more defined growth cones and become elongating neurites. More recently, Gertler and colleagues elegantly showed that filopodia facilitate neurite formation in cortical neurons.[25] Treatments that blocked filopodia formation inhibited neuritogenesis. For example, neurons from mouse mutants lacking all three isoforms of the Ena family of ABPs (Ena, Mena, Vasp) fail to form neurites in culture in a substratum-dependent manner. While the ablation of Ena/Mena/Vasp is essential for neurite formation on polylysine, neurites grow when these neurons are plated on laminin. The key difference is that the neurons were able to form filopodia on the laminin. In fact, essentially any treatment that induced filopodia formation rescued neuritogenesis, suggesting that filopodia are essential for neurite formation.

The ablation of Ena/Vasp activity revealed two "modes" of neuritogenesis: one dependent on the elongation of actin bundles mediated by Ena/Vasp and one dependent on the growth of actin filaments mediated by Arp2/3 complex for neurons cultured on laminin.[1] The authors took advantage of the fact that laminin rescues neurite formation to "impel" neurons to assemble neurites using the second mode of neurite formation. Blocking Arp2/3 complex activity with the CA domain of the Arp2/3 complex activator N-Wasp abolished the ability for laminin to rescue neurites in Ena/Vasp inhibited neurons. These data suggest that Arp2/3 complex can mediate neurite formation only when integrin signaling engages other factors to coordinate with Arp2/3 complex to form actin structures that facilitate neurite formation. Blocking Arp2/3 complex in neurons cultured on polylysine has no effect on neuritogenesis, suggesting that other actin nucleators coordinate with Ena/Vasp to facilitate to actin bundle formation and neuritogenesis. These data argues for different sets of ABPs at work during neuritogenesis; sets of ABPs for connecting certain extracellular cues to actin rearrangements (filopodia) and other sets for the intrinsic triggering mechanism of these actin rearrangements.

At the plasma membrane, the BAR (Bin, Amphiphysin, RVS) superfamily of proteins can regulate neuronal morphogenesis by forming banana shaped dimers that can bind underneath phosphatdylinositol-rich membranes and promoting positive or negative membrane curvature.[93] In addition, BAR proteins can recruit actin and actin regulators like WASP/WAVE to the membrane which facilitates the protrusion of the membrane. I-Bar (inverted BAR) domain bearing proteins such as Irs-58 were proposed to regulate neurite formation and growth by inducing negative curvature and filopodia.[94] Another BAR protein of the F-Bar variety (Fer/CIP4 homology [FCH] domain and BAR), srGAP2 acts like I-Bar proteins to induce filopodia and neurite growth, seemingly at the expense of bipolar neuronal migration.[95] Conversely, the F-Bar domain containing protein, Cdc42-interacting protein 4 (CIP4), has recently been shown to define sites of neurite initiation by limiting filopodial protrusions in favor of lamellipodia[96].. In neurons, the downregulation of CIP4 coincides with neurite formation and its upregulation promotes broad lamellipodial protrusions and inhibits neuritogenesis.[96] Thus, BAR domain proteins can coordinate membrane remodeling and F-actin dynamics to regulate neurite protrusions. In the case of srGAP2, enhanced filopodia formation promotes neuritogenesis whereas CIP4 activity favors lamellipodia formation at the expense of filopodia thereby inhibiting neurite formation, supporting the notion that filopodia are crucial for neuritogenesis.

It is possible that formins and/or tandem actin nucleators such as cordon blue can also coordinate with Ena/Vasp to mediate filopodia formation and neuritogenesis. A recent study, however, suggested that formins are not necessary for neuritogenesis as the ablation of the mDia1 and mDia3 did not affect the development of pyramidal neurons although tangential migration of interneurons was perturbed.[97] It is possible that mDia2, additional proteins with formin homology domains, or other actin nucleators, such as Arp2/3 complex and Spire, which shows similar expression patterns as formins, can compensate for the loss of mDia1 and mDia3 to mediate neurite extension. In addition to Arp2/3 complex and Spire, the expression of the neuron-enriched tandem actin nucleator, cordon bleu, drives the formation of filopodia-like protrusions and supernumerary neurites.[70] Since all these actin nucleators are potentially targeted to the leading edge membrane and can generate actin filaments at an angle perpendicular or orthogonal to the membrane,[37] the actin organization initiated by any actin nucleator may be sufficient to facilitate neurite initiation as long as the actin dynamics and organization are maintained after nucleation.

A malleable peripheral actin network—one that is dynamic and rapidly turning over—is essential for neuritogenesis. This assertion is supported by experiments that use toxins affecting the assembly and disassembly of F-actin and microtubules. Treatment of stage 1 neurons with the actin stabilizing drug, jasplakinolide freezes actin turnover leading to an inhibition of neurite initiation.[23] Conversely, increasing actin destabilization with the actin monomer sequestering drug latrunculin B accelerates neurite formation. These experiments suggest that maintaining or increasing actin malleability and dynamic turnover is essential to driving neurite formation. In addition, evidence suggests that the peripheral actin network keeps uncontrolled microtubule growth at bay[37,98]. Indeed, treating AC KO neurons with latrunculin B slowly destabilizes the rigid actin network as microtubule bundles concomitantly grow out of the soma and form neurite protrusions.[23] However, it is not enough to simply have dynamic actin in the periphery of the neuronal soma to initiate neuritogenesis. The organization of the actin superstructures is also important, as the absence of radially oriented actin filaments also inhibits neurite growth even if filaments are still dynamic.[25] However, radial oriented F-actin bundles like those in filopodia are not sufficient to induce neurites in the absence

of actin turnover and retrograde flow.[23] Thus two key properties of the actin network are essential for neuritogenesis: 1. The organization of radial actin filament arrays and bundles and 2. Maintaining or increasing actin turnover dynamics—assembly, retrograde flow and depolymerization.

In growth cones, it is widely accepted that actin retrograde flow is driven by the combined effects of the contractility of myosin II on the actin network and the pushing force that actin exerts on the leading edge membrane as it is polymerizing[60,61,63,99]. The Brownian ratchet model describes how polymerizing actin filaments undergo thermal motions, flexing, bending and straightening. As they are bent away from the membrane, actin monomers squeeze in and the filaments elongate. When the filaments straighten again, the rectifying force can push the leading edge forward.[100] In order to push the leading edge forward, the balance of actin polymerization and retrograde flow needs to be biased toward increased polymerization. Conversely, when retrograde flow exceeds actin polymerization at the membrane, then retraction occurs.[3] One way to shift the balance to actin polymerization-driven protrusion is to decrease retrograde flow by increasing the coupling of the actin network to substratum via transmembrane adhesions.[101] Alternatively, increasing actin assembly and retrograde flow can also lead to increased protrusion in the absence of adhesion, at least in non-neuronal cells.[102] One aspect of retrograde flow that is often overlooked is the contribution of actin depolymerization and turnover at the pointed end of the filaments, since without it actin filaments would continue elongating and moving inward until the actin monomer pool was depleted. As discussed below, ADF/cofilin proteins are essential to actin retrograde flow and overall actin turnover dynamics in neurons. Thus, whether it is modyifying actin assembly/disassembly kinetics or coupling actin network to adhesions, retrograde flow is like an idle engine upon which ABPs engage to drive neurite growth.[3]

While profilin and Ena/Vasp are aiding in the assembly of actin at the barbed end, ADF/cofilin are disassembling filaments through severing the ADP-actin regions near the minus end. A combination of overexpression studies, RNA interference, and genetic knockout and rescue experiments indicate that ADF/cofilin is essential for actin retrograde flow and neuritogenesis in forebrain neurons.[23] The effects of ADF/cofilin ablation are devastating to actin architecture and dynamics, with an increase in abnormal F-actin aggregates and transversely oriented filaments, a loss of radial actin bundles and filopodia, and nearly a complete immobilization of actin retrograde flow. As a result, neurons largely remain stuck in stage 0/1 with an inability to form neurites. Expression of a cofilin mutant that can only sever actin filaments and treatment with actin destabilizing drugs rescue neurite formation, indicating that actin turnover is essential to neurite formation.[23] From this work, it appears other actin severing proteins cannot compensate for the loss of ADF/cofilin. Furthermore, the ability for myosin II to drive actin retrograde flow seems to be dependent on an F-actin network modified by ADF/cofilin. ADF/cofilin directly competes with myosin II for binding to F-actin[103] and this competition may play a role in myosin II release and recycling. Future work will be required to determine how the interplay of ADF/Cofilin, myosin II and substrate coupling regulate retrograde flow and neurite formation.

In motile cells and growth cones, actin dynamics is also greatly affected by the myosin motors. There are 17 classes of the myosin superfamily of motor proteins which use ATP for energy to produce force and move along actin filaments. In addition to walking along F-actin to transport cargo, myosins can cross-link actin filaments, propel the sliding of filaments along each other, produce tension and regulate the assembly and function of cell adhesions. Recently, it has become clear that non-muscle myosin II (NM II) is particularly important in regulating actin during cell motility.[104] In motile non-neuronal cells, myosin II plays a role in the retrograde movement of actin filaments in the lamella, which is a spatially distinct actin network posterior to the lamellipodia.[105,106] In neurons this distinction is less clear (as is the presence of lamella) and although myosin II accumulates a few micrometers behind the leading edge and is especially enriched at actin arcs, it seems to help drive the retrograde movement of actin filaments throughout the growth cone.[61] However, in other systems increased myosin II activity may act to slow actin retrograde flow.[107,108] Regardless of these discrepancies, it is generally accepted that myosin II activity drives the formation and compression of actin arcs which can impede microtubule advance into the periphery of the growth cone and along filopodia.[61,109]

During the early phases of neuritogenesis increasing myosin II activity by overexpressing an active version of the myosin II regulatory light chain kinase significantly decreases neurite outgrowth.[110] On the other hand, inhibiting myosin II with blebbistatin accelerates neuritogenesis, increasing the percentage of neurons extending neurites by over 10-fold at early time-points in culture. Since microtubule engorgement of filopodia is a potent mechanism to induce neurite formation, myosin II activity may impede the stage1–2 transition by increasing actin arc formation and compression in the transitional zone which obstructs microtubule advance into filopodia. The action of myosin II on retrograde flow is also important for the engagement of focal contact (neuronal focal adhesion) based tethering to the actin cytoskeleton, which can accelerate cell protrusion and overall motility by generating traction force against the substrate and allowing actin polymerization to overcome membrane tension (clutch hypothesis[104]). If myosin II inactivation decreases retrograde flow and increases coupling to focal contacts in this system, then actin polymerization could result in increased membrane protrusion and neuritogenesis.

To date, the role of the molecular clutch during neuritogenesis remains unclear. However, there are a few lines of evidence that suggest the engagement of transmembrane actin-substrate interactions may be important for neurite formation. As previously discussed, laminin can rescue neuritogenesis in Ena/Vasp KO neurons and this requires functional integrin receptors[1,25]. Although Arp2/3 complex is required for neuritogenesis on laminin, it is unclear if the integrin-mediated neuritogenesis (or that of other adhesion molecules) is due to simply to biochemical signaling pathways or if the actin-substratum coupling alters the balance of actin polymerization and retrograde flow. In addition, in the engagement of N-cadherin based adhesions

increases actin-based traction forces and growth cone advance in more mature neurons.[111] The neuronal cell adhesion molecule, L1CAM, has recently been shown to mediate the linkage of F-actin retrograde flow to the substrate to accelerate growth cone advance.[112] This study showed that phosphorylation of shootin1 enhanced clutch engagement of the actin retrograde flow with LCAM adhesions, thereby slowing retrograde flow, increasing traction force, and promoting growth cone protrusions. It remains unknown what role cadherin or L1CAM based adhesions play during neuritogenesis.

An important aspect of actin dynamics and organization is this cooperation and competition among various ABPs.[53] A notable player in modulating ABP interactions pertinent for neuronal actin is the tropomyosin (Tm) family of proteins.[113] Tropomyosins form rod-like dimers that bind along the long axis of F-actin, generally stabilizing actin filaments and regulating the access of ABPs to the filament. The latter activity is robust and, as a result, Tm's have been called "gatekeepers" or "bouncers" since they allow or restrict access of other ABPs to actin filaments. For example, certain tropomyosins encourage the binding and activity of myosin motors.[114,115] In other cases tropomyosins compete with ABPs, as observed with the Tm5NM1 isoform and ADF/Cofilin[115,116]. However, not all Tms compete with ADF/Cofilin, as shown for TmBr3 which recruits ADF/cofilin to actin filaments.[114] Given that there are over 40 isoforms of Tms in mammals from alternative splicing of four genes, the marked diversity of Tms and their individual and cumulative effects on actin and ABPs is remarkably complex. Although a few isoforms have been shown to be important for the regulation of neuronal morphogenesis,[117] the temporal and spatial expression patterns and function of Tms in neurons remains a future challenge for neuronal cell biology.

One class of ABPs modulated by tropomyosins important for neurite outgrowth are tropomodulins (Tmods). Tmods bind cooperatively to tropomyosin-decorated actin filaments and cap the minus end of actin filaments thereby inhibiting their disassembly. It is likely because of this attenuation of pointed end dynamics and/or due to their actin monomer binding activity, that tropomodulins exhibit negative effects on cell motility.[118] Of the four members of this protein family expressed in vertebrates, three members (Tmod1–3) are expressed in neurons.[119] A direct role for tropomodulins as negative regulators of neurite formation was illustrated with knockodown studies in N2A cells whereby neurite formation and length was increased following knockdown of Tmod1 and 2, respectively.[119] Overexpression of Tmod mutants in PC12 cells showed that alteration of Tmod1 interactions with tropomyosin isoforms is critical for neurite outgrowth.[120] These data indicate that tropomodulin regulates the early phases of neurite outgrowth by modulating the stability of actin pointed ends.

In addition to proteins that directly regulate the kinetics of actin turnover, other ABPs influence actin architecture, dynamics and viscoelasticity by cross-linking actin filaments. These "organizational" ABPs help determine the arrangement of actin filaments into higher order networks or superstructures. Generally speaking, there is a correlative effect on molecular mass and the type of actin network generated by ABPs such that smaller cross-linkers organize F-actin into parallel bundle while larger ABPs tend to promote branched filament networks.[121] For example smaller molecules such as fascin, fimbrin and epsin cross link actin filaments into tight bundles such as those found in filopodia whereas larger proteins such as filamin and α-actinin support gel-like (i.e., meshwork) filament networks. Depending on concentration and the presence of other ABPs, cross linkers can also modify actin turnover dynamics. For example, fascin acts synergistically with cofilin to promote actin filament severing in filopodia.[122]

In summary, specific repertoires of actin regulating proteins are engaged in a spatio-temporal manner to form and regulate the distinct actin structures that drive the protrusion of the leading edge membrane and the formation of nascent growth cones. This involves the active assembly of actin filaments at the membrane, retrograde flow and disassembly of these actin structures. Since actin nucleation is initiated at the membrane and the initial vector of actin growth is perpendicular to the leading edge of the cell, any actin nucleator should be sufficient to generate neurites, as long as other ABPs are present to generate or maintain the radial organization and retrograde flow. If this supposition is true, then the fact that the ablation of mDia1 and mDia3 has no effect on neuritogenesis in the cortex is not surprising,[97] as the Arp2/3 complex, other formin domain containing proteins and tandem actin nucleators are also present. Regulators of actin polymerization, such as Ena/Vasp proteins, and disassembly, such as ADF/cofilin proteins, are crucial for the organization and dynamics of these radially oriented actin filaments. Tethering these actin bundles to the substratum may be a mechanism to drive the protrusion of neurites, but this remains to be demonstrated conclusively. Importantly, at the leading edge membrane deformations mediated by BAR proteins also help define where the actin can push the membrane forward. From the data thus far, the most accurate conclusion is that neurons can use different means to achieve dynamic radially-oriented actin filament bundles such as those in filopodia. It is still questionable if filopodia themselves, as thin protrusions extending beyond the bundles of actin protruding from the leading edge are essential or if having underlying radially-oriented actin filament bundles is sufficient to facilitate leading edge protrusion. The formation and initial protrusion of the growth cone is just the first step in neurite formation. The extension and consolidation of the neurite requires microtubules.

