Cytoskeletons in neuronal development

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Abstract Neuronal networks in our brain and body are fundamental for sensation, behavior, and all other functions based on neuronal activity. To construct precisely wired networks, the development of neurons is tightly controlled. During development, neurons must alter their shape so as to be able to migrate toward the destination from their origin and to make contact, called a synapse, with a defined target a certain distance away from them. Like the skeleton in our body, neurons have a cytoskeleton to maintain their shape. The cytoskeleton not only supports this shape, but also helps regulate the motility of neurons; and in this regard, the cytoskeleton is comparable to muscle. Therefore, orchestrating the reorganization of the cytoskeleton is of great importance in allowing extensive changes in neuronal morphology during development. In this review, we highlight the crucial roles of the cytoskeleton and associated proteins in each phase of neuronal development, including neurogenesis, neuronal migration, neurite genesis and growth, and synapse formation.

Keywords : cytoskeleton, actin filament, microtubule, neuronal development

Introduction

An intricately and precisely wired neural network consisting of billions of neurons is essential for proper functioning of the nervous system. The process of establishing functional neural networks is called neuronal development. There are several phases of neuronal development, including neurogenesis, neuronal migration, neurite genesis and growth, and synaptogenesis. During development, neurons must undergo extensive morphological changes. For example, in the cerebral cortex, neurons just after birth, i.e., those generated by the asymmetric division of radial glial cells, take on a multipolar morphology, and subsequently, these cells convert into a bipolar morphology (with both leading and trailing processes) (Fig. 1). These neurons then start migration, for which the coordinated movement of both processes and the soma is required. After reaching the destination, the leading process differentiates into branched dendrites, and the trailing process develops into a long axon^{1,2)}. To achieve such significant changes in cell shape, cytoskeletal dynamics must be appropriately regulated.

Eukaryotic cells contain three main types of cytoskeletal filaments. Of these, microfilaments (or actin filaments) and microtubules have been extensively studied in neuronal cells. Globular actin polymerizes to form actin filaments. This linear filament has structural polarity: the filament grows at the plus end (or barbed end) and shrinks at the minus end (or pointed end). Microtubules consist

of α - and β -tubulin heterodimers and are also structurally polarized filaments: the growth rate is significantly more rapid at the plus end than the minus end. A tubulin polymer is easily disrupted at the minus end without minus end capping proteins such as the γ -tubulin ring complex. Actin filaments and microtubules function as “skeletons” for the cell, which establish and maintain cell shape. Furthermore, these cytoskeletons, in concert with associated proteins, play crucial roles in dynamic cellular events including cell division, cell motility, and organelle movement. Defects in genes encoding proteins required for the cytoskeleton can cause severe brain malformations such as lissencephaly and a double cortex, clearly demonstrating that cytoskeletal regulation is of great importance in neuronal development.

This review summarizes crucial roles of cytoskeletons and their associated proteins in each phase of neuronal development, including neurogenesis, neuronal migration, neurite genesis and growth, and synaptogenesis.

Neurogenesis

Neurons and macro glial cells in the central nervous system are derived from ectoderm, and more specifically, from neuroepithelial cells in the neural tube. At the onset of neurogenesis, neuroepithelial cells differentiate into radial glial cells in the cerebral cortex. Asymmetric division of radial glial cells produces two daughter cells, one of which retains the identity of the mother cell and the other develops into a neuron (Fig. 1).

Defects in proteins that regulate mitosis and cytokinesis

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Fig. 1 Morphological changes of newborn neurons in the cerebral cortex.

Asymmetric division of a radial glial cell (RG) generates a daughter radial glia and neuron that takes on multipolar morphology (MP). Multipolar cells then transform into bipolar cells (BP), and start migrating toward their destination.

result in brain disorders such as lissencephaly and microcephaly probably because of a decreased number of neuroepithelial cells and thus of neurons and glial cells^{3,4}. Microtubules and actin filaments, together with their associated proteins, play indispensable roles in cell proliferation. For example, perturbing the function of LIS1, a regulator of the microtubule-associated motor protein dynein, disrupts cell division by inducing random cleavage planes^{5,6}.