Building a Neurite—Microtubules

The core of a cylindrical neurite is defined by its microtubules. In addition to their roles as the superhighways of intracellular trafficking, distinct patterns of microtubule organization and dynamics are essential to nearly all aspects of morphogenesis including neurite initiation.[123] In the first phases of neuritogenesis, microtubules become reconfigured from disordered radial orientations, with microtubules emanating out of the centrosome, into parallel bundles which protrude out of the neuronal sphere.[22] Without a core of bundled microtubules, there is

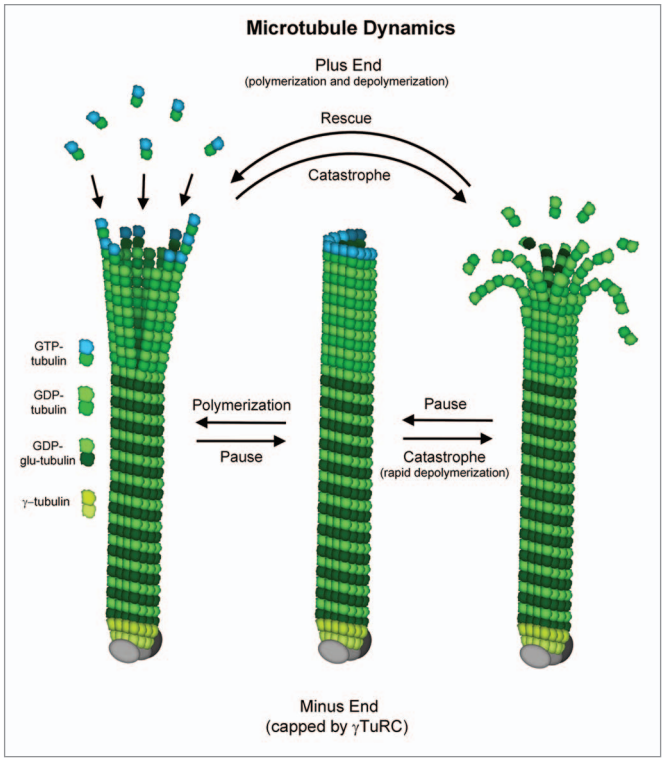

Figure 4. (Continued from page 11) Microtubules are inherently polarized polymers with a "plus" end a "minus" end. In vitro, isolated microtubules are surprisingly similar to actin filaments, with the minus ends exhibiting overall tubulin dimer loss or depolymerization and the plus ends showing net dimer addition and growth. Since the majority of minus ends are "capped" in vivo, thedynamics at the plus end are illustrated here . It is important to note that in contrast to other somatic cells, that neurons contain many microtubules that are not anchored to the centrosome, but are "free" throughout the cytoplasm which are funneled into microtubule bundles in the neurite processes 219. However, in the context of neuritogenesis, the majority of microtubule nucleation occurs at the centrosome, as observed with microtubule repolymerization assays following a washout with the microtubule depolymerizing drug nocodazole220. After the rate-limiting nucleation phase, which is supported by MTOCs in vivo, and rapid polymerization of microtubules, there is a steady-state phase of microtubule dynamics marked by the dynamic properties important for neurite extension124. At steady-state, the plus ends of microtubules undergo phases of growth and rapid depolymerization known as dynamic instability132. Similar to actin, the dynamic behavior of microtubules is dependent of status of the nucleotide bound to the tubulin dimer (β-tubulin subunit), but in this case the nucleotide is guanosine. When local concentrations are high, guanosine triphosphate (GTP) bound β-tubulin is added to the growing plus ends of microtubules. Immediately following polymerization, the GTP is hydrolyzed to GDP. This results in a plus end GTP-tubulin cap on microtubule otherwise consisting of GDP-tubulin dimers. The GTP tubulin provides stability to the plus end, allowing the microtubules to grow. When the supply of GTP-tubulin dimers is limited and polymerization is slower than GTP hydrolysis, the GTP cap is lost and microtubule plus ends become highly unstable and depolymerize rapidly in a process coined a catastrophe. Catastrophes can be "rescued" as GTP-tubulin heterodimers become available and microtubule growth reinitiated. Thus, microtubules are typically in a dynamic state of growth or shrinkage. In cells, microtubules also exhibit pausing behavior, which can be due to stabilization from structural MAPs221 or result from plus end "capture" at the cortex of the cell 222 or peripheral actin structures and filopodia223. The capture of microtubules at the cell cortex or in spatially defined regions of the growth cone is also associated with stimulating cell polarity and growth cone turning, respectively222, 224. The conversion of polymerization, pausing, and catastrophe phases of microtubule instability occur constantly in neurons. Since many microtubule binding proteins and other biochemical and biophysical cues impact these dynamics, a complex picture arises from the potential regulation of microtubules during neuronal morphogenesis. This is certainly true at the early stages of neurite initiation during which the regulation of microtubule dynamics and organization is essential for building the core of the neurite.

simply no neurite. But how is the extended microtubule lattice constructed out of the spherical cell body to build up the neurite shaft? Why are neurons special in their ability to extend neurites? The answer to these questions lies in the repertoire of microtubule binding proteins expressed in neurons and how they modify microtubule organization and dynamics. Microtubule isoform expression patterns, regulation of microtubule dynamics, and several important microtubule binding proteins (MBPs) that influence neuronal morphogenesis are discussed below.

Microtubules are cylindrical polymers constructed of 13 protofilaments with a diameter of 25 nm, each composed of α- and β- (α β) tubulin heterodimers (**Fig.** 4). A third isoform, γ-tubulin, is associated with microtubule organizing centers (MTOCs), such as the centrosome, and forms the γ-tubulin ring complex (γTuRC), which serves as a template for the nucleation of microtubules constructed from α,β tubulin dimers.[124] There are multiple α- (six) and β-tubulin (seven) isotypes expressed in mammals, with notable sequence differences in the C-terminal 15 amino acids. These differences are conserved across species, suggesting functional significance. Indeed, tubulin isoforms exhibit differences in vitro and in post-translational modifications, which mostly occur within these C-terminal residues.[125] In

mammals, tubulin expression patterns are complex; for example, in the brain, various α- and β- isoforms are expressed at different levels and at different times but with significant overlap.[126] Thus any single microtubule may contain heterodimers consisting of α- and β-I, β-II, β-III or β-IV. Only one isoform, β-III tubulin, shows almost exclusive neuronal expression which is high during development but decreases in adulthood. The heterodimer composition may also be important for microtubule dynamics, as microtubules composed of different heterodimers display different dynamics in vitro.[127] For example, microtubules enriched with β-III tubulin are more stable than those of other β-tubulin isotypes, suggesting functional importance in neuronal development.[128] Moreover, the expression of β-III tubulin is one of the earliest markers for post-mitotic neurons and coincides with neuritogenesis.[126] Recent studies suggest that mutations in different tubulin isoforms can cause similar neurodevelopmental disorders in humans including microcephaly and lissencephaly.[129,130] However, function altering mutations in β-III tubulin, which are distinct from other tubulin mutations described thus far, are more specific for axon guidance-related diseases such as congenital fibrosis of the extraocular muscles type 3.[131] Nevertheless, it is still unclear if β-III tubulin, or any of the other isoforms serve specific functions in neuritogenesis.

Microtubules share with actin filaments the property of being a polarized polymer with a "plus" end and a "minus" end (**Fig. 4**). Since the minus ends of microtubules are typically capped in cells, either by the γTuRC at the centrosome or by hitherto unknown minus end capping proteins, most of the interesting dynamics relevant for neurite growth occurs at the plus ends, which undergo phases of growth, rapid disassembly, and pausing. These phases of growth and rapid depolymerization are known as dynamic instability[132] and have important consequences for neuronal morphogenesis. It allows microtubules to rapidly reorganize when presented with biochemical or physical cues, generate pushing forces during polymerization phases or render microtubules unable to resist compressive actin-based forces during depolymerization phases which can lead to neurite retraction[28,124,133,134]. The dynamic behavior of microtubules is dependent on the status of the guanosine nucleotide bound to the β-tubulin subunit of the tubulin dimer. Guanosine triphosphate (GTP) bound β-tubulin subunits are added to the growing plus ends of microtubules. Immediately following polymerization, the GTP is hydrolyzed to GDP which is non-exchangeable on the microtubule resulting in a GTP-tubulin cap on the plus end of growing microtubules. The GTP tubulin provides stability to the plus end, allowing the microtubules to grow. When the GTP cap is lost, microtubule plus ends become highly unstable and depolymerize rapidly in a process coined a catastrophe[132] (**Fig. 4**). The state of the bound guanosine nucleotide on the tubulin dimer is also important for the binding of MBPs. For example, the plus-end tracking proteins protein, EB1 binds preferentially to GTP-tubulin, thus explaining its ability to track plus ends.[135]

As neurites emerge from the cell body, microtubules need to assume different characteristics in different regions of the neuron. In the soma, microtubules splay out from the centrosome in all directions and are relatively stable to accommodate the growing need for microtubule based transport from ER and golgi. In the developing neurites, microtubules coalesce together to form bundles which make up the neurite shaft (**Fig. 2**). Neurite microtubules are also more stable, with stability increasing as neurons grow and become polarized.[28] Studies focused on axonogenesis suggested that microtubules exert a "pushing" force that facilitates the rapid growth of the axon.[28] Microtubules can theoretically produce force by concerted polymerization and/or by microtubule motors such as kinesin or dynein. In neuroblastoma cells and stage 1 neurons, actin depolymerization induces accelerated neurite formation and the protrusion of growing microtubules out of the neuronal sphere[22,23], suggesting some force exerted by the microtubules is sufficient to induce neurite initiation. Low levels of the microtubule stabilizing drug, taxol redistributes polymerizing microtubules to the tips of growing neurites, and this is correlated with increased axon extension.[28] However, at least in primary pyramidal neurons, these doses of taxol do not augment neurite formation.[23] Reducing microtubule polymerization with low doses of nocodazole does inhibit neuritogenesis[23,25], which together with the taxol data suggests that microtubule polymerization is necessary for generating neurites, but the force generated by polymerization alone is not sufficient to initiate neurite formation. The microtubule minus end motor, dynein, may induce neurites by directly applying force on microtubules to push against the cell cortex.[136] In this study, microtubule repolymerization assays were used to observe that newly formed microtubule bundles were transported uni-directionally from the center of soma radially to the cell periphery and this delivery correlated with neurite initiation in neuroblastoma cells. Inhibition of dynein with function blocking antibodies impeded this transport and RNAi-mediated knockdown of dynein inhibited the formation of new neurites, suggesting that a dynein mediated force may "push" microtubule bundles against the cell cortex and/or counteract actin retrograde flow to help induce neuritogenesis[136,137] Although the authors suggest that dynein propels microtubule bundles to the periphery via a "cortical microtubule" gliding mechanism, other work offers an alternative explanation, whereby dynein may directly tether and stabilize microtubule to the cell cortex at orthogonal angles and generate an anaphase-like force.[138,139]

Another interesting observation from the study by Dehmelt and colleagues[136] was the formation of short polymerized microtubules that were transported radially in the soma. During neuronal morphogenesis, the microtubule severing protein, katanin, has been postulated to sever centrosome nucleated microtubules, which are then transported into growing neurites[124,140]. This katanin-based mechanism may aid in delivering microtubules to sites of newly forming neurites. Expression of a dominant-negative katanin reduces axon growth,[141] while moderately increasing katanin activity increases neurite number.[140] It is likely that the level of microtubule severing needs to be tightly regulated during neurite growth since excessive increases of katanin activity is also deleterious to neurite growth.[141]

The dynamic properties of the microtubules can be modulated by posttranslational modifications to the tubulin heterodimers and by various microtubule binding proteins that affect

dynamics at the plus end of the microtubule or affect stability along the microtubule lattice.[125] Microtubules can be modified by an ever expanding list that includes detyrosination, glutamylation, glycylation, phosphorylation and acetylation. These modifications can affect microtubule stability, the binding of structural MBPs, which influences microtubule bundling, the binding of +TIPs, and the recruitment of microtubule motors. For example, α-tubulin is synthesized with a tyrosine at the C-terminus and the presence of the tyrosine promotes the binding of certain members of the plus-end tracking proteins (+TIPs), such as Clip 170/115 and p150 Glued.[142] This, in turn, can affect microtubule plus end dynamics and interactions with the actin cytoskeleton (see below). The α-tubulin C-terminal tyrosine is removed in intact microtubules by a carboxypeptidase activity such that long lived microtubules have the penultimate residue (glutamate) exposed. Microtubule acetylation is another prominent modification that occurs on stable microtubules in neurons and may influence the binding of microtubule motors.[143]

Microtubule modifications may be important during neuritogenesis. Immunostaining experiments have indicated that the induction of neurite formation is correlated with microtubule detyrosination.[25] In support of this, neuronal development occurs more rapidly in neurons lacking tyrosine tubulin ligase (TTL), the enzyme that catalyzes tyrosine addition to the C-terminus of α-tubulin in the tubulin dimer, liberated after microtubule depolymerization.[144] In the brains of TTL KO mice, there are also complications with axon guidance, as the corpus callosum and commissures are malformed. Thus, the ability to re-tyrosinate the depolymerized tubulin subunits seem to aid in exploratory behavior of microtubules necessary for axon guidance, but is not essential to neurite initiation. Other work suggests that the control of microtubule acetylation may also regulate neuritogenesis as the expression of the microtubule binding protein DDA3 prevents acetylation and inhibits neurite initiation.[145]

An ensemble of microtubule-binding proteins influences microtubule organization and dynamics in neurons[3,124]. These can be subdivided into five broad classes: (1) microtubule stabilizing proteins, including the classical structural microtubule binding proteins which were originally coined microtubule associated proteins (MAPs), (2) plus-end tracking proteins (+TIPs), (3) tubulin dimer binding proteins, (4) microtubule motor proteins, and (5) microtubule severing proteins. Only a few major microtubule binding proteins that are particularly relevant for neuronal morphogenesis will be discussed below.

In vitro microtubule isolation by assembly/disassembly cycling led to the identification of "classical" MAPs that co-assembled with the microtubules. These MAPs consist of the distinct and unrelated Map1A/Map1B (type I) and Map2/Tau (type II) families of proteins which are both highly expressed in neurons.[146-148] Although structurally distinct, members of both families stabilize microtubule structure, facilitate cross-linking of microtubule bundles, influence the binding of other proteins to microtubules and act as molecular scaffolds for multiple proteins. These activities are principally important for neuronal morphogenesis, as neurons highly express a few specific MAPs that endow neurons with the ability to form tightly bundled, linear microtubule arrays. Indeed, some of the earliest markers for post-mitotic neurons are MAP isoforms,[149] suggesting a fundamental requirement for MAPs to begin neuronal morphogenesis. There are three main neuronal MAPs, Map1B, Map2 and Tau, which in spite of exhibiting some functional overlap, also have distinct localizations and slight differences in how they bind and bundle microtubules.[150] Although these proteins are highly expressed in neurons, they are nearly absent in other tissues.[148,150] It is likely for good reason, as their main activity is to form microtubule bundles, which provide the core of the neurite. Other somatic cells have no need for long arrays of bundled microtubules. The Map2/Tau family MAPs have repeating domains which allow them to bind to more than one tubulin dimer along the sides of microtubules, which likely facilitates their influence on microtubule stability. The expression of both type I and type II MAPs induce the formation of microtubule bundles in cells.[2,148] In vivo, Map1B, Map2 and Tau all regulate the spacing between microtubule bundles,[151,152] which may determine the size of the cargo that can be transported. In spite of these results, there is no direct evidence that type I and type II MAPs can bundle microtubules alone[147,148] suggesting that ancillary proteins may be needed to mediate microtubule cross-linking. Although MAPs bind along the sides of the microtubule lattice, they can also affect microtubule assembly and disassembly kinetics at the plus ends. For example, the binding of Map2 and Tau can prevent catastrophies[150] and Map1B can directly bind the +Tips EB1/3 and modulate their access to microtubule plus ends where they regulate microtubule growth.[153]

Map2 is particularly important for the earliest phases of neurite formation as suppressing its expression blocks the extension of neurites in cerebellar neurons, which remain in stage 1, whereas suppressing Tau expression only blocks axon extension.[154,155] Although another study alluded to the specific importance of the Map 2c isoform in neuritogenesis,[156] work from the Halpain lab confirmed the importance of Map 2c in mediating neurite formation by facilitating the extension of bundled microtubules out of the neuronal sphere, along F-actin bundles.[22] Genetic knockout studies have shown that the type I Map, Map1B can compensate for the loss of Map2[152] and Tau,[157] suggesting functional redundancy of Maps that promote microtubule bundling during neurite growth.

Other work contends, however, that the canonical type I and type II Maps may not be necessary for neuritogenesis themselves, but any factor that promotes microtubule bundling may facilitate neuritogenesis. A study of Cath.a-differentiated (CAD) cells, a mouse brain derived neuronal cell line, showed that neurite formation and outgrowth occurs as rapidly as in primary neurons.[158] Interestingly, these CAD cells do not express Maps normally associated with microtubule bundling in neurons such as Map1B, Map2, Tau, or doublecortin. The expression of LIS1 was the only Map capable of bundling microtubules expressed in CAD cells. The presence of other Maps capable of bundling microtubules such as Map1A and PRC1 were not examined. Furthermore, not all microtubule bundling proteins are necessarily beneficial for neurite formation. The overexpression of DDA3, which strongly bundles microtubules and interacts with EB3 and APC, causes

a marked reduction in neurite initiation and growth whereas its depletion causes the opposite in N2A cells and hippocampal neurons.[145] It is unclear, however, if the neuritogenesis-inhibiting effect of DDA3 is via its effect on microtubule bundling or on the posttranslational modifications of tubulin as DDA3 expression blocks detyrosination and acetylation.

Seminal drug studies have indicated that modulation of microtubule plus end assembly and disassembly kinetics is crucial for neurite extension[159,160] particularly at the distal tips of growing neurites.[161] Low concentration of the microtubule destabilizing drug, nocodazole, which disrupts microtubule polymerization but does not induce depolymerization[28,160], inhibits neurite formation indicating that plus-end microtubule growth is necessary for neuritogenesis[23,25]. Neuronal microtubule plus end assembly and disassembly can be regulated in multiple ways including the activity of microtubule plus end binding proteins that aid polymerization or facilitate catastrophies, and proteins that sequester free tubulin (destabilizing proteins) or help deliver it to plus ends.

Binding at the plus ends of microtubules, a large number of disparate plus end binding proteins (+ Tips) modify microtubule assembly and disassembly. These include the end binding proteins, EB1–3, cytoplasmic linker proteins, Clip170 and Clip115, dynactin large subunit p150^{glued}, adenomatous polyposis coli (APC), and CLIP-associating proteins (Clasps).[162] In addition to their effects on microtubule plus end dynamics, these proteins can impact microtubule stability, organization into bundles, and interactions with the peripheral actin cytoskeleton. The end binding proteins (EB1/3) are essential core element of the plus-end binding complex, as the binding of the other plus end tracking proteins seem to depend on a functional EB protein.[162] Interestingly, although EB proteins themselves promote microtubule nucleation and protofilament sheet closure, they also increase all aspects of dynamic instability including catastophies (depolymerization) and rescues (reestablishment of the GTP-tubulin cap and polymerization).[163] The overall effects of EB proteins in promoting microtubule polymerization may require a particular ensemble of proteins recruited to the plus ends.[164,165] These interactions are likely important during neurite growth.[166] EB-1 is highly expressed in early stages of neuronal development and its depletion reduces microtubule growth rate and persistence correlating with decreases in neurite outgrowth in neuronal cell lines.[166] EB-3 also may be important during neuritogenesis by regulating interactions between microtubules and actin (see below). Other +Tips may play modulator roles in microtubule growth during neuritogenesis, but their presence may not be absolutely necessary. For example, in TTL knockout mice, members of the cytoplasmic linker protein family, Clip 170/115, do not associate with microtubules, yet neurite growth occurs at even faster rates than in wild type mice.[144] Clips, adenomatous polyposis coli (APC) and Clasps have all been linked to the regulation of later stages of neuronal morphogenesis, including polarization and axonal branching but not to neuritogenesis per se.[167-169]

Other MBPs that localize to microtubule plus ends include Lis1 and DCX, two proteins linked with human neuronal migration disorders. Lissenencephaly is a human disease characterized by a severe disruption of cortical lamination.[170] Mutations in Lis 1 gene can lead to this developmental disorder. Lis1 is a MAP and a centrosome-associated protein influencing nucleokinesis and neurite growth, presumably via its interactions with dynein.[137] Deficiency of Lis1 in mice results in severely reduced neuronal migration and decreased neurite outgrowth and branching.[171] Lis1 may affect microtubule organization and dynamics during neuritogenesis via direct effects on microtubules or via interactions with other MBPs such as Clip170, Map1B, or DCX.[170] Another gene discovered to be mutated in X-linked forms of lissencephaly is doublecortin (DCX), a MBP with implications in neurite growth.[170] DCX localizes throughout the microtubule lattice but with particular enrichment near the plus ends. It is a multifunctional MBP promoting the nucleation, assembly, and stabilization of microtubules. During neuritogenesis it may be linked with the consolidation of the neurite shaft by facilitating the bundling of microtubules[172] (see below).

Microtubule dimer binding proteins include destabilizing proteins such as stathmin and polymerization promoting proteins such as CRMP2. The microtubule destabilizing factors, stathmin, superior cervical ganglia neural-specific 10 (SCG10), and SCG10-like protein (SCLIP) are highly expressed in nascent neurons, and represent a target of cellular signaling pathways to modulate microtubule dynamics in neurons.[173-176] Stathmin/SCG10 family of proteins can destabilize microtubules and increase catastrophes by sequestering free tubulin and preventing its association with microtubule plus ends or by associating with tubulin on microtubule plus ends.[177,178] It appears an intricate balance of stathmin/SCG10 expression/activity is necessary for optimal neurite growth since neurons depleted of SCG10 or highly overexpressing it show reduced neurite growth.[174,175] These results suggest that the cycle of dynamic instability is important to neurite formation and some level of microtubule catastrophe benefits the spatial and temporal dynamics of microtubules required for neurite growth. Regulation of catastrophe frequency and rescue also may be crucial to allow microtubules to reorient toward a more appropriate site for microtubule growth and neurite initiation. In support of this, when SCG10 function is impaired, there is a reduction of neurite extension and increased looping microtubules,[174] which may signify inappropriate microtubule growth patterns. Although generally termed "destabilizing factors", SCG10, stathmin and SCLIP may have different effects on microtubule growth and neuronal morphogenenesis.[179,180] For example, in contrast to stathmin, under certain conditions SCG10 may actually promote microtubule stability and tubulin polymerization to regulate neuronal development.[181]

Other free heterotubulin dimer binding proteins act to enhance microtubule assembly. Collapsin-response mediator protein-2 (CRMP-2) was shown to increase microtubule assembly by binding free tubulin dimers and "delivering" them to the plus ends of microtubules.[182] CRMP-2 was first identified for mediating semaphorin-mediated growth cone collapse[183] and later shown to promote axonogenesis.[184] During development, CRMP-2 is highly expressed in the brain and localizes mainly to growth cones. It is inhibited by GSK 3β-mediated phosphorylation, which prevents its binding to tubulin dimers.[185] Indeed,

the expression of a phosphorylation resistant CRMP-2 mutant has even more robust effects on neurite growth than wild-type CRMP-2. Thus, the regulation of CRMP-2 activity likely promotes neurite extension by promoting microtubule assembly at their plus ends in developing growth cones.

In addition to their roles as long-range transporters, microtubule motors also serve other important functions such as the positioning and organization of microtubules themselves, microtubule stability, and the interaction of microtubules with the cell cortex. All of these activities could impinge upon neuronal development. The traditional "transport" motors include the plus end-directed kinesins (e.g., Kinesin 1 and 2) as well as the minus end-directed dynein motor which forms a complex with dynactin.[186] Microtubule-based transport of organelles and other cargo is not an issue during the early phases of neuritogenesis, and most transport is simply diffusion based or utilizes actin-based mechanisms[1] (Flynn et al., in press) although movements of short microtubules by dynein may be utilized.[136] There are over 45 kinesins, some of which are involved in generating forces between microtubules (i.e., mitotic kinesins) and others that can actively depolymerize microtubules (i.e., KIF2A) and thus potentially generating changes in cell shape and motility.[187] KIF2A ablation in neurons results in excessive collateral branch growth and decreased microtubule depolymerization.[188] KIF2A is thought to use ATP hydrolysis for energy to depolymerize microtubules from their plus ends in growth cones.[124] Furthermore, certain kinesins and dynein-dynactin can link microtubules with actin, providing another mechanism to influence cell morphology.[189,190]

A final class of microtubule regulating proteins that can influence neuronal morphology is the microtubule severing MSP1/katanin/spastin subgroup of AAA family of proteins.[140,191] Although their exact mechanism of action is unknown, katanin is postulated to bind to the sides of microtubules and uses ATP hydrolysis to cause structural bending of the microtubule lattice and remove microtubule dimers eventually leading to severing.[192] Microtubule severing by spastin and katanin generate short microtubules, which can be reconfigured and transported more easily to respond to environmental signals and alter microtubule organization. It is also important to note that the presence of microtubule binding proteins such as Tau or specific posttranslational modifications such as acetylation and polyglutamylation can inhibit or facilitate microtubule severing by spastin and katanin, respectively.[192]

Overall, the data suggest that a balance of microtubule bundling, dynamics and stability is required for neuritogenesis. The neuron-specific expression of various microtubule bundling proteins, such as canonical type I (Map1B) and type II (Map2, Tau) Maps as well as DCX and LIS1, are indicative of a necessity to regulate the organization of microtubule bundles during neuronal morphogenesis. The seminal studies overexpressing Maps in non-neuronal cells showed that microtubule bundling factors (along with dynamic actin) were sufficient to induce neuritogenesis.[2,156] However, too much microtubule bundling and stabilization also seem refractory to neurite growth. As with many biological processes, balance is the key. This balance is achieved with an arsenal of microtubule binding proteins whereby the regulation of microtubule organization and dynamics during neurite initiation and growth becomes complex. Compensatory mechanisms and functional redundancy ensure that neurites can grow under a variety of situations. For example, in the absence of Map1B, Map2 and Tau, LIS1 and/or other MBPs may be sufficient for the microtubule organization in neurite-like processes of CAD cells.[158] The regulation of plus end dynamics by +Tips and microtubule dimer binding proteins also appear to be important for directing neurite formation. Dynamic instability, both catastrophies and rescue events, are important for microtubules to explore potential sites of neurite formation. This exploratory behavior may facilitate the proper targeting of growing microtubules to the actin at the cell cortex by +Tips (and other proteins) to designate and reaffirm sites for neurite initiation. This will be discussed in more detail below, with an emphasis on how the linkages between microtubules and actin serve to guide microtubule growth to sites of neurite formation.

Putting the Neurite Together: Actin and Microtubule Interactions

The coordination of the growth of microtubule bundles and an advancing actin-driven leading edge is essential for neurite initiation. This coordination is regulated on multiple levels. First, cell signaling pathways, such as those mediated by the Rho GTPases, diverge and impinge upon both ABPs and MBPs.[193] For example, the Rac1 can mediate divergent signals to coordinate increased actin dynamics via Wave complex and facilitate microtubule polymerization by inhibiting stathmin. Second, microtubules and actin can physically interact via protein complexes that tether these cytoskeletal elements together.[194] In neurons, this was shown with elegant imaging experiments while treating with cytoskeleton-disrupting drugs. When the F-actin in growth cones was depolymerized, microtubules, which are normally confined to the central domain, extend out into the peripheral domain in an uncontrolled fashion all the way up to the leading edge.[195] These observations lead to the idea that actin filaments constrain the growth of microtubules, confining them to the central domain of growth cones. Later work extended these findings showing that microtubules penetrating into the peripheral domain of growth cones can bend, buckle and even depolymerize when caught in actin retrograde flow.[196] The coupling of the actin retrograde flow to the substratum can also induce changes in microtubule organization. When the coupling is strong and retrograde flow is attenuated, corridors of actin free zones facilitate the growth of microtubules further into the periphery of growth cones.[101] These types of interactions have been proposed to be important for axon growth and guidance and even during the early stages of neurite formation.[3,25,123]

Live cell imaging of neuroblastoma cells clearly shows that microtubules grow out along F-actin bundles at sites of neurite formation,[22] a finding confirmed in primary hippocampal and cortical neurons[23,25] (**Fig. 5**). There are essentially two modes of neurite formation involving the coordination of actin and microtubules: 1. Neurites form as a broad F-actin based lamellipodia increases its dynamics and advances away from the cell

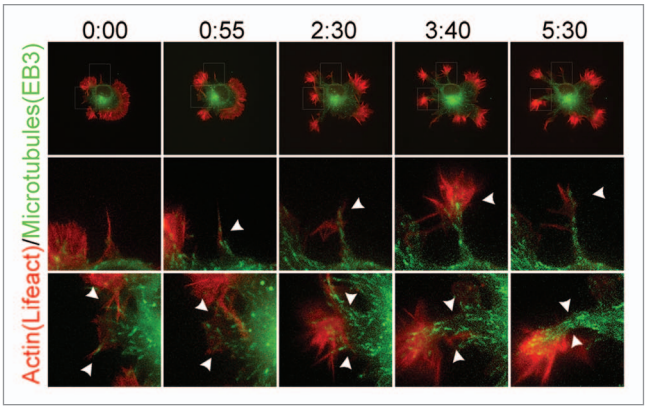

Figure 5. Actin and microtubule organization during neuritogenesis as observed with live-cell imaging. Single frames from a live-cell imaging series are shown of a neuron expressing Lifeact (labels F-actin) and EB3 (labels growing microtubule plus ends). Time is indicated above the images in hours:minutes. The lower two rows of panels are magnified views of the indicated regions from the top row. The first frame shows a neuron in stage 1 with broad, circumferential lamellipodia and filopodia. As neuritogenesis commences, a stable filopodium extends, becomes engorged with microtubules, develops a growth cone and begins to transform into a neurite (middle panels, white arrowheads). Concomitantly, broad lamellipodia segment and begin extending away from the cell body to form nascent neurites. Initially discrete microtubules follow the advancing actin and begin to compact into bundles (lower panels; white arrowheads).

body while polymerizing microtubules follow and bundle to stabilize the neurite shaft, and 2. Stable filopodia become engorged with microtubules, distending the filopodial structure which then develops a growth cone and becomes a neurite.[23] Dent et al., showed that Ena/Mena/Vasp ablation caused a loss of filopodia and radial actin bundles in stage 1 neurons and this in turn caused abnormal curving trajectories of microtubules.[25] In ADF/cofilin knockout neurons the abnormal composition, aggregation and inertia of actin filaments not only caused abnormal looping microtubule trajectories, but also decreased microtubule plus end velocity in the periphery of the neurons.[23] Since actin destabilization with latrunculin reverses this effect and increases microtubule plus end velocity, the abnormal actin network likely caused the perturbation in microtubule growth patterns. From these studies, it is clear that microtubule-actin interactions are regulated early in neuronal development and this interaction is important to neurite initiation.