Abnormal spindle-like microcephaly-associated (ASPM) protein is enriched at the poles of mitotic spindles and localizes to the minus ends of central spindle microtubules⁷. Mutations in ASPM cause microcephaly, because ASPM is involved in microtubule organization during spindle formation and cytokinesis⁸. Other proteins regulating spindle formation are also implicated in microcephaly^{2,4}.

Recently, we identified a novel mitotic spindle protein, radmis⁹. This novel protein contributes to mitotic spindle formation via regulating microtubule stability. Gain-of-function mutation of radmis results in shrinkage of the embryonic subventricular zone by inhibiting proliferation of neuronal precursor cells. This inhibition is because of hyperstabilization of microtubules that causes mitotic arrest. Conversely, loss-of-function mutation of radmis disturbs proliferation of neuronal precursor cells *in vivo*, most likely by decreasing microtubule stability leading to abnormal multipolar spindles. These observations clearly demonstrate the necessity of a strictly regulated level of radmis in early neuronal development.

At the stage of cytokinesis, actin and myosin accumulate at the cell division plane called the cleavage furrow. The contraction force generated by actin and myosin is essential for splitting the membrane and thus for complet-

ing cell division. One example demonstrating the importance of myosin in neurogenesis comes from mice lacking citron kinase, a Rho-dependent kinase that regulates myosin activity^{10,11}. Citron kinase knock out mice show ataxia and epilepsy. Their brain shows specific loss of neuronal populations including hippocampal and cerebellar granule cells¹¹. Many other actin- and myosin-associated proteins including small GTPases are also implicated in cell division¹², indicating the importance of proper regulation of actin filaments for neurogenesis.

Neuronal Migration

Neuroepithelial cells and radial glial cells exhibit polarity, with distinctive apical and basal processes. Most of the pyramidal neurons in the cerebral cortex are derived from intermediate progenitor cells generated by the asymmetric division of radial glial cells^{13,14}. These intermediate progenitor cells have to delaminate from the ventricular zone for basal migration and differentiation. A recent study using a chicken and mouse neural tube as a model system showed that actomyosin-dependent contractility mediates apical abscission and delamination¹⁵, resulting in brain malformation if the intermediate progenitors fail to delaminate¹⁶. Differentiated neurons in the cerebral cortex first take on a multipolar morphology and then a bipolar morphology, with both leading and trailing processes. After establishing a bipolar morphology, neurons start their migration toward their destination either dependent or independent upon basal processes of the radial glial cells^{17,18}.

Neuronal migration consists of several phases: neurons extend the leading process; cytoplasmic dilation (or swelling) is formed at the proximal region of the leading process; the nucleus migrates toward the cytoplasmic dilation; and finally the cell soma moves forward (Fig. 2A). Coordinated cytoskeletal reorganization is necessary in all phases. For example, filamin A, an actin cross-linking protein, and LIS1 are implicated in formation of the leading process^{19,20}. DCX (XLIS) and p21^{Kip1} are required for dilation formation²¹, indicative of its dependence upon both actin filaments and microtubules. In addition, nucleokinesis and soma movement rely on actin filaments and microtubules and their associated motors. The centrosome is often positioned ahead of the nucleus²². Microtubules expanded out from the centrosome direct their plus ends toward the cell rear encompassing the nucleus (Fig. 2B). This microtubule "cage" holds the nucleus and dynein motors are assumed to function as a force generator for nucleokinesis²². However, several studies have reported that the centrosome can position at the rear of the nucleus, suggesting a mixed orientation of microtubules surrounding the nucleus²³⁻²⁵. In this case, nucleokinesis should be powered by protein motors other than dynein, with the actomyosin system as the likely candidate. High myosin activity is observed at three different locations in

Fig. 2 Schematic drawing of neuronal migration.

(A) Neuronal migration can be divided into several phases as follows: neurons extend the leading process; cytoplasmic dilation (or swelling) occurs at the proximal region of the leading process; the nucleus migrates toward the dilation; and the cell soma moves forward. (B) Microtubules expand out from the centrosome, positioned ahead of the nucleus to form a microtubule “cage” to hold the nucleus. High myosin activity is observed at three different locations in migrating neurons: the distal and proximal parts of the leading process, and the trailing process. Contraction at the leading process pulls the nucleus, whereas force at the trailing process is assumed to push the nucleus.

migrating neurons: the distal and proximal parts of the leading process, and the trailing process²⁶⁻²⁸). While contraction centers at the leading process pulling the nucleus, the trailing process is assumed to push the nucleus. A recent study using traction force microscopy revealed a temporal correlation between these contraction activities and soma translocation²⁹). This study also reported that myosin-dependent contractility needs microtubules, demonstrating the importance of the coordinated regulation of two cytoskeletal elements in neuronal migration.