What proteins/protein complexes mediate these interactions during neuritogenesis? Thus far, a few potential ones have been identified. The microtubule +Tip protein EB3 forms a complex with the actin binding protein drebrin during neuritogenesis.[197] EB3-drebrin complex promoted microtubule penetration into filopodia and the suppression of EB3 and drebrin reduced growth cone formation and neurite outgrowth. The structural MAPs, Map1B and Map2, can also potentially mediate direct actin-microtubule linkages since these proteins have microtubule and F-actin binding domains, can bind F-actin in vitro while in some cases induce F-actin bundling, and localize to microtubules and actin in non-neuronal cells.[123] Map2c expression induces growth of microtubules along F-actin bundles thereby promoting neurite formation.[22] Members of the Plakin family, especially the spektraplakin subgroup, represent a novel class of adaptor proteins that can bind many proteins including actin, microtubules, and components of focal adhesions.[198] Spektraplakins have been proposed to influence neurite growth by acting both as structural MAPs and as +Tips, helping stabilize microtubules and guide their growth along F-actin, respectively.[199] The +Tip, Clip170 can interact with the IQ-motif containing GTPase activating protein (IQGAP) to transiently capture microtubule plus ends at the actin cortex and induce polarization in non-neuronal cells.[200] In neurons, it was recently shown that mTOR mediates the interaction of Clip170 with IQGAP to promote dendritic growth and complexity and this effect is mediated via actin dynamics.[201] Another potential mediator of microtubule-actin linkages is a complex formed by Lis1, dynein and dynactin. In growth cones, interfering with this complex attenuates axon growth and microtubule advance into the periphery of the growth cone. Furthermore, microtubules are less able to resist actin retrograde flow, suggesting an alteration of actin-microtubule linkages.[137] This could be due to direct interactions of Lis1-dynein-dynactin with actin filaments[138,202]or an alteration of dynein-mediated force production counteracting actin retrograde flow. The force elicited by dynein may also be necessary to counteract the tendency for myosin II contractility in the growth cone to induce neurite retraction; the balance of these forces may be crucial to appropriately guide neurite outgrowth.[133,136] It remains unknown if Clip170-IQGAP or Lis1-dynein interactions are important for neurite initiation.

As the growth cone advances away from the soma, the contiguous membrane must contract and consolidate the neurite shaft. This involves both the contraction of the cell cortex and the tethering of splayed microtubules together into bundles. The control of the consolidation of a protruding lamellopodium into a neurite shaft involves both the action of myosin II on the actin network and the phophorylation-dependent interaction of doublecortin (DCX) with microtubules at the "neck" of the neurite. Microtubules are closely aligned with myosin II-decorated actin filaments at the growth cone neck. Contractile forces generated by myosin II compress laterally oriented actin arcs toward the center of the neurite shaft and this corrals microtubules together promoting the formation of bundles at the neck of the growth cone.[203] Inhibition of myosin II with blebbistatin blocks this bundling, and microtubules become more splayed at the growth cone neck. At the same time, a sphinophilin-protein phosphatase 1 complex mediates the dephosphorylation of DCX at the growth cone neck, thereby increasing its interaction with microtubules enhancing the stability, the bundling of microtubules and, perhaps linking to actin via dynein or other adaptors.[172] Although these studies were performed in neurons that already had neurites, it is likely that these same mechanisms are employed during neurite consolidation. In filopodia-mediated neurite formation, there is no consolidation phase required to form the initial shaft since this is present as the filopodium into which microtubules have penetrated, but consolidation occurs at the base of the growth cone as it extends away from the soma.

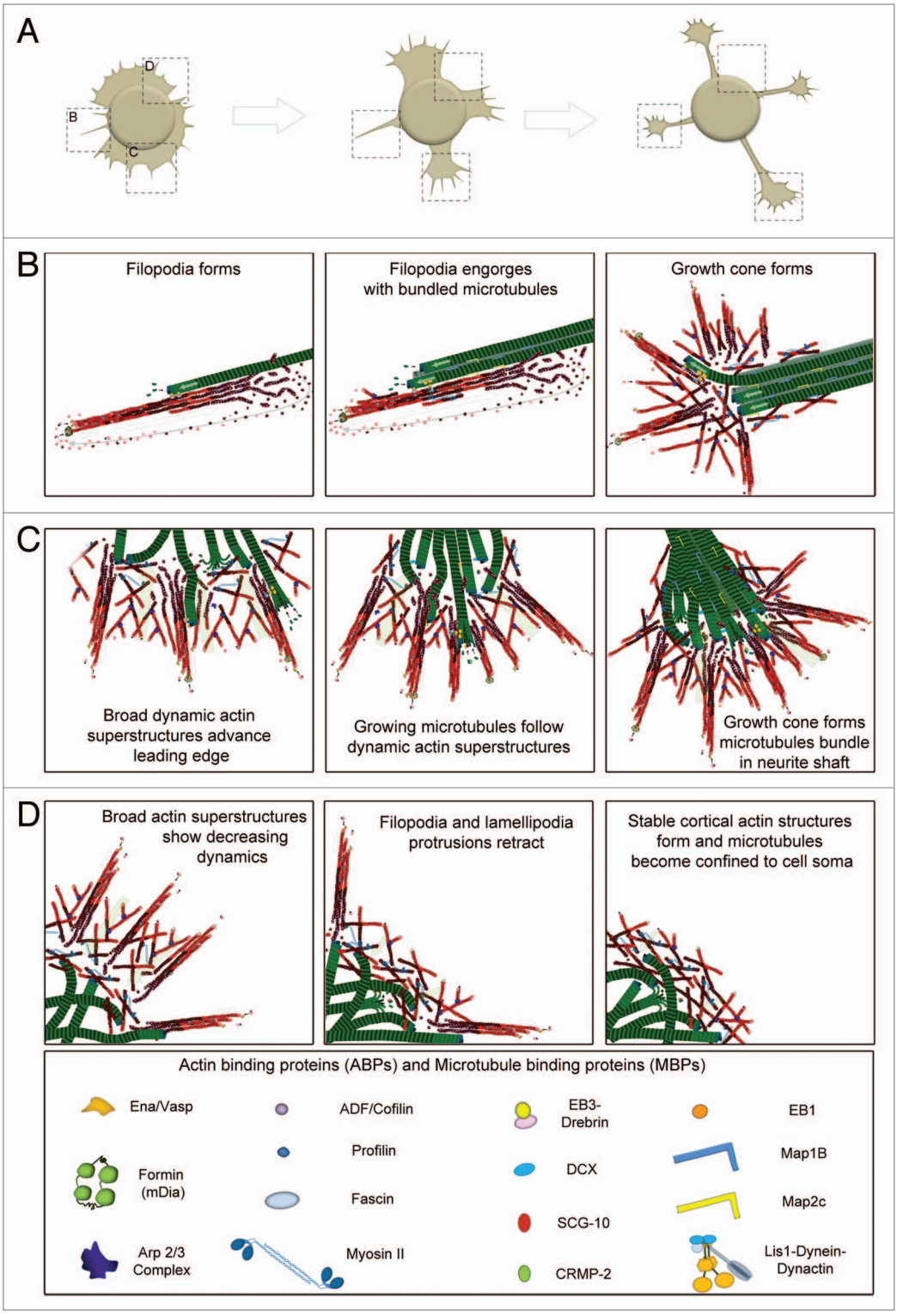

Figure 6. For figure legend see page 103.

After a neurite forms, the cortical actin may act to support microtubule bundles. A recent study using high resolution microscopy showed that actin organizes into periodic actin-spectrin ring structures encircling the axon shaft at regular (180–190nm) intervals along its length.[204] In minor neurites there is also cortical actin structure, but with less elegant organization. In cultured Cos7 cells and cardiac myocytes, the lateral interaction of microtubules with the actin network reinforces individual microtubules allowing them to bear higher compressive loads.[205] In neurites, it is probable that cortical actin-microtubule

Figure 6 (See previous page). The cytoskeletal mechanisms of neuritogenesis. (A) An overview of neuritogenesis is shown depicting the major morphological changes that occur as neurites extend out of the cell body. A broad membrane with lamellipodia and filopodia can extend away from the cell body as contiguous regions collapse and become inactive. As the lamellipodia protrudes forward, individual microtubules grow radially into the periphery along F-actin bundles. The neurite is formed as the microtubules become progressively bundled and the peripheral actin superstructures continues advancing and becomes a growth cone. Filopodia can also become stable, engorge with microtubules, develop a growth cone and transform into a neurite. (B-D) The highlighted areas in (A) are magnified to point to regions, in which changes in F-actin (red tones) and microtubules (green) occur during neuritogenesis. Some of the major actin binding proteins (ABPs) and microtubule binding proteins (MBPs) regulating these structural changes are also depicted. (B) Filopodia consist of radial actin bundles oriented with their barbed ends (growing) toward the membrane. Actin regulators such as formins and Cordon blue may help regulate the formation of F-actin in the filopodia. Ena/Vasp proteins also regulate the polymerization of actin in filopodia while ADF/cofilin disassembles the actin by severing ADP-actin near the pointed ends of the filaments. The polymerization of actin against the membrane and disassembly of actin further away from the membrane help drive retrograde flow, which can couple to the substratum to allow more protrusion. Actin monomers are also required for further polymerization and profilin can interact with actin monomers to enhance polymerization. Crosslinking proteins such as fascin may help stabilize filopodia. Microtubules can grow along these F-actin bundles and invade the filopodia. Protein complexes such as EB3-drebrin, Lis1-dynein, or Map2c may aid in this process. +Tip proteins, SCG10, and CRMP2 may all aid in regulating microtubule growth while Maps such as Map2c or Map1B bundle microtubules together. As the neurite forms more microtubules populate the filopodia and actin becomes dynamic building a growth cone at the tip. (C) The broad advance of a growth cone marks the second means to generate a neurite. This advancing structure contains both lamellipodia and filopodial actin. Therefore, actin nucleators like Arp2/3 complex, formins, and cordon blue are likely involved in actin filament growth. As in filopodia, membrane advance is driven by actin polymerization at the leading edge, but with the coordinated assembly of a multitude of actin filaments. ABPs such as Ena/Vasp and profilin help polymerize actin while ADF/cofilin still drives disassembly toward the minus ends. Myosin II also contributes to retrograde flow and drives the compression of actin into arcs proximally, which can provide obstacles for microtubule growth. Splayed microtubules occasionally grow into the peripheral zone, often along actin filaments. Protein complexes such as Lis1/dynein/dynactin may help these microtubules resist retrograde flow and maintain their presence in the periphery. +Tips (EB1), SCG10 and CRMP2 regulate the plus end dynamics of the growing microtubules. As neurite formation occurs, microtubules become progressively more bundled and the coordination of multiple MBPs helps in this. MBPs such as DCX, Map1B, and Map2c all likely participate in bundling microtubules as the neurite forms. Myosin II also aids in the compression of adjoining actin to consolidate the formation of the neurite. (D) As the neurites form at distinct locales, the other regions around the soma develop different cytoskeleton structures and become quiescent. The actin becomes less dynamic with a collapse of filopodia and lamellipodia forming into stable cortical actin while microtubule advance is restrained, only occurring in a looping pattern within the soma.

interactions may also be essential for reinforcing microtubule bundles and maintaining the neurite's cylindrical structure.

Concluding Remarks and Perspectives for Neuritogenesis

Although our understanding of neuritogenesis is still incomplete, we can summarize some of the steps that transform a spherical cell into a neuron (**Fig. 6**). Dynamic, radially oriented F-actin superstructures reorganize the periphery and push the membrane forward powered by actin polymerization facilitated by Ena/Vasp proteins and the actin retrograde flow "engine" powered by the action of ADF/cofilin proteins and myosin II. Membrane deformations and actin linkages regulated by F-Bar proteins influence sites of neurite initiation. As the actin retrograde flow is coupled to the substrate at focal contacts, membrane protrusion occurs more efficiently (although this remains to be definitively shown during neuritogenesis). Concomitantly, dynamic microtubules grow along F-actin tracks in a radial fashion and follow the "lead" of the advancing actin network which begins forming the nascent growth cone. The neurite is consolidated as the contiguous membrane contracts with myosin II activity compressing actin arcs and corralling the advancing microtubules closer together. As the microtubules become more tightly packed, microtubule binding proteins such as Lis-1, DCX, and Map2c help bundle the microtubules. Stable filopodia also can give rise to a neurite when it becomes progressively engorged with bundled microtubules and then develops a growth cone. As neurites extend, microtubules continue to bundle and grow in a coordinated manner. Although neurite extension can occur simply through a microtubule elongation process[206] such elongating neurites fail to follow pathfinding cues either in vitro[207] or in vivo.[208] Thus, the coordinated dynamics of actin and microtubules is required for physiological neuritogenesis and guidance.

Although the basic cytoskeletal reorganization events for neuritogenesis have been thoroughly characterized in culture, their physiological relevance to neuritogenesis in vivo largely remains to be determined. This review extrapolated liberally from the cell biology literature to postulate the possible roles of many different proteins in neurite formation. These need to be verified in vivo, as does the exact progression of steps for the morphological changes leading to neurite initiation. In recent years, many proteins anticipated to play fundamental roles in actin organization and dynamics have turned out to seemingly play little if any role in neuronal morphogenesis. For example, mice with genetic knockouts of the cross-linkers fascin and filamin show no perturbations in neurite formation.[209,210] The ablation of the major formin isoforms expressed in the brain likewise only affects interneuron migration, while other neurons develop completely normally.[97] Most likely, these results do not indicate that the protein in question plays no role but rather that the role it plays is so fundamental in development that functional redundancy of ABPs can compensate for the loss of any single one of the proteins. Thus, the roles of different ABPs during neurite formation need to be carefully explored in vivo, probably using a conditional multiple knock out strategy such as has been used for ADF/cofilin.[23]

It is clear from a few recent studies that when actin and microtubule dynamics become uncoupled neuritogenesis is attenuated[23,25]. However, it is unknown what proteins/protein complexes are key to guiding the advancing microtubules to the actin bundles in developing neurites. As alluded to throughout this review, the interactions between actin binding proteins are

essential to determining the overall effect on actin dynamics and organization. This can entail collaboration or competition. The levels and activities of a milieu of ABPs orchestrate the formation and turnover of different actin superstructures[53,211]. Recent work has begun to examine the interactions of different ABPs in vitro and in cells, but little is known how different ABPs collaborate and compete to drive specific changes in actin organization during neuritogenesis. For example, in the absence of ADF/cofilin, radially oriented actin bundles and filopodia formation are attenuated, dense networks of circumferential actin are increased, and neurite formation is largely inhibited.[23] Since ADF/cofilin can compete with tropomyosins,[212] myosin II,[103] and Arp2/3 complex,[213] the question remains if some of the perturbations in neuronal morphogenesis are due to altered activities of other actin binding proteins.

Beyond the cytoskeleton, it is unclear if specialized membrane domains specify sites of neurite initiation in the spherical cell body of a new born neuron. F-Bar recruitment to the membrane may be one mechanism to segregate sites for neurite initiation (or inhibition), but how are F-Bar proteins and other actin regulators recruited to the membrane? Phosphoinosides, such as phosphotidylinositol bis- and tri- phosphates, can directly recruit various actin regulating proteins, modulate their activity, and trigger signaling cascades,[214] locally affecting membrane deformation and leading edge protrusion which could play a directive role during neuritogenesis. Lipid rafts coalescence leads to amplification of signal transduction during cell polarization in Leukocytes[215] and CAM mediated neurite growth.[216] Lipid raft domain clustering and/or phosphoinositide enrichment may not only locally amplify extracellular signaling but also capture cytoskeletal machinery and thereby help direct neurite initiation at discrete regions of the neuron. Specifically, it will be interesting if and how membrane domains localize actin nucleators or aid in the capture of microtubules to determine sites of the future neurites.

Some of these questions are basic biological questions. Others are specific to neurons and neurite initiation.[217-224] But the answers will provide new insights about what distinguishes neurons from other cells in the brain (i.e., neurogenesis and neuronal identity), how aberrant neurite growth contributes to developmental diseases and even offer clues how to rebuild brain and spinal cord circuitry following disease or injury.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

Acknowledgments

I would like to thank Drs James R Bamburg, Sebastian Dupraz, and Claudia Laskowski for critically reading this manuscript. Gratitude is also extended to Frank Bradke, Farida Hellal, Sina Stern and Joerg Rueschel for helpful discussions. I gratefully acknowledge the German Center for Neurodegenerative Diseases and the Max Planck Society for financial support. Although I endeavored to include as much relevant information as possible, I was unable to include information on all potential players in the regulation of neuritogenesis due to space restrictions. Many important cytoskeletal regulators of later stages of neuronal morphogenesis have been left out in this article if no direct link to neurite initiation was previously demonstrated.

References

1. Gupton SL, Gertler FB. Integrin signaling switches the cytoskeletal and exocytic machinery that drives neuritogenesis. Dev Cell 2010; 18:725-36; PMID:20493807; http://dx.doi.org/10.1016/j.devcel.2010.02.017
2. Edson K, Weisshaar B, Matus A. Actin depolymerisation induces process formation on MAP2-transfected non-neuronal cells. Development 1993; 117:689-700; PMID:8392463
3. Dent EW, Gupton SL, Gertler FB. The growth cone cytoskeleton in axon outgrowth and guidance. Cold Spring Harb Perspect Biol 2011; 3:3; PMID:21106647; http://dx.doi.org/10.1101/cshperspect.a001800
4. Dotti CG, Sullivan CA, Banker GA. The establishment of polarity by hippocampal neurons in culture. J Neurosci 1988; 8:1454-68; PMID:3282038
5. Craig AM, Banker G. Neuronal polarity. Annu Rev Neurosci 1994; 17:267-310; PMID:8210176; http://dx.doi.org/10.1146/annurev.ne.17.030194.001411
6. Heidemann SR, Reynolds M, Ngo K, Lamoureux P. The culture of chick forebrain neurons. Methods Cell Biol 2003; 71:51-65; PMID:12884686; http://dx.doi.org/10.1016/S0091-679X(03)01004-5
7. Tahirovic S, Bradke F. Neuronal polarity. Cold Spring Harb Perspect Biol 2009; 1:a001644; PMID:20066106; http://dx.doi.org/10.1101/cshperspect.a001644
8. Bradke F, Dotti CG. Establishment of neuronal polarity: lessons from cultured hippocampal neurons. Curr Opin Neurobiol 2000; 10:574-81; PMID:11084319; http://dx.doi.org/10.1016/S0959-4388(00)00124-0
9. Stiess M, Bradke F. Neuronal polarization: the cytoskeleton leads the way. Dev Neurobiol 2011; 71:430-44; PMID:21557499; http://dx.doi.org/10.1002/dneu.20849
10. Georges PC, Hadzimichalis NM, Sweet ES, Firestein BL. The yin-yang of dendrite morphology: unity of actin and microtubules. Mol Neurobiol 2008; 38:270-84; PMID:18987787; http://dx.doi.org/10.1007/s12035-008-8046-8
11. Shirao T, González-Billault C. Actin filaments and microtubules in dendritic spines. J Neurochem 2013; 126:155-64; PMID:23692384; http://dx.doi.org/10.1111/jnc.12313
12. Noctor SC, Martínez-Cerdeño V, Ivic L, Kriegstein AR. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. Nat Neurosci 2004; 7:136-44; PMID:14703572; http://dx.doi.org/10.1038/nn1172
13. Hand R, Bortone D, Mattar P, Nguyen L, Heng JI, Guerrier S, Boutt E, Peters E, Barnes AP, Parras C, et al. Phosphorylation of Neurogenin2 specifies the migration properties and the dendritic morphology of pyramidal neurons in the neocortex. Neuron 2005; 48:45-62; PMID:16202708; http://dx.doi.org/10.1016/j.neuron.2005.08.032
14. Barnes AP, Polleux F. Establishment of axon-dendrite polarity in developing neurons. Annu Rev Neurosci 2009; 32:347-81; PMID:19400726; http://dx.doi.org/10.1146/annurev.neuro.31.060407.125536
15. Zolessi FR, Poggi L, Wilkinson CJ, Chien CB, Harris WA. Polarization and orientation of retinal ganglion cells in vivo. Neural Dev 2006; 1:2; PMID:17147778; http://dx.doi.org/10.1186/1749-8104-1-2
16. Tabata H, Nakajima K. Multipolar migration: the third mode of radial neuronal migration in the developing cerebral cortex. J Neurosci 2003; 23:9996-10001; PMID:14602813
17. Kwiatkowski AV, Rubinson DA, Dent EW, Edward van Veen J, Leslie JD, Zhang J, Mebane LM, Philippar U, Pinheiro EM, Burds AA, et al. Ena/VASP Is Required for neuritogenesis in the developing cortex. Neuron 2007; 56:441-55; PMID:17988629; http://dx.doi.org/10.1016/j.neuron.2007.09.008
18. Hand R, Polleux F. Neurogenin2 regulates the initial axon guidance of cortical pyramidal neurons projecting medially to the corpus callosum. Neural Dev 2011; 6:30; PMID:21864333; http://dx.doi.org/10.1186/1749-8104-6-30
19. Heng JI, Chariot A, Nguyen L. Molecular layers underlying cytoskeletal remodelling during cortical development. Trends Neurosci 2010; 33:38-47; PMID:19837469; http://dx.doi.org/10.1016/j.tins.2009.09.003
20. Lei WL, Xing SG, Deng CY, Ju XC, Jiang XY, Luo ZG. Laminin/β1 integrin signal triggers axon formation by promoting microtubule assembly and stabilization. Cell Res 2012; 22:954-72; PMID:22430151; http://dx.doi.org/10.1038/cr.2012.40
21. Goldberg DJ, Burmeister DW. Stages in axon formation: observations of growth of Aplysia axons in culture using video-enhanced contrast-differential interference contrast microscopy. J Cell Biol 1986; 103:1921-31; PMID:3782290; http://dx.doi.org/10.1083/jcb.103.5.1921
22. Dehmelt L, Smart FM, Ozer RS, Halpain S. The role of microtubule-associated protein 2c in the reorganization of microtubules and lamellipodia during neurite initiation. J Neurosci 2003; 23:9479-90; PMID:14573527

23. Flynn KC, Hellal F, Neukirchen D, Jacob S, Tahirovic S, Dupraz S, Stern S, Garvalov BK, Gurniak C, Shaw AE, et al. ADF/cofilin-mediated actin retrograde flow directs neurite formation in the developing brain. Neuron 2012; 76:1091-107; PMID:23259946; http://dx.doi.org/10.1016/j.neuron.2012.09.038
24. Smith CL. The initiation of neurite outgrowth by sympathetic neurons grown in vitro does not depend on assembly of microtubules. J Cell Biol 1994; 127:1407-18; PMID:7962099; http://dx.doi.org/10.1083/jcb.127.5.1407
25. Dent EW, Kwiatkowski AV, Mebane LM, Philippar U, Barzik M, Rubinson DA, Gupton S, Van Veen JE, Furman C, Zhang J, et al. Filopodia are required for cortical neurite initiation. Nat Cell Biol 2007; 9:1347-59; PMID:18026093; http://dx.doi.org/10.1038/ncb1654
26. Flynn KC, Pak CW, Shaw AE, Bradke F, Bamburg JR. Growth cone-like waves transport actin and promote axonogenesis and neurite branching. Dev Neurobiol 2009; 69:761-79; PMID:19513994; http://dx.doi.org/10.1002/dneu.20734
27. da Silva JS, Dotti CG. Breaking the neuronal sphere: regulation of the actin cytoskeleton in neuritogenesis. Nat Rev Neurosci 2002; 3:694-704; PMID:12209118; http://dx.doi.org/10.1038/nrn918
28. Witte H, Neukirchen D, Bradke F. Microtubule stabilization specifies initial neuronal polarization. J Cell Biol 2008; 180:619-32; PMID:18268107; http://dx.doi.org/10.1083/jcb.200707042
29. Dennerll TJ, Joshi HC, Steel VL, Buxbaum RE, Heidemann SR. Tension and compression in the cytoskeleton of PC-12 neurites. II: Quantitative measurements. J Cell Biol 1988; 107:665-74; PMID:3417767; http://dx.doi.org/10.1083/jcb.107.2.665
30. Fass JN, Odde DJ. Tensile force-dependent neurite elicitation via anti-beta1 integrin antibody-coated magnetic beads. Biophys J 2003; 85:623-36; PMID:12829516; http://dx.doi.org/10.1016/S0006-3495(03)74506-8
31. Lamoureux P, Ruthel G, Buxbaum RE, Heidemann SR. Mechanical tension can specify axonal fate in hippocampal neurons. J Cell Biol 2002; 159:499-508; PMID:12417580; http://dx.doi.org/10.1083/jcb.200207174
32. Yuan A, Rao MV, Veeranna, Nixon RA. Neurofilaments at a glance. J Cell Sci 2012; 125:3257-63; PMID:22956720; http://dx.doi.org/10.1242/jcs.104729
33. Pollard TD, Cooper JA. Actin, a central player in cell shape and movement. Science 2009; 326:1208-12; PMID:19965462; http://dx.doi.org/10.1126/science.1175862
34. Wegner A. Head to tail polymerization of actin. J Mol Biol 1976; 108:139-50; PMID:1003481; http://dx.doi.org/10.1016/S0022-2836(76)80100-3
35. Loisel TP, Boujemaa R, Pantaloni D, Carlier MF. Reconstitution of actin-based motility of Listeria and Shigella using pure proteins. Nature 1999; 401:613-6; PMID:10524632; http://dx.doi.org/10.1038/44183
36. Michelot A, Berro J, Guérin C, Boujemaa-Paterski R, Staiger CJ, Martiel JL, Blanchoin L. Actin-filament stochastic dynamics mediated by ADF/cofilin. Curr Biol 2007; 17:825-33; PMID:17493813; http://dx.doi.org/10.1016/j.cub.2007.04.037
37. Pak CW, Flynn KC, Bamburg JR. Actin-binding proteins take the reins in growth cones. Nat Rev Neurosci 2008; 9:136-47; PMID:18209731; http://dx.doi.org/10.1038/nrn2236
38. Choo QL, Bray D. Two forms of neuronal actin. J Neurochem 1978; 31:217-24; PMID:566783; http://dx.doi.org/10.1111/j.1471-4159.1978.tb12451.x
39. Pardee JD, Bamburg JR. Actin from embryonic chick brain. Isolation in high yield and comparison of biochemical properties with chicken muscle actin. Biochemistry 1979; 18:2245-52; PMID:444452; http://dx.doi.org/10.1021/bi00578a017
40. Marsick BM, Flynn KC, Santiago-Medina M, Bamburg JR, Letourneau PC. Activation of ADF/cofilin mediates attractive growth cone turning toward nerve growth factor and netrin-1. Dev Neurobiol 2010; 70:565-88; PMID:20506164; http://dx.doi.org/10.1002/dneu.20800
41. Kislauskis EH, Zhu X, Singer RH. Sequences responsible for intracellular localization of beta-actin messenger RNA also affect cell phenotype. J Cell Biol 1994; 127:441-51; PMID:7929587; http://dx.doi.org/10.1083/jcb.127.2.441
42. Terman JR, Kashina A. Post-translational modification and regulation of actin. Curr Opin Cell Biol 2013; 25:30-8; PMID:23195437; http://dx.doi.org/10.1016/j.ceb.2012.10.009
43. Bassell GJ, Zhang H, Byrd AL, Femino AM, Singer RH, Taneja KL, Lifshitz LM, Herman IM, Kosik KS. Sorting of beta-actin mRNA and protein to neurites and growth cones in culture. J Neurosci 1998; 18:251-65; PMID:9412505
44. Bergeron SE, Zhu M, Thiem SM, Friderici KH, Rubenstein PA. Ion-dependent polymerization differences between mammalian beta- and gamma-nonmuscle actin isoforms. J Biol Chem 2010; 285:16087-95; PMID:20308063; http://dx.doi.org/10.1074/jbc.M110.110130
45. Belyantseva IA, Perrin BJ, Sonnemann KJ, Zhu M, Stepanyan R, McGee J, Frolenkov GI, Walsh EJ, Friderici KH, Friedman TB, et al. Gamma-actin is required for cytoskeletal maintenance but not development. Proc Natl Acad Sci U S A 2009; 106:9703-8; PMID:19497859; http://dx.doi.org/10.1073/pnas.0900221106
46. Cheever TR, Li B, Ervasti JM. Restricted morphological and behavioral abnormalities following ablation of β-actin in the brain. PLoS One 2012; 7:e32970; PMID:22403730; http://dx.doi.org/10.1371/journal.pone.0032970
47. Larsson H, Lindberg U. The effect of divalent cations on the interaction between calf spleen profilin and different actins. Biochim Biophys Acta 1988; 953:95-105; PMID:3342244; http://dx.doi.org/10.1016/0167-4838(88)90013-1
48. Prochniewicz E, Janson N, Thomas DD, De la Cruz EM. Cofilin increases the torsional flexibility and dynamics of actin filaments. J Mol Biol 2005; 353:990-1000; PMID:16213521; http://dx.doi.org/10.1016/j.jmb.2005.09.021
49. Ramon y Cajal S. À quelle époque apparaissent les expansions des cellules nerveuses de la moëlle épinière du poulet? Anat Anz 1890; 21–22:609-39
50. Dent EW, Tang F, Kalil K. Axon guidance by growth cones and branches: common cytoskeletal and signaling mechanisms. Neuroscientist 2003; 9:343-53; PMID:14580119; http://dx.doi.org/10.1177/1073858403252683
51. Mattila PK, Lappalainen P. Filopodia: molecular architecture and cellular functions. Nat Rev Mol Cell Biol 2008; 9:446-54; PMID:18464790; http://dx.doi.org/10.1038/nrm2406
52. Faix J, Rottner K. The making of filopodia. Curr Opin Cell Biol 2006; 18:18-25; PMID:16337369; http://dx.doi.org/10.1016/j.ceb.2005.11.002
53. Michelot A, Drubin DG. Building distinct actin filament networks in a common cytoplasm. Curr Biol 2011; 21:R560-9; PMID:21783039; http://dx.doi.org/10.1016/j.cub.2011.06.019
54. Svitkina TM, Bulanova EA, Chaga OY, Vignjevic DM, Kojima S, Vasiliev JM, Borisy GG. Mechanism of filopodia initiation by reorganization of a dendritic network. J Cell Biol 2003; 160:409-21; PMID:12566431; http://dx.doi.org/10.1083/jcb.200210174
55. Tseng Y, An KM, Esue O, Wirtz D. The bimodal role of filamin in controlling the architecture and mechanics of F-actin networks. J Biol Chem 2004; 279:1819-26; PMID:14594947; http://dx.doi.org/10.1074/jbc.M306090200
56. Small JV, Rottner K, Kaverina I, Anderson KI. Assembling an actin cytoskeleton for cell attachment and movement. Biochim Biophys Acta 1998; 1404:271-81; PMID:9739149; http://dx.doi.org/10.1016/S0167-4889(98)00080-9
57. Small JV, Resch GP. The comings and goings of actin: coupling protrusion and retraction in cell motility. Curr Opin Cell Biol 2005; 17:517-23; PMID:16099152; http://dx.doi.org/10.1016/j.ceb.2005.08.004
58. Svitkina TM, Verkhovsky AB, McQuade KM, Borisy GG. Analysis of the actin-myosin II system in fish epidermal keratocytes: mechanism of cell body translocation. J Cell Biol 1997; 139:397-415; PMID:9334344; http://dx.doi.org/10.1083/jcb.139.2.397
59. Hotulainen P, Lappalainen P. Stress fibers are generated by two distinct actin assembly mechanisms in motile cells. J Cell Biol 2006; 173:383-94; PMID:16651381; http://dx.doi.org/10.1083/jcb.200511093
60. Lowery LA, Van Vactor D. The trip of the tip: understanding the growth cone machinery. Nat Rev Mol Cell Biol 2009; 10:332-43; PMID:19373241; http://dx.doi.org/10.1038/nrm2679
61. Medeiros NA, Burnette DT, Forscher P. Myosin II functions in actin-bundle turnover in neuronal growth cones. Nat Cell Biol 2006; 8:215-26; PMID:16501565; http://dx.doi.org/10.1038/ncb1367
62. Firat-Karalar EN, Welch MD. New mechanisms and functions of actin nucleation. Curr Opin Cell Biol 2011; 23:4-13; PMID:21093244; http://dx.doi.org/10.1016/j.ceb.2010.10.007
63. Andrianantoandro E, Pollard TD. Mechanism of actin filament turnover by severing and nucleation at different concentrations of ADF/cofilin. Mol Cell 2006; 24:13-23; PMID:17018289; http://dx.doi.org/10.1016/j.molcel.2006.08.006
64. Chen Q, Pollard TD. Actin filament severing by cofilin dismantles actin patches and produces mother filaments for new patches. Curr Biol 2013; 23:1154-62; PMID:23727096; http://dx.doi.org/10.1016/j.cub.2013.05.005
65. Schumacher N, Borawski JM, Leberfinger CB, Gessler M, Kerkhoff E. Overlapping expression pattern of the actin organizers Spir-1 and formin-2 in the developing mouse nervous system and the adult brain. Gene Expr Patterns 2004; 4:249-55; PMID:15053972; http://dx.doi.org/10.1016/j.modgep.2003.11.006
66. Strasser GA, Rahim NA, VanderWaal KE, Gertler FB, Lanier LM. Arp2/3 is a negative regulator of growth cone translocation. Neuron 2004; 43:81-94; PMID:15233919; http://dx.doi.org/10.1016/j.neuron.2004.05.015
67. Carlsson AE. Actin dynamics: from nanoscale to microscale. Annu Rev Biophys 2010; 39:91-110; PMID:20462375; http://dx.doi.org/10.1146/annurev.biophys.093008.131207
68. Chesarone MA, DuPage AG, Goode BL. Unleashing formins to remodel the actin and microtubule cytoskeletons. Nat Rev Mol Cell Biol 2010; 11:62-74; PMID:19997130; http://dx.doi.org/10.1038/nrm2816
69. Kerkhoff E. Cellular functions of the Spir actin-nucleation factors. Trends Cell Biol 2006; 16:477-83; PMID:16901698; http://dx.doi.org/10.1016/j.tcb.2006.07.005
70. Ahuja R, Pinyol R, Reichenbach N, Custer L, Klingensmith J, Kessels MM, Qualmann B. Cordon-bleu is an actin nucleation factor and controls neuronal morphology. Cell 2007; 131:337-50; PMID:17956734; http://dx.doi.org/10.1016/j.cell.2007.08.030