Polarization

In general, a single long axon and several branched dendrites emerge from one neuron. These morphological differences may be because of microtubule organization; axonal microtubules are uniformly plus end distal, whereas those in dendrites exhibit a mixed orientation. Axons function as output devices sending information to the target cell. Dendrites receive and process the information, determining whether the neuron generates an action potential or not. Establishing these morphologically and functionally distinct neurites is called neuronal polarization. Till date, numerous studies have been conducted to understand the mechanisms of polarization, with many of those utilizing a dissociated hippocampal neuron culture as a model system. Morphogenesis of hippocampal neurons *in vitro* is divided into five stages (Fig. 3): neurons form several thin filopodia (stage 1), neurons form several immature neurites, so-called minor processes (stage 2), one long neurite (future axon) and several minor processes (stage 3), rapid growth of axon and several dendrites (stage 4), neurons differentiate as a branched

axon and dendrites with premature spines (stage 5). According to this classification, axon formation occurring at stage 3 is the initial event of polarization. Regulating cytoskeletal dynamics at the tip of the neurite seems to be important for axon specification. There is a highly motile structure called the growth cone at the tip of a developing neurite. The growth cone is subdivided into two domains, the central (C)- and peripheral (P)-domains, and the boundary is called the transition (T)-zone. The C-domain contains microtubules, and the P-domain is enriched in actin filaments that form both a meshwork in veil-like lamellipodia and bundles in finger-like filopodia (Fig. 4). In growth cones, cytoskeletal components, actin filaments and microtubules, are highly dynamic. Myosin motor-dependent actin retrograde flow in both filopodia and lamellipodia³⁰) is continual, and supported by treadmilling, i.e., cycles of net assembly of actin filaments at the leading edge, followed by retrograde movement of actin filament networks and then disassembly of actin filaments proximally³¹). While many microtubules reside within the C-domain, some of them occasionally extend into the P-domain. Microtubules which advance into the P-domain are controlled by the balance of several different factors: polymerization, depolymerization, and motor proteins at the T-zone pushing microtubules antero- or retrogradely. Cytoplasmic dynein pushes microtubules toward the leading edge, supporting distal translocation³²), whereas myosin motors push back microtubules toward the C-domain because microtubules associate with actin filaments³³).

Studies utilizing drug-induced localized changes affecting the dynamics of actin filaments and microtubules in growth cones revealed that appropriate regulation of those cytoskeletons are of great importance in axon for-

Fig. 3 Schematic drawing of neuronal polarization *in vitro*.

Neurons exhibit several thin filopodia (stage 1), then extend several immature processes (stage 2). One neurite grows faster than the other, which will then develop into an axon (stage 3). The remaining several immature processes develop into dendrites (stage 4). Finally, neurons develop a branched axon and the dendrites with premature spines (stage 5).

Fig. 4 Schematic drawing of growth cone.

The growth cone is subdivided into two domains, the central (C)- and the peripheral (P)-domains, with the boundary known as the transition (T)-zone. The C-domain contains microtubules, and the P-domain is enriched in actin filaments, which form both a meshwork in veil-like lamellipodia and bundles in finger-like filopodia.

mation. Local application of cytochalasin D, an actin-depolymerizing drug, to the growth cone of a neurite causes the neurite to develop into an axon³⁴). Microtubule stabilization in a growth cone induced by uncaging of caged taxol is sufficient for axon formation³⁵). In addition, many proteins that regulate cytoskeletal dynamics have been identified as polarization regulators. Rac1, Cdc42, and RhoA are well-studied members of the Rho family of small GTPases. Rac1 activation likely causes axon formation by facilitating actin remodeling in growth cones in a manner dependent on WAVE complex and Arp2/3³⁶). Hippocampal neurons from Cdc42 knock out mice can sprout neurites but exhibit severe defects in axon formation, whereas cofilin, an actin-depolymerizing protein, is probably involved in Cdc42-dependent axon formation³⁷). Based on the following findings, a positive feedback loop between Rac1 and Cdc42 is proposed for axon specification: Rac1 can bind to and activate phosphatidylinositol 3-kinase (PI3K)^{38,39}), PI3K activates Cdc42 via its product phosphatidylinositol-3,4,5-trisphosphate⁴⁰); whereas, Cdc42 activates Rac GEFs⁴¹). Inhibition of Rho-kinase, a downstream effector of RhoA, results in the formation of multiple axons⁴²). Because expression of either constitutively active or dominant negative forms of these Rho GTPases causes defects in polarization, the balance of activity is essential.