71. Lebrand C, Dent EW, Strasser GA, Lanier LM, Krause M, Svitkina TM, Borisy GG, Gertler FB. Critical role of Ena/VASP proteins for filopodia formation in neurons and in function downstream of netrin-1. Neuron 2004; 42:37-49; PMID:15066263; http://dx.doi.org/10.1016/S0896-6273(04)00108-4
72. Bear JE, Gertler FB. Ena/VASP: towards resolving a pointed controversy at the barbed end. J Cell Sci 2009; 122:1947-53; PMID:19494122; http://dx.doi.org/10.1242/jcs.038125
73. Barzik M, Kotova TI, Higgs HN, Hazelwood L, Hanein D, Gertler FB, Schafer DA. Ena/VASP proteins enhance actin polymerization in the presence of barbed end capping proteins. J Biol Chem 2005; 280:28653-62; PMID:15939738; http://dx.doi.org/10.1074/jbc.M503957200
74. Ono S. Mechanism of depolymerization and severing of actin filaments and its significance in cytoskeletal dynamics. Int Rev Cytol 2007; 258:1-82; PMID:17338919; http://dx.doi.org/10.1016/S0074-7696(07)58001-0
75. Hung RJ, Yazdani U, Yoon J, Wu H, Yang T, Gupta N, Huang Z, van Berkel WJ, Terman JR. Mical links semaphorins to F-actin disassembly. Nature 2010; 463:823-7; PMID:20148037; http://dx.doi.org/10.1038/nature08724
76. Haviv L, Gillo D, Backouche F, Bernheim-Groswasser A. A cytoskeletal demolition worker: myosin II acts as an actin depolymerization agent. J Mol Biol 2008; 375:325-30; PMID:18021803; http://dx.doi.org/10.1016/j.jmb.2007.09.066
77. Bernstein BW, Bamburg JR. ADF/cofilin: a functional node in cell biology. Trends Cell Biol 2010; 20:187-95; PMID:20133134; http://dx.doi.org/10.1016/j.tcb.2010.01.001
78. Elam WA, Kang H, De la Cruz EM. Biophysics of actin filament severing by cofilin. FEBS Lett 2013; 587:1215-9; PMID:23395798; http://dx.doi.org/10.1016/j.febslet.2013.01.062
79. Carlier MF, Laurent V, Santolini J, Melki R, Didry D, Xia GX, Hong Y, Chua NH, Pantaloni D. Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover: implication in actin-based motility. J Cell Biol 1997; 136:1307-22; PMID:9087445; http://dx.doi.org/10.1083/jcb.136.6.1307
80. dos Remedios CG, Chhabra D, Kekic M, Dedova IV, Tsubakihara M, Berry DA, Nosworthy NJ. Actin binding proteins: regulation of cytoskeletal microfilaments. Physiol Rev 2003; 83:433-73; PMID:12663865
81. Gremm D, Wegner A. Gelsolin as a calcium-regulated actin filament-capping protein. Eur J Biochem 2000; 267:4339-45; PMID:10880956; http://dx.doi.org/10.1046/j.1432-1327.2000.01463.x
82. Mazur AJ, Gremm D, Dansranjavin T, Litwin M, Jockusch BM, Wegner A, Weeds AG, Mannherz HG. Modulation of actin filament dynamics by actin-binding proteins residing in lamellipodia. Eur J Cell Biol 2010; 89:402-13; PMID:20133009; http://dx.doi.org/10.1016/j.ejcb.2009.10.017
83. Lee CW, Vitriol EA, Shim S, Wise AL, Velayutham RP, Zheng JQ. Dynamic localization of G-actin during membrane protrusion in neuronal motility. Curr Biol 2013; 23:1046-56; PMID:23746641; http://dx.doi.org/10.1016/j.cub.2013.04.057
84. Heidemann SR, Joshi HC, Schechter A, Fletcher JR, Bothwell M. Synergistic effects of cyclic AMP and nerve growth factor on neurite outgrowth and microtubule stability of PC12 cells. J Cell Biol 1985; 100:916-27; PMID:2982887; http://dx.doi.org/10.1083/jcb.100.3.916
85. Knöll B. Actin-mediated gene expression in neurons: the MRTF-SRF connection. Biol Chem 2010; 391:591-7; PMID:20370316; http://dx.doi.org/10.1515/bc.2010.061
86. Knöll B, Kretz O, Fiedler C, Alberti S, Schütz G, Frotscher M, Nordheim A. Serum response factor controls neuronal circuit assembly in the hippocampus. Nat Neurosci 2006; 9:195-204; PMID:16415869; http://dx.doi.org/10.1038/nn1627
87. Witke W. The role of profilin complexes in cell motility and other cellular processes. Trends Cell Biol 2004; 14:461-9; PMID:15308213; http://dx.doi.org/10.1016/j.tcb.2004.07.003
88. van Kesteren RE, Carter C, Dissel HM, van Minnen J, Gouwenberg Y, Syed NI, Spencer GE, Smit AB. Local synthesis of actin-binding protein beta-thymosin regulates neurite outgrowth. J Neurosci 2006; 26:152-7; PMID:16399682; http://dx.doi.org/10.1523/JNEUROSCI.4164-05.2006
89. Da Silva JS, Medina M, Zuliani C, Di Nardo A, Witke W, Dotti CG. RhoA/ROCK regulation of neuritogenesis via profilin IIa-mediated control of actin stability. J Cell Biol 2003; 162:1267-79; PMID:14517206; http://dx.doi.org/10.1083/jcb.200304021
90. Xu J, Casella JF, Pollard TD. Effect of capping protein, CapZ, on the length of actin filaments and mechanical properties of actin filament networks. Cell Motil Cytoskeleton 1999; 42:73-81; PMID:9915586; http://dx.doi.org/10.1002/(SICI)1097-0169(1999)42:1<73::AID-CM7>3.0.CO;2-Z
91. Carlier MF, Pantaloni D. Control of actin dynamics in cell motility. J Mol Biol 1997; 269:459-67; PMID:9217250; http://dx.doi.org/10.1006/jmbi.1997.1062
92. Akin O, Mullins RD. Capping protein increases the rate of actin-based motility by promoting filament nucleation by the Arp2/3 complex. Cell 2008; 133:841-51; PMID:18510928; http://dx.doi.org/10.1016/j.cell.2008.04.011
93. Fricke R, Gohl C, Bogdan S. The F-BAR protein family Actin' on the membrane. Commun Integr Biol 2010; 3:89-94; PMID:20585497; http://dx.doi.org/10.4161/cib.3.2.10521
94. Govind S, Kozma R, Monfries C, Lim L, Ahmed S. Cdc42Hs facilitates cytoskeletal reorganization and neurite outgrowth by localizing the 58-kD insulin receptor substrate to filamentous actin. J Cell Biol 2001; 152:579-94; PMID:11157984; http://dx.doi.org/10.1083/jcb.152.3.579
95. Guerrier S, Coutinho-Budd J, Sassa T, Gresset A, Jordan NV, Chen K, Jin WL, Frost A, Polleux F. The F-BAR domain of srGAP2 induces membrane protrusions required for neuronal migration and morphogenesis. Cell 2009; 138:990-1004; PMID:19737524; http://dx.doi.org/10.1016/j.cell.2009.06.047
96. Saengsawang W, Mitok K, Viesselmann C, Pietila L, Lumbard DC, Corey SJ, Dent EW. The F-BAR protein CIP4 inhibits neurite formation by producing lamellipodial protrusions. Curr Biol 2012; 22:494-501; PMID:22361215; http://dx.doi.org/10.1016/j.cub.2012.01.038
97. Shinohara R, Thumkeo D, Kamijo H, Kaneko N, Sawamoto K, Watanabe K, Takebayashi H, Kiyonari H, Ishizaki T, Furuyashiki T, et al. A role for mDia, a Rho-regulated actin nucleator, in tangential migration of interneuron precursors. Nat Neurosci 2012; 15:373-80, S1-2; PMID:22246438; http://dx.doi.org/10.1038/nn.3020
98. Bradke F, Dotti CG. The role of local actin instability in axon formation. Science 1999; 283:1931-4; PMID:10082468; http://dx.doi.org/10.1126/science.283.5409.1931
99. Vitriol EA, Zheng JQ. Growth cone travel in space and time: the cellular ensemble of cytoskeleton, adhesion, and membrane. Neuron 2012; 73:1068-81; PMID:22445336; http://dx.doi.org/10.1016/j.neuron.2012.03.005
100. Franze K, Gerdelmann J, Weick M, Betz T, Pawlizak S, Lakadamyali M, Bayer J, Rillich K, Gögler M, Lu YB, et al. Neurite branch retraction is caused by a threshold-dependent mechanical impact. Biophys J 2009; 97:1883-90; PMID:19804718; http://dx.doi.org/10.1016/j.bpj.2009.07.033
101. 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; PMID:10934315; http://dx.doi.org/10.1002/1097-4695(200008)44:2<97::AID-NEU2>3.0.CO;2-U
102. Renkawitz J, Sixt M. Mechanisms of force generation and force transmission during interstitial leukocyte migration. EMBO Rep 2010; 11:744-50; PMID:20865016; http://dx.doi.org/10.1038/embor.2010.147
103. Wiggan O, Shaw AE, DeLuca JG, Bamburg JR. ADF/cofilin regulates actomyosin assembly through competitive inhibition of myosin II binding to F-actin. Dev Cell 2012; 22:530-43; PMID:22421043; http://dx.doi.org/10.1016/j.devcel.2011.12.026
104. Vicente-Manzanares M, Ma X, Adelstein RS, Horwitz AR. Non-muscle myosin II takes centre stage in cell adhesion and migration. Nat Rev Mol Cell Biol 2009; 10:778-90; PMID:19851336; http://dx.doi.org/10.1038/nrm2786
105. Ponti A, Machacek M, Gupton SL, Waterman-Storer CM, Danuser G. Two distinct actin networks drive the protrusion of migrating cells. Science 2004; 305:1782-6; PMID:15375270; http://dx.doi.org/10.1126/science.1100533
106. Cai Y, Biais N, Giannone G, Tanase M, Jiang G, Hofman JM, Wiggins CH, Silberzan P, Buguin A, Ladoux B, et al. Nonmuscle myosin IIA-dependent force inhibits cell spreading and drives F-actin flow. Biophys J 2006; 91:3907-20; PMID:16920834; http://dx.doi.org/10.1529/biophysj.106.084806
107. Diefenbach TJ, Latham VM, Yimlamai D, Liu CA, Herman IM, Jay DG. Myosin 1c and myosin IIB serve opposing roles in lamellipodial dynamics of the neuronal growth cone. J Cell Biol 2002; 158:1207-17; PMID:12356865; http://dx.doi.org/10.1083/jcb.200202028
108. Brown ME, Bridgman PC. Retrograde flow rate is increased in growth cones from myosin IIB knockout mice. J Cell Sci 2003; 116:1087-94; PMID:12584251; http://dx.doi.org/10.1242/jcs.00335
109. Ketschek AR, Jones SL, Gallo G. Axon extension in the fast and slow lanes: substratum-dependent engagement of myosin II functions. Dev Neurobiol 2007; 67:1305-20; PMID:17638383; http://dx.doi.org/10.1002/dneu.20455
110. Kollins KM, Hu J, Bridgman PC, Huang YQ, Gallo G. Myosin-II negatively regulates minor process extension and the temporal development of neuronal polarity. Dev Neurobiol 2009; 69:279-98; PMID:19224562; http://dx.doi.org/10.1002/dneu.20704
111. Bard L, Boscher C, Lambert M, Mège RM, Choquet D, Thoumine O. A molecular clutch between the actin flow and N-cadherin adhesions drives growth cone migration. J Neurosci 2008; 28:5879-90; PMID:18524892; http://dx.doi.org/10.1523/JNEUROSCI.5331-07.2008
112. Toriyama M, Kozawa S, Sakumura Y, Inagaki N. Conversion of a signal into forces for axon outgrowth through Pak1-mediated shootin1 phosphorylation. Curr Biol 2013; 23:529-34; PMID:23453953; http://dx.doi.org/10.1016/j.cub.2013.02.017
113. Schevzov G, Curthoys NM, Gunning PW, Fath T. Functional diversity of actin cytoskeleton in neurons and its regulation by tropomyosin. Int Rev Cell Mol Biol 2012; 298:33-94; PMID:22878104; http://dx.doi.org/10.1016/B978-0-12-394309-5.00002-X