Several lines of evidence indicate a crucial role of glycogen synthase kinase (GSK) 3 β , a kinase regulating sev-

eral microtubule-associated proteins (MAPs), in neuronal polarization. Inhibition of GSK3 β causes the formation of multiple axons, whereas expression of the constitutively active form of GSK3 β prevents axon formation⁴³. Moreover, phosphorylated GSK3 β that distributes evenly throughout every tip of immature neurites at stage 2, concentrates at growth cones of future axons in stage 3 neurons⁴³⁻⁴⁵, demonstrating the importance of microtubule regulation in growth cones. GSK3 β substrates, including collapsing response mediator protein 2 (CRMP2) and adenomatous polyposis coli (APC), have been implicated in neuronal polarization. CRMP2 binds tubulin heterodimers and promotes microtubule polymerization⁴⁵. This polymerizing activity is inhibited by GSK3 β -mediated phosphorylation⁴⁶. Overexpression of CRMP2 is sufficient for inducing multiple axons⁴⁷. Furthermore, the non-phosphorylated form of CRMP2, that alone causes multiple axons, counteracts axon formation failure induced by constitutively active GSK3 β , suggesting the involvement of GSK3 β -dependent phosphorylation of CRMP2 in neuronal polarity formation⁴⁵. APC also promotes microtubule polymerization, and GSK3 β -mediated phosphorylation negatively regulates microtubule binding of APC⁴⁸. Accumulation of APC at the tip of one neurite may be essential for axon specification, because perturbing the polarized distribution of APC results in multiple axon formation⁴⁴. Polarized targeting of APC is suggested to be regulated by GSK3 β . Although these findings indicate a necessity for APC, a more recent study claims that APC is dispensable for neuronal polarity formation⁴⁹. Other microtubule-associated proteins such as tau and MAP1B are also implicated in the polarization pathway⁵⁰⁻⁵², although the involvement of GSK3 β remains to be revealed.

In contrast to neurons in culture, neuronal polarization *in vivo* does not always begin with axon formation⁵³. For example, retinal ganglion cells inherit apico-basal polarity from their mother cells because they are directly generated from neuroepithelial cells without a transition from their multipolar morphology⁵⁴. The basal and apical processes of neuroepithelial cells develop, respectively, into axons and dendrites of retinal ganglion cells⁵⁵. In the cerebral cortex, neurons exhibit a morphological change from a multipolar to bipolar shape, corresponding to a stage 2 to stage 3 transition *in vitro*. As in the case of a dissociated culture, many multipolar cells extend the trailing process first, which will become axons, and then the leading process, developing into dendrites⁵⁶. Another population of multipolar cells, fewer than 30% in proportion, initially generates the leading process followed by formation of the trailing process. Although studies of neuronal polarization *in vivo* remain under development, many molecules such as Rac, Cdc42, and CRMP2 that have been shown to regulate axon formation *in vitro*, have also been implicated in a multipolar to bipolar transition *in vivo*^{36,57,58}. Therefore, similar machinery may operate in *in vitro* and *in vivo*.

Neurite growth

Following migration, neurons extend axons and dendrites. To establish functional connections to target cells, axons not only elongate but also navigate toward the defined target, a process known as axon guidance. Also, dendrites must elongate and arborize to allow thousands of axon terminals to synapse onto dendrites of a single neuron.