114. Bryce NS, Schevzov G, Ferguson V, Percival JM, Lin JJ, Matsumura F, Bamburg JR, Jeffrey PL, Hardeman EC, Gunning P, et al. Specification of actin filament function and molecular composition by tropomyosin isoforms. Mol Biol Cell 2003; 14:1002-16; PMID:12631719; http://dx.doi.org/10.1091/mbc.E02-04-0244
115. Lees JG, Bach CT, O'Neill GM. Interior decoration: tropomyosin in actin dynamics and cell migration. Cell Adh Migr 2011; 5:181-6; PMID:21173575; http://dx.doi.org/10.4161/cam.5.2.14438
116. Bernstein BW, Bamburg JR. Tropomyosin binding to F-actin protects the F-actin from disassembly by brain actin-depolymerizing factor (ADF). Cell Motil 1982; 2:1-8; PMID:6890875; http://dx.doi.org/10.1002/cm.970020102
117. Schevzov G, Bryce NS, Almonte-Baldonado R, Joya J, Lin JJ, Hardeman E, Weinberger R, Gunning P. Specific features of neuronal size and shape are regulated by tropomyosin isoforms. Mol Biol Cell 2005; 16:3425-37; PMID:15888546; http://dx.doi.org/10.1091/mbc.E04-10-0951
118. Fischer RS, Fowler VM. Tropomodulins: life at the slow end. Trends Cell Biol 2003; 13:593-601; PMID:14573353; http://dx.doi.org/10.1016/j.tcb.2003.09.007
119. Fath T, Fischer RS, Dehmelt L, Halpain S, Fowler VM. Tropomodulins are negative regulators of neurite outgrowth. Eur J Cell Biol 2011; 90:291-300; PMID:21146252; http://dx.doi.org/10.1016/j.ejcb.2010.10.014
120. Moroz N, Guillaud L, Desai B, Kostyukova AS. Mutations changing tropomodulin affinity for tropomyosin alter neurite formation and extension. PeerJ 2013; 1:e7; PMID:23638401; http://dx.doi.org/10.7717/peerj.7
121. Lieleg O, Schmoller KM, Claessens MM, Bausch AR. Cytoskeletal polymer networks: viscoelastic properties are determined by the microscopic interaction potential of cross-links. Biophys J 2009; 96:4725-32; PMID:19486695; http://dx.doi.org/10.1016/j.bpj.2009.03.038
122. Breitsprecher D, Koestler SA, Chizhov I, Nemethova M, Mueller J, Goode BL, Small JV, Rottner K, Faix J. Cofilin cooperates with fascin to disassemble filopodial actin filaments. J Cell Sci 2011; 124:3305-18; PMID:21940796; http://dx.doi.org/10.1242/jcs.086934
123. Dehmelt L, Halpain S. Actin and microtubules in neurite initiation: are MAPs the missing link? J Neurobiol 2004; 58:18-33; PMID:14598367; http://dx.doi.org/10.1002/neu.10284
124. Conde C, Cáceres A. Microtubule assembly, organization and dynamics in axons and dendrites. Nat Rev Neurosci 2009; 10:319-32; PMID:19377501; http://dx.doi.org/10.1038/nrn2631
125. Janke C, Kneussel M. Tubulin post-translational modifications: encoding functions on the neuronal microtubule cytoskeleton. Trends Neurosci 2010; 33:362-72; PMID:20541813; http://dx.doi.org/10.1016/j.tins.2010.05.001
126. Katsetos CD, Herman MM, Mörk SJ. Class III beta-tubulin in human development and cancer. Cell Motil Cytoskeleton 2003; 55:77-96; PMID:12740870; http://dx.doi.org/10.1002/cm.10116
127. Joshi HC, Cleveland DW. Diversity among tubulin subunits: toward what functional end? Cell Motil Cytoskeleton 1990; 16:159-63; PMID:2194680; http://dx.doi.org/10.1002/cm.970160302
128. Schwarz PM, Liggins JR, Ludueña RF. Beta-tubulin isotypes purified from bovine brain have different relative stabilities. Biochemistry 1998; 37:4687-92; PMID:9521790; http://dx.doi.org/10.1021/bi972763d
129. Jaglin XH, Chelly J. Tubulin-related cortical dysgeneses: microtubule dysfunction underlying neuronal migration defects. Trends Genet 2009; 25:555-66; PMID:19864038; http://dx.doi.org/10.1016/j.tig.2009.10.003
130. Poirier K, Keays DA, Francis F, Saillour Y, Bahi N, Manouvrier S, Fallet-Bianco C, Pasquier L, Toutain A, Tuy FP, et al. Large spectrum of lissencephaly and pachygyria phenotypes resulting from de novo missense mutations in tubulin alpha 1A (TUBA1A). Hum Mutat 2007; 28:1055-64; PMID:17584854; http://dx.doi.org/10.1002/humu.20572
131. Tischfield MA, Baris HN, Wu C, Rudolph G, Van Maldergem L, He W, Chan WM, Andrews C, Demer JL, Robertson RL, et al. Human TUBB3 mutations perturb microtubule dynamics, kinesin interactions, and axon guidance. Cell 2010; 140:74-87; PMID:20074521; http://dx.doi.org/10.1016/j.cell.2009.12.011
132. Howard J, Hyman AA. Dynamics and mechanics of the microtubule plus end. Nature 2003; 422:753-8; PMID:12700769; http://dx.doi.org/10.1038/nature01600
133. Ahmad FJ, Hughey J, Wittmann T, Hyman A, Greaser M, Baas PW. Motor proteins regulate force interactions between microtubules and microfilaments in the axon. Nat Cell Biol 2000; 2:276-80; PMID:10806478; http://dx.doi.org/10.1038/35010544
134. Joshi HC, Chu D, Buxbaum RE, Heidemann SR. Tension and compression in the cytoskeleton of PC 12 neurites. J Cell Biol 1985; 101:697-705; PMID:2863274; http://dx.doi.org/10.1083/jcb.101.3.697
135. Zanic M, Stear JH, Hyman AA, Howard J. EB1 recognizes the nucleotide state of tubulin in the microtubule lattice. PLoS One 2009; 4:e7585; PMID:19851462; http://dx.doi.org/10.1371/journal.pone.0007585
136. Dehmelt L, Nalbant P, Steffen W, Halpain S. A microtubule-based, dynein-dependent force induces local cell protrusions: Implications for neurite initiation. Brain Cell Biol 2006; 35:39-56; PMID:17940912; http://dx.doi.org/10.1007/s11068-006-9001-0
137. Grabham PW, Seale GE, Bennecib M, Goldberg DJ, Vallee RB. Cytoplasmic dynein and LIS1 are required for microtubule advance during growth cone remodeling and fast axonal outgrowth. J Neurosci 2007; 27:5823-34; PMID:17522326; http://dx.doi.org/10.1523/JNEUROSCI.1135-07.2007
138. Hendricks AG, Lazarus JE, Perlson E, Gardner MK, Odde DJ, Goldman YE, Holzbaur EL. Dynein tethers and stabilizes dynamic microtubule plus ends. Curr Biol 2012; 22:632-7; PMID:22445300; http://dx.doi.org/10.1016/j.cub.2012.02.023
139. Gusnowski EM, Srayko M. Visualization of dynein-dependent microtubule gliding at the cell cortex: implications for spindle positioning. J Cell Biol 2011; 194:377-86; PMID:21825072; http://dx.doi.org/10.1083/jcb.201103128
140. Yu W, Solowska JM, Qiang L, Karabay A, Baird D, Baas PW. Regulation of microtubule severing by katanin subunits during neuronal development. J Neurosci 2005; 25:5573-83; PMID:15944385; http://dx.doi.org/10.1523/JNEUROSCI.0834-05.2005
141. Karabay A, Yu W, Solowska JM, Baird DH, Baas PW. Axonal growth is sensitive to the levels of katanin, a protein that severs microtubules. J Neurosci 2004; 24:5778-88; PMID:15215300; http://dx.doi.org/10.1523/JNEUROSCI.1382-04.2004
142. Peris L, Thery M, Fauré J, Saoudi Y, Lafanechère L, Chilton JK, Gordon-Weeks P, Galjart N, Bornens M, Wordeman L, et al. Tubulin tyrosination is a major factor affecting the recruitment of CAP-Gly proteins at microtubule plus ends. J Cell Biol 2006; 174:839-49; PMID:16954346; http://dx.doi.org/10.1083/jcb.200512058
143. Reed NA, Cai D, Blasius TL, Jih GT, Meyhofer E, Gaertig J, Verhey KJ. Microtubule acetylation promotes kinesin-1 binding and transport. Curr Biol 2006; 16:2166-72; PMID:17084703; http://dx.doi.org/10.1016/j.cub.2006.09.014
144. Erck C, Peris L, Andrieux A, Meissirel C, Gruber AD, Vernet M, Schweitzer A, Saoudi Y, Pointu H, Bosc C, et al. A vital role of tubulin-tyrosine-ligase for neuronal organization. Proc Natl Acad Sci U S A 2005; 102:7853-8; PMID:15899979; http://dx.doi.org/10.1073/pnas.0409626102
145. Hsieh PC, Chiang ML, Chang JC, Yan YT, Wang FF, Chou YC. DDA3 stabilizes microtubules and suppresses neurite formation. J Cell Sci 2012; 125:3402-11; PMID:22467851; http://dx.doi.org/10.1242/jcs.099150
146. Maccioni RB, Cambiazo V. Role of microtubule-associated proteins in the control of microtubule assembly. Physiol Rev 1995; 75:835-64; PMID:7480164
147. Dehmelt L, Halpain S. The MAP2/Tau family of microtubule-associated proteins. Genome Biol 2005; 6:204; PMID:15642108; http://dx.doi.org/10.1186/gb-2004-6-1-204
148. Halpain S, Dehmelt L. The MAP1 family of microtubule-associated proteins. Genome Biol 2006; 7:224; PMID:16938900; http://dx.doi.org/10.1186/gb-2006-7-6-224
149. Ma D, Nothias F, Boyne LJ, Fischer I. Differential regulation of microtubule-associated protein 1B (MAP1B) in rat CNS and PNS during development. J Neurosci Res 1997; 49:319-32; PMID:9260743; http://dx.doi.org/10.1002/(SICI)1097-4547(19970801)49:3<319::AID-JNR7>3.0.CO;2-F
150. Dehmelt L, Halpain S. The MAP2/Tau family of microtubule-associated proteins. Genome Biol 2005; 6:204; PMID:15642108; http://dx.doi.org/10.1186/gb-2004-6-1-204
151. Chen J, Kanai Y, Cowan NJ, Hirokawa N. Projection domains of MAP2 and tau determine spacings between microtubules in dendrites and axons. Nature 1992; 360:674-7; PMID:1465130; http://dx.doi.org/10.1038/360674a0
152. Teng J, Takei Y, Harada A, Nakata T, Chen J, Hirokawa N. Synergistic effects of MAP2 and MAP1B knockout in neuronal migration, dendritic outgrowth, and microtubule organization. J Cell Biol 2001; 155:65-76; PMID:11581286; http://dx.doi.org/10.1083/jcb.200106025
153. Tortosa E, Galjart N, Avila J, Sayas CL. MAP1B regulates microtubule dynamics by sequestering EB1/3 in the cytosol of developing neuronal cells. EMBO J 2013; 32:1293-306; PMID:23572079; http://dx.doi.org/10.1038/emboj.2013.76
154. Caceres A, Mautino J, Kosik KS. Suppression of MAP2 in cultured cerebellar macroneurons inhibits minor neurite formation. Neuron 1992; 9:607-18; PMID:1389180; http://dx.doi.org/10.1016/0896-6273(92)90025-9
155. Caceres A, Potrebic S, Kosik KS. The effect of tau antisense oligonucleotides on neurite formation of cultured cerebellar macroneurons. J Neurosci 1991; 11:1515-23; PMID:1904479
156. Leclerc N, Baas PW, Garner CC, Kosik KS. Juvenile and mature MAP2 isoforms induce distinct patterns of process outgrowth. Mol Biol Cell 1996; 7:443-55; PMID:8868472; http://dx.doi.org/10.1091/mbc.7.3.443
157. Takei Y, Teng J, Harada A, Hirokawa N. Defects in axonal elongation and neuronal migration in mice with disrupted tau and map1b genes. J Cell Biol 2000; 150:989-1000; PMID:10973990; http://dx.doi.org/10.1083/jcb.150.5.989
158. Bisig CG, Chesta ME, Zampar GG, Purro SA, Santander VS, Arce CA. Lack of stabilized microtubules as a result of the absence of major maps in CAD cells does not preclude neurite formation. FEBS J 2009; 276:7110-23; PMID:19878302; http://dx.doi.org/10.1111/j.1742-4658.2009.07422.x
159. Letourneau PC, Ressler AH. Inhibition of neurite initiation and growth by taxol. J Cell Biol 1984; 98:1355-62; PMID:6143759; http://dx.doi.org/10.1083/jcb.98.4.1355