Axon growth and guidance

Forward migration of the growth cone is essential for axon elongation, and a growth cone migration model has been proposed based on findings mainly from *in vitro* studies. The main engine for migration is the myosin motor: once adhesion molecules expressed on the plasma membrane associate with actin filaments via the molecular clutch, actin filament networks are coupled to the extracellular environment. This association transmits the myosin-generated retrograde force into a driving force for growth cone forward migration³⁰. For continuous migration, actin filament treadmilling, microtubule polymerization, the attach-and-detach cycle, and plasma membrane expansion by insertion of intracellular vesicles should be appropriately organized. There are several types of molecular clutch that specifically contribute to the association of actin filaments to certain types of adhesion molecules. In integrin-dependent adhesion, the molecular complex, called "focal contact" or "focal adhesion", works as a clutch that is composed of many proteins including focal adhesion kinase, src kinase, paxillin, and vinculin⁵⁹. For L1 or N-cadherin, shootin-1 or catenin, respectively, are reported to be involved in the clutch in growth cones^{60,61}. Consistent with this model, perturbation of the above-mentioned molecules hinders axon growth. In addition, this model of growth cone migration leads to the hypothesis that spatially asymmetric changes in cytoskeletal and/or adhesion dynamics cause turning of the growth cone. In fact, pharmacological perturbation of actin filaments or microtubule dynamics on one side of the growth cone elicits its turning. An artificially-generated extracellular gradient of cytochalasin B, an inhibitor of actin filament polymerization, or of ML-7, a myosin motor inhibitor, elicits repulsive turning^{62,63}, whereas a gradient of taxol, an MT stabilizer, or of nocodazole, an MT destabilizer, induces attraction or repulsion, respectively⁶⁴. Furthermore, local disruption of focal complex makes the growth cone turn toward the opposite side⁶⁵. Taken collectively, these findings demonstrate that regulating the dynamics of cytoskeletal and cytoskeleton-extracellular environment coupling is of great importance in the navigation of a growth cone.

Our recent findings proposed a novel interaction between microtubules and actin filaments (Akiyama and Kamiguchi, unpublished observation). We found that con-

tact of an extending microtubule with the leading edge of growth cones induces localized membrane protrusion at the site of microtubule contact, suggesting microtubule-dependent activation of a growing actin filament network. We further revealed that such contact-induced protrusion requires delivery of intracellular membranous vesicles that have been centrifugally transported along the microtubule. Therefore, these vesicles are assumed to contain molecules that promote actin filament growth, such as small GTPases. Because Rac1 and Cdc42 have prenylation sequences⁶⁶, they can associate with vesicles after being modified. Upon delivery of a vesicle to the leading edge, these GTPases can locally facilitate actin filament polymerization, which would result in membrane protrusion. Axon growth slows when delivery of intracellular vesicles is inhibited, supporting the importance of membrane trafficking in elongation⁶⁷. Moreover, asymmetric facilitation or inhibition of microtubule-induced lamellipodial protrusion across the growth cone is sufficient for inducing attractive or repulsive turning, respectively (Akiyama and Kamiguchi, unpublished observation).

Growth cones *in vivo* must sense and respond to environmental guidance cues to follow a correct path toward a particular destination. Numerous studies have demonstrated that guidance cues modulate cytoskeletal dynamics in growth cones, and aberrant regulation causes misguidance in many brain and spinal regions^{68,69}. For example, netrin-1, which attracts axons by activating its receptor deleted in colorectal cancer, requires several actin-regulating molecules including Rac1, Cdc42, and N-WASP⁷⁰. Also, the regulation of microtubule dynamics is involved in signaling pathway mediating growth cone attraction^{64,71,72}. Similar to attractive guidance cues, repulsive cues regulate cytoskeletal dynamics. Slit, involved in commissural axon guidance, decreases actin filaments in growth cones, likely in a cofilin-dependent manner, causing repulsion and collapse of the growth cone⁷³. Activation of plexin receptors by binding of Semaphorin 3A results in depolymerization of the actin filament and microtubule in a manner dependent on myosin motors, RhoA, and CRMP2^{74,75}. Collectively, these findings indicate the critical importance of cytoskeletal regulation in cue-induced growth cone navigation.

Dendrite growth and arborization

Dendrites extending from the soma must arborize to fill up the target field. Therefore, branching occurs not once, but several times. Dendrite branching occurs primarily by interstitial branching, whereby branches emerge from the side of existing dendrite shafts. Such side branches initially appear as filopodia, and then develop into growth cone-like structures which extend to develop into stable branches⁷⁶. Similar to axon elongation, small GTPases are implicated in dendrite development. Less-branched dendrites were observed in neurons expressing either in-

active Rac or constitutively active Rho^{77,78}. Hyperactivation of Cdc42, because of a mutation in a GAP for Cdc42, shortened dendrites of cortical neurons⁷⁹. Knock down of Tiam1, a GEF for Rac1, resulted in aberrant dendritic arborization⁸⁰. These results demonstrate that the balanced activity of Rac1, Cdc42, and RhoA is essential for proper development of dendrites.