160. Rochlin MW, Wickline KM, Bridgman PC. Microtubule stability decreases axon elongation but not axoplasm production. J Neurosci 1996; 16:3236-46; PMID:8627361
161. Bamburg JR, Bray D, Chapman K. Assembly of microtubules at the tip of growing axons. Nature 1986; 321:788-90; PMID:2872595; http://dx.doi.org/10.1038/321788a0
162. Akhmanova A, Steinmetz MO. Tracking the ends: a dynamic protein network controls the fate of microtubule tips. Nat Rev Mol Cell Biol 2008; 9:309-22; PMID:18322465; http://dx.doi.org/10.1038/nrm2369
163. Vitre B, Coquelle FM, Heichette C, Garnier C, Chrétien D, Arnal I. EB1 regulates microtubule dynamics and tubulin sheet closure in vitro. Nat Cell Biol 2008; 10:415-21; PMID:18364701; http://dx.doi.org/10.1038/ncb1703
164. Hayashi I, Wilde A, Mal TK, Ikura M. Structural basis for the activation of microtubule assembly by the EB1 and p150Glued complex. Mol Cell 2005; 19:449-60; PMID:16109370; http://dx.doi.org/10.1016/j.molcel.2005.06.034
165. Nakamura M, Zhou XZ, Lu KP. Critical role for the EB1 and APC interaction in the regulation of microtubule polymerization. Curr Biol 2001; 11:1062-7; PMID:11470413; http://dx.doi.org/10.1016/S0960-9822(01)00297-4
166. Stepanova T, Smal I, van Haren J, Akinci U, Liu Z, Miedema M, Limpens R, van Ham M, van der Reijden M, Poot R, et al. History-dependent catastrophes regulate axonal microtubule behavior. Curr Biol 2010; 20:1023-8; PMID:20471267; http://dx.doi.org/10.1016/j.cub.2010.04.024
167. Neukirchen D, Bradke F. Cytoplasmic linker proteins regulate neuronal polarization through microtubule and growth cone dynamics. J Neurosci 2011; 31:1528-38; PMID:21273437; http://dx.doi.org/10.1523/JNEUROSCI.3983-10.2011
168. Chen Y, Tian X, Kim WY, Snider WD. Adenomatous polyposis coli regulates axon arborization and cytoskeleton organization via its N-terminus. PLoS One 2011; 6:e24335; PMID:21915313; http://dx.doi.org/10.1371/journal.pone.0024335
169. Hur EM, Saijilafu, Lee BD, Kim SJ, Xu WL, Zhou FQ. GSK3 controls axon growth via CLASP-mediated regulation of growth cone microtubules. Genes Dev 2011; 25:1968-81; PMID:21937714; http://dx.doi.org/10.1101/gad.17015911
170. Reiner O. LIS1 and DCX: Implications for Brain Development and Human Disease in Relation to Microtubules. Scientifica 2013; 2013:1-17.
171. Youn YH, Pramparo T, Hirotsune S, Wynshaw-Boris A. Distinct dose-dependent cortical neuronal migration and neurite extension defects in Lis1 and Ndel1 mutant mice. J Neurosci 2009; 29:15520-30; PMID:20007476; http://dx.doi.org/10.1523/JNEUROSCI.4630-09.2009
172. Bielas SL, Serneo FF, Chechlacz M, Deerinck TJ, Perkins GA, Allen PB, Ellisman MH, Gleeson JG. Spinophilin facilitates dephosphorylation of doublecortin by PP1 to mediate microtubule bundling at the axonal wrist. Cell 2007; 129:579-91; PMID:17482550; http://dx.doi.org/10.1016/j.cell.2007.03.023
173. Stein R, Mori N, Matthews K, Lo LC, Anderson DJ. The NGF-inducible SCG10 mRNA encodes a novel membrane-bound protein present in growth cones and abundant in developing neurons. Neuron 1988; 1:463-76; PMID:3272176; http://dx.doi.org/10.1016/0896-6273(88)90177-8
174. Morii H, Shiraishi-Yamaguchi Y, Mori N. SCG10, a microtubule destabilizing factor, stimulates the neurite outgrowth by modulating microtubule dynamics in rat hippocampal primary cultured neurons. J Neurobiol 2006; 66:1101-14; PMID:16838365; http://dx.doi.org/10.1002/neu.20295
175. Watabe-Uchida M, John KA, Janas JA, Newey SE, Van Aelst L. The Rac activator DOCK7 regulates neuronal polarity through local phosphorylation of stathmin/Op18. Neuron 2006; 51:727-39; PMID:16982419; http://dx.doi.org/10.1016/j.neuron.2006.07.020
176. Li YH, Ghavampur S, Bondallaz P, Will L, Grenningloh G, Püschel AW. Rnd1 regulates axon extension by enhancing the microtubule destabilizing activity of SCG10. J Biol Chem 2009; 284:363-71; PMID:18996843; http://dx.doi.org/10.1074/jbc.M808126200
177. Grenningloh G, Soehrman S, Bondallaz P, Ruchti E, Cadas H. Role of the microtubule destabilizing proteins SCG10 and stathmin in neuronal growth. J Neurobiol 2004; 58:60-9; PMID:14598370; http://dx.doi.org/10.1002/neu.10279
178. Wittmann T, Bokoch GM, Waterman-Storer CM. Regulation of microtubule destabilizing activity of Op18/stathmin downstream of Rac1. J Biol Chem 2004; 279:6196-203; PMID:14645234; http://dx.doi.org/10.1074/jbc.M307261200
179. Manna T, Thrower D, Miller HP, Curmi P, Wilson L. Stathmin strongly increases the minus end catastrophe frequency and induces rapid treadmilling of bovine brain microtubules at steady state in vitro. J Biol Chem 2006; 281:2071-8; PMID:16317007; http://dx.doi.org/10.1074/jbc.M510661200
180. Poulain FE, Sobel A. The "SCG10-LIke Protein" SCLIP is a novel regulator of axonal branching in hippocampal neurons, unlike SCG10. Mol Cell Neurosci 2007; 34:137-46; PMID:17145186; http://dx.doi.org/10.1016/j.mcn.2006.10.012
181. Westerlund N, Zdrojewska J, Padzik A, Komulainen E, Björkblom B, Rannikko E, Tararuk T, Garcia-Frigola C, Sandholm J, Nguyen L, et al. Phosphorylation of SCG10/stathmin-2 determines multipolar stage exit and neuronal migration rate. Nat Neurosci 2011; 14:305-13; PMID:21297631; http://dx.doi.org/10.1038/nn.2755
182. Fukata Y, Itoh TJ, Kimura T, Ménager C, Nishimura T, Shiromizu T, Watanabe H, Inagaki N, Iwamatsu A, Hotani H, et al. CRMP-2 binds to tubulin heterodimers to promote microtubule assembly. Nat Cell Biol 2002; 4:583-91; PMID:12134159
183. Goshima Y, Nakamura F, Strittmatter P, Strittmatter SM. Collapsin-induced growth cone collapse mediated by an intracellular protein related to UNC-33. Nature 1995; 376:509-14; PMID:7637782; http://dx.doi.org/10.1038/376509a0
184. Inagaki N, Chihara K, Arimura N, Ménager C, Kawano Y, Matsuo N, Nishimura T, Amano M, Kaibuchi K. CRMP-2 induces axons in cultured hippocampal neurons. Nat Neurosci 2001; 4:781-2; PMID:11477421; http://dx.doi.org/10.1038/90476
185. Yoshimura T, Kawano Y, Arimura N, Kawabata S, Kikuchi A, Kaibuchi K. GSK-3beta regulates phosphorylation of CRMP-2 and neuronal polarity. Cell 2005; 120:137-49; PMID:15652488; http://dx.doi.org/10.1016/j.cell.2004.11.012
186. Caviston JP, Ross JL, Antony SM, Tokito M, Holzbaur EL. Huntingtin facilitates dynein/dynactin-mediated vesicle transport. Proc Natl Acad Sci U S A 2007; 104:10045-50; PMID:17548833; http://dx.doi.org/10.1073/pnas.0610628104
187. Hirokawa N, Noda Y, Tanaka Y, Niwa S. Kinesin superfamily motor proteins and intracellular transport. Nat Rev Mol Cell Biol 2009; 10:682-96; PMID:19773780; http://dx.doi.org/10.1038/nrm2774
188. Homma N, Takei Y, Tanaka Y, Nakata T, Terada S, Kikkawa M, Noda Y, Hirokawa N. Kinesin superfamily protein 2A (KIF2A) functions in suppression of collateral branch extension. Cell 2003; 114:229-39; PMID:12887924; http://dx.doi.org/10.1016/S0092-8674(03)00522-1
189. Vallee RB, Seale GE, Tsai JW. Emerging roles for myosin II and cytoplasmic dynein in migrating neurons and growth cones. Trends Cell Biol 2009; 19:347-55; PMID:19524440; http://dx.doi.org/10.1016/j.tcb.2009.03.009
190. Liu M, Nadar VC, Kozielski F, Kozlowska M, Yu W, Baas PW. Kinesin-12, a mitotic microtubule-associated motor protein, impacts axonal growth, navigation, and branching. J Neurosci 2010; 30:14896-906; PMID:21048148; http://dx.doi.org/10.1523/JNEUROSCI.3739-10.2010
191. Yu W, Qiang L, Solowska JM, Karabay A, Korulu S, Baas PW. The microtubule-severing proteins spastin and katanin participate differently in the formation of axonal branches. Mol Biol Cell 2008; 19:1485-98; PMID:18234839; http://dx.doi.org/10.1091/mbc.E07-09-0878
192. Ghosh DK, Debdeep D, Guha A. Models, Regulations, and Functions of Microtubule Severing by Katanin. ISRN Molecular Biology 2012; 2012; http://dx.doi.org/10.5402/2012/596289
193. Hall A, Lalli G. Rho and Ras GTPases in axon growth, guidance, and branching. Cold Spring Harb Perspect Biol 2010; 2:a001818; PMID:20182621; http://dx.doi.org/10.1101/cshperspect.a001818
194. Rodriguez OC, Schaefer AW, Mandato CA, Forscher P, Bement WM, Waterman-Storer CM. Conserved microtubule-actin interactions in cell movement and morphogenesis. Nat Cell Biol 2003; 5:599-609; PMID:12833063; http://dx.doi.org/10.1038/ncb0703-599
195. 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; PMID:3170637; http://dx.doi.org/10.1083/jcb.107.4.1505
196. Schaefer AW, Schoonderwoert VT, Ji L, Mederios N, Danuser G, Forscher P. Coordination of actin filament and microtubule dynamics during neurite outgrowth. Dev Cell 2008; 15:146-62; PMID:18606148; http://dx.doi.org/10.1016/j.devcel.2008.05.003
197. Geraldo S, Khanzada UK, Parsons M, Chilton JK, Gordon-Weeks PR. Targeting of the F-actin-binding protein drebrin by the microtubule plus-tip protein EB3 is required for neuritogenesis. Nat Cell Biol 2008; 10:1181-9; PMID:18806788; http://dx.doi.org/10.1038/ncb1778
198. Sonnenberg A, Rojas AM, de Pereda JM. The structure of a tandem pair of spectrin repeats of plectin reveals a modular organization of the plakin domain. J Mol Biol 2007; 368:1379-91; PMID:17397861; http://dx.doi.org/10.1016/j.jmb.2007.02.090
199. Alves-Silva J, Sánchez-Soriano N, Beaven R, Klein M, Parkin J, Millard TH, Bellen HJ, Venken KJ, Ballestrem C, Kammerer RA, et al. Spectraplakins promote microtubule-mediated axonal growth by functioning as structural microtubule-associated proteins and EB1-dependent +TIPs (tip interacting proteins). J Neurosci 2012; 32:9143-58; PMID:22764224; http://dx.doi.org/10.1523/JNEUROSCI.0416-12.2012
200. Fukata M, Watanabe T, Noritake J, Nakagawa M, Yamaga M, Kuroda S, Matsuura Y, Iwamatsu A, Perez F, Kaibuchi K. Rac1 and Cdc42 capture microtubules through IQGAP1 and CLIP-170. Cell 2002; 109:873-85; PMID:12110184; http://dx.doi.org/10.1016/S0092-8674(02)00800-0
201. Swiech L, Blazejczyk M, Urbanska M, Pietruszka P, Dortland BR, Malik AR, Wulf PS, Hoogenraad CC, Jaworski J. CLIP-170 and IQGAP1 cooperatively regulate dendrite morphology. J Neurosci 2011; 31:4555-68; PMID:21430156; http://dx.doi.org/10.1523/JNEUROSCI.6582-10.2011
202. Kardon JR, Vale RD. Regulators of the cytoplasmic dynein motor. Nat Rev Mol Cell Biol 2009; 10:854-65; PMID:19935668; http://dx.doi.org/10.1038/nrm2804

203. Burnette DT, Ji L, Schaefer AW, Medeiros NA, Danuser G, Forscher P. Myosin II activity facilitates microtubule bundling in the neuronal growth cone neck. Dev Cell 2008; 15:163-9; PMID:18606149; http://dx.doi.org/10.1016/j.devcel.2008.05.016
204. Xu K, Zhong G, Zhuang X. Actin, spectrin, and associated proteins form a periodic cytoskeletal structure in axons. Science 2013; 339:452-6; PMID:23239625; http://dx.doi.org/10.1126/science.1232251
205. Brangwynne CP, MacKintosh FC, Kumar S, Geisse NA, Talbot J, Mahadevan L, Parker KK, Ingber DE, Weitz DA. Microtubules can bear enhanced compressive loads in living cells because of lateral reinforcement. J Cell Biol 2006; 173:733-41; PMID:16754957; http://dx.doi.org/10.1083/jcb.200601060
206. Marsh L, Letourneau PC. Growth of neurites without filopodial or lamellipodial activity in the presence of cytochalasin B. J Cell Biol 1984; 99:2041-7; PMID:6389568; http://dx.doi.org/10.1083/jcb.99.6.2041
207. 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; PMID:8856499
208. Bentley D, Toroian-Raymond A. Disoriented pathfinding by pioneer neurone growth cones deprived of filopodia by cytochalasin treatment. Nature 1986; 323:712-5; PMID:3773996; http://dx.doi.org/10.1038/323712a0
209. Feng Y, Chen MH, Moskowitz IP, Mendonza AM, Vidali L, Nakamura F, Kwiatkowski DJ, Walsh CA. Filamin A (FLNA) is required for cell-cell contact in vascular development and cardiac morphogenesis. Proc Natl Acad Sci U S A 2006; 103:19836-41; PMID:17172441; http://dx.doi.org/10.1073/pnas.0609628104
210. Yamakita Y, Matsumura F, Yamashiro S. Fascin1 is dispensable for mouse development but is favorable for neonatal survival. Cell Motil Cytoskeleton 2009; 66:524-34; PMID:19343791; http://dx.doi.org/10.1002/cm.20356
211. Insall RH, Machesky LM. Actin dynamics at the leading edge: from simple machinery to complex networks. Dev Cell 2009; 17:310-22; PMID:19758556; http://dx.doi.org/10.1016/j.devcel.2009.08.012
212. Kuhn TB, Bamburg JR. Tropomyosin and ADF/cofilin as collaborators and competitors. Adv Exp Med Biol 2008; 644:232-49; PMID:19209826; http://dx.doi.org/10.1007/978-0-387-85766-4_18
213. Chan C, Beltzner CC, Pollard TD. Cofilin dissociates Arp2/3 complex and branches from actin filaments. Curr Biol 2009; 19:537-45; PMID:19362000; http://dx.doi.org/10.1016/j.cub.2009.02.060
214. Saarikangas J, Zhao H, Lappalainen P. Regulation of the actin cytoskeleton-plasma membrane interplay by phosphoinositides. Physiol Rev 2010; 90:259-89; PMID:20086078; http://dx.doi.org/10.1152/physrev.00036.2009
215. Gómez-Moutón C, Lacalle RA, Mira E, Jiménez-Baranda S, Barber DF, Carrera AC, Martínez-A C, Mañes S. Dynamic redistribution of raft domains as an organizing platform for signaling during cell chemotaxis. J Cell Biol 2004; 164:759-68; PMID:14981096; http://dx.doi.org/10.1083/jcb.200309101
216. Kamiguchi H. The region-specific activities of lipid rafts during axon growth and guidance. J Neurochem 2006; 98:330-5; PMID:16805828; http://dx.doi.org/10.1111/j.1471-4159.2006.03888.x
217. Bugyi B, Carlier MF. Control of actin filament treadmilling in cell motility. Annu Rev Biophys 2010; 39:449-70; PMID:20192778; http://dx.doi.org/10.1146/annurev-biophys-051309-103849
218. Pollard TD, Borisy GG. Cellular motility driven by assembly and disassembly of actin filaments. Cell 2003; 112:453-65; PMID:12600310; http://dx.doi.org/10.1016/S0092-8674(03)00120-X
219. Baas PW. Microtubules and neuronal polarity: lessons from mitosis. Neuron 1999; 22:23-31; PMID:10027286; http://dx.doi.org/10.1016/S0896-6273(00)80675-3
220. Stiess M, Maghelli N, Kapitein LC, Gomis-Rüth S, Wilsch-Bräuninger M, Hoogenraad CC, Tolić-Nørrelykke IM, Bradke F. Axon extension occurs independently of centrosomal microtubule nucleation. Science 2010; 327:704-7; PMID:20056854; http://dx.doi.org/10.1126/science.1182179
221. Dhamodharan R, Wadsworth P. Modulation of microtubule dynamic instability in vivo by brain microtubule associated proteins. J Cell Sci 1995; 108:1679-89; PMID:7615685
222. Siegrist SE, Doe CQ. Microtubule-induced cortical cell polarity. Genes Dev 2007; 21:483-96; PMID:17344411; http://dx.doi.org/10.1101/gad.1511207
223. Gordon-Weeks PR. Microtubules and growth cone function. J Neurobiol 2004; 58:70-83; PMID:14598371; http://dx.doi.org/10.1002/neu.10266
224. Buck KB, Zheng JQ. Growth cone turning induced by direct local modification of microtubule dynamics. J Neurosci 2002; 22:9358-67; PMID:12417661