To develop into stable branches, actin-based protrusions are assumed to be supported by microtubules after formation. A result consistent with this idea is from a study using *Drosophila*: centrosomin suppresses dendritic arborization by inhibiting microtubule innervation into nascent branches⁸¹. A knock out of microtubule-actin crosslinking factor 1 decreased dendritic branching⁸², further supporting the notion that both actin filaments and microtubules are essential for stable branch formation. Microtubule motor functions are also implicated in dendrite development. Neurons derived from LIS1 mutant mice demonstrated less branched dendrites^{83,84}. Besides dynein, kinesin motors may also contribute to branch formation via the transportation of molecules mediating dendritic growth^{85,86}. Dependence on both plus- and minus-end directed motors may be explained by the mixed orientation of microtubules in dendrites.

As in the case of axon guidance, extracellular cues control the direction of dendrite elongation, although their effects on dendrites are not always the same as that on axons⁸⁷. Semaphorin 3A, a repellent for axons, attracts dendrites of cortical neurons, and also facilitates branch formation^{88,89}. Dendritic growth mediated by Semaphorin 3A is likely to include Rac1-dependent regulation of actin filaments⁹⁰. Abnormal orientation of dendrites in neurons lacking GSK3 suggests that microtubule-regulating proteins such as CRMP2 are also involved in dendrite development⁹¹.

Dendrites stop growing upon contact with dendritic branches, which have emerged from the same neuron or from neighboring neurons, allowing dendrites to spread non-redundantly. This dendritic "tiling" is mediated through a contact-dependent inhibition mechanism, sometimes accompanying retraction of either branch. Contact-induced branch retraction requires protein kinase D activity⁹², suggesting the involvement of actin-regulating molecules such as cofilin and cortactin^{93,94}.

Synaptogenesis

A typical chemical synapse consists of a presynaptic terminal, postsynaptic structure such as a dendritic spine, and an astrocyte process surrounding the synaptic cleft. Although numerous papers have demonstrated crucial roles of astrocytes in synaptic development and function⁹⁵⁻⁹⁷, here, we only examine the neuronal structure. Filopodia emerging from axons develop into presynaptic boutons which contain particular synaptic proteins^{98,99} (Fig. 5). On the other hand, dendritic filopodia are precursors of postsynaptic spines^{78,100-102}. Upon contact with

Fig. 5 Schematic drawing of synapse formation. After making contact, two filopodia emerge with the axon and dendrite developing into a presynaptic terminal and postsynaptic spine, respectively.

a presynaptic bouton, dendritic filopodia convert into spines¹⁰³⁻¹⁰⁶. This association critically relies on adhesive molecules. For example, heterophilic adhesion between neuroligin at the presynapse and neuroligin at the postsynapse stimulates synapse formation¹⁰⁷. In addition, homophilic adhesion through cadherins mediates spinogenesis in developmental and postnatal neurons¹⁰⁸. Knock down of β -catenin prevents morphological changes of the spine, demonstrating the involvement of actin filaments in cadherin-dependent spinogenesis. Defects in these adhesive molecules result in neuropsychiatric disorders including autism and schizophrenia, most likely because of aberrant synapse formation^{109,110}.

The first step of synaptogenesis is the formation of filopodia from both axons and dendrites. A filopodium is supported by bundled actin filaments with the rapidly polymerizing barbed ends pointed toward the tip of the filopodium. To form a filopodium, actin nucleation, filament elongation, and filament bundling are all necessary.

Molecules that regulate these events are thus required for filopodia formation. Formins and Arp2/3 are responsible for nucleation, although the precise mechanisms by which these nucleators provide actin seeds for bundles supporting filopodia remain controversial¹¹¹⁻¹¹³. Anti-capping proteins such as Ena/VASP promote filament elongation by shielding the polymerizing barbed ends from capping proteins; and Ena/VASP loss-of-function interferes with filopodia formation¹¹⁴⁻¹¹⁶. Eps8 is another class of protein that exhibits barbed end-binding ability. In primary hippocampal neurons, genetic removal of *Eps8* increases the formation of axonal and dendritic filopodia¹¹⁷, as expected from its capping activity. The polymerized long actin filaments are stabilized by bundling proteins such as fascin, and thus able to support filopodia¹¹⁸. The axonal filopodia contain fascin, whereas the dendritic filopodia do not¹¹⁹, which might explain why more branched actin filaments are observed in dendritic filopodia. Drebrin is probably responsible for actin bundling in dendritic filopodia¹²⁰, and thereby plays a crucial role in spine morphogenesis¹²¹. Although filopodia are indispensable structures for synaptogenesis, filopodial motility seems to inversely correlate with axo-dendritic contact formation¹⁰⁶, suggesting the importance of balanced control of actin filament dynamics; indeed, higher stability would favor contact formation rather than filopodia initiation.

After contact formation between two filopodia from an axon and a dendrite, particular proteins should be transported toward and accumulated at the nascent contact so that the contact develops into a mature synapse. Because synaptic proteins are translated at the soma, microtubule-dependent long distance transport is necessary^{122,123}. Mice lacking either KIF1A or KIF1B exhibit a reduced number of presynaptic terminals and a reduced density of synaptic vesicles at the terminals^{124,125}. Transport of SNARE proteins, SNAP25 and syntaxin-1, which mediate exocytosis of synaptic vesicles, depends on KIF5 motors. SNAP25 can directly bind to KIF5, whereas syntaxin-1 needs an adaptor protein, syntabulin for its binding^{126,127}. KIF5 also contributes to the transport of postsynaptic proteins. Overexpression of dominant negative KIF5, that lacks the motor domain, reduced the density of GluA2 at synapses, suggesting the necessity of KIF5 for AMPA receptor function¹²⁸. Unlike KIF5, which is expressed in both axons and dendrites, KIF17 is relatively enriched in dendrites and regulates synaptic targeting of GluN2B, an NMDA receptor subunit¹²⁹. Collectively, these findings demonstrate that microtubule-associated motors are indispensable for transport of both pre- and post-synaptic proteins, and thus for synapse maturation.

In addition, it has been reported that actin-based motors are also involved in synaptic cargo transport^{122,123}. Actin depolymerizing drugs inhibit the accumulation of synaptic vesicles and other presynaptic components¹³⁰. Consistent with this observation, myosin V was found to associate with synaptic vesicles, and co-immunoprecipitated with

the synaptic vesicle proteins synaptobrevin-2 and synaptophysin. Furthermore, the inhibition of myosin ATPase activity reduced neurotransmitter release^{131,132}. More direct evidence indicating the involvement of myosin motors in synaptic cargo trafficking comes from a study conducted on hippocampal neurons in culture¹³³. Overexpression of motor-domain lacking myosin Va significantly impairs the movement of large dense core vesicles in both dendrites and axons of cultured hippocampal neurons. Myosin V is also implicated in the post-synaptic trafficking of receptors for neurotransmitters, which may have a role in the maintenance and plastic changes of functional synapses¹²². Because myosin V can directly interact with a kinesin motor to form a hetero-motor complex¹³⁴, the dual motor complex may facilitate the coordination of long-range transport along microtubules and short-range movement on actin filaments.

Concluding remarks

A large number of studies have revealed crucial roles of cytoskeletons and their associated proteins in all phases of neuronal development. Cytoskeletal reorganization is essential for both the morphological changes of developing neurons and for accurately coordinating motility among subregions in a single neuron that is required for migration, polarization, and subsequent neurite growth. Furthermore, cytoskeletons act as tracks, supporting appropriate delivery of particular molecules to defined sites. Defects in any of these functions can result in brain malformations and neuropsychiatric diseases. Although numerous molecules have been shown to be involved in signaling cascades that mediate neuronal development, much of this knowledge is provided by studies *in vitro* or *ex vivo*. Recent advances in live cell imaging techniques have allowed us to measure molecular activities of cells *in vivo*. Findings from studies employing these new techniques will provide a more complete picture of neuronal development, thus contributing to a better understanding of pathogenesis of brain diseases and also the development of new therapeutic approaches.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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