

Actin in dendritic spines: connecting dynamics to function

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Dendritic spines are small actin-rich protrusions from neuronal dendrites that form the postsynaptic part of most excitatory synapses and are major sites of information processing and storage in the brain. Changes in the shape and size of dendritic spines are correlated with the strength of excitatory synaptic connections and heavily depend on remodeling of its underlying actin cytoskeleton. Emerging evidence suggests that most signaling pathways linking synaptic activity to spine morphology influence local actin dynamics. Therefore, specific mechanisms of actin regulation are integral to the formation, maturation, and plasticity of dendritic spines and to learning and memory.

Introduction

The human brain consists of a hundred billion neurons interconnected into functional neuronal circuits that underlie all our behaviors, thoughts, emotions, dreams, and memories. The capacity of neurons to function within neuronal circuits is mediated via specialized cell junctions called synapses. Chemical synapses regulate the electric communication within neural networks and pass information directly from presynaptic axon terminals to postsynaptic dendritic regions. Precise control of the development and connectivity of synapses is critical for accurate neural network activity and normal brain function. Most excitatory synapses in the mammalian brain are formed at tiny dendritic protrusions, named dendritic spines (Bourne and Harris, 2008). Experimental evidence has shown that changes in spine morphology account for functional differences at the synaptic level (Yuste and Bonhoeffer, 2001; Kasai et al., 2003). It is now widely believed that information in the brain can be stored by strengthening or weakening existing synapses, as well as appearance or disappearance of dendritic spines, which subsequently leads to the formation or elimination of synapses. These functional and structural changes at spines and synapses are believed to be the basis

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Abbreviations used in this paper: CaMKII β , Ca²⁺-calmodulin-dependent protein kinase II β ; Ena, Enabled; LIMK1, LIM kinase 1; LTP, long-term potentiation; PSD, postsynaptic density; VASP, vasodilator-stimulated phosphoprotein.

of learning and memory in the brain (Holtmaat and Svoboda, 2009; Kasai et al., 2010).

The primary function of dendritic spines is to compartmentalize local synaptic signaling pathways and restrict the diffusion of postsynaptic molecules (Nimchinsky et al., 2002; Newpher and Ehlers, 2009). Because the actin cytoskeleton is central to numerous cellular processes involving membrane dynamics such as cell motility and morphogenesis (Pollard and Borisy, 2003; Carlier and Pantaloni, 2007), it is not surprising that dendritic spine formation and dynamics are determined by the actin cytoskeleton. During the last decade, numerous studies on postsynaptic signaling pathways demonstrated that the actin cytoskeleton plays a pivotal role in the formation and elimination, motility and stability, and size and shape of dendritic spines (Halpain, 2000; Luo, 2002; Ethell and Pasquale, 2005; Tada and Sheng, 2006; Schubert and Dotti, 2007). In addition, modulation of actin dynamics drives the morphological changes in dendritic spines that are associated with alteration in synaptic strength (Matus, 2000; Cingolani and Goda, 2008). At synapses, the actin cytoskeleton does not only contribute to overall structure of synapses but also plays important roles in synaptic activities that range from organizing the postsynaptic density (Sheng and Hoogenraad, 2007) and anchoring postsynaptic receptors (Renner et al., 2008) to facilitating the trafficking of synaptic cargos (Schlager and Hoogenraad, 2009) and localizing the translation machinery (Bramham, 2008). It has also been shown that various memory disorders involve defects in the regulation of the actin cytoskeleton (Newey et al., 2005).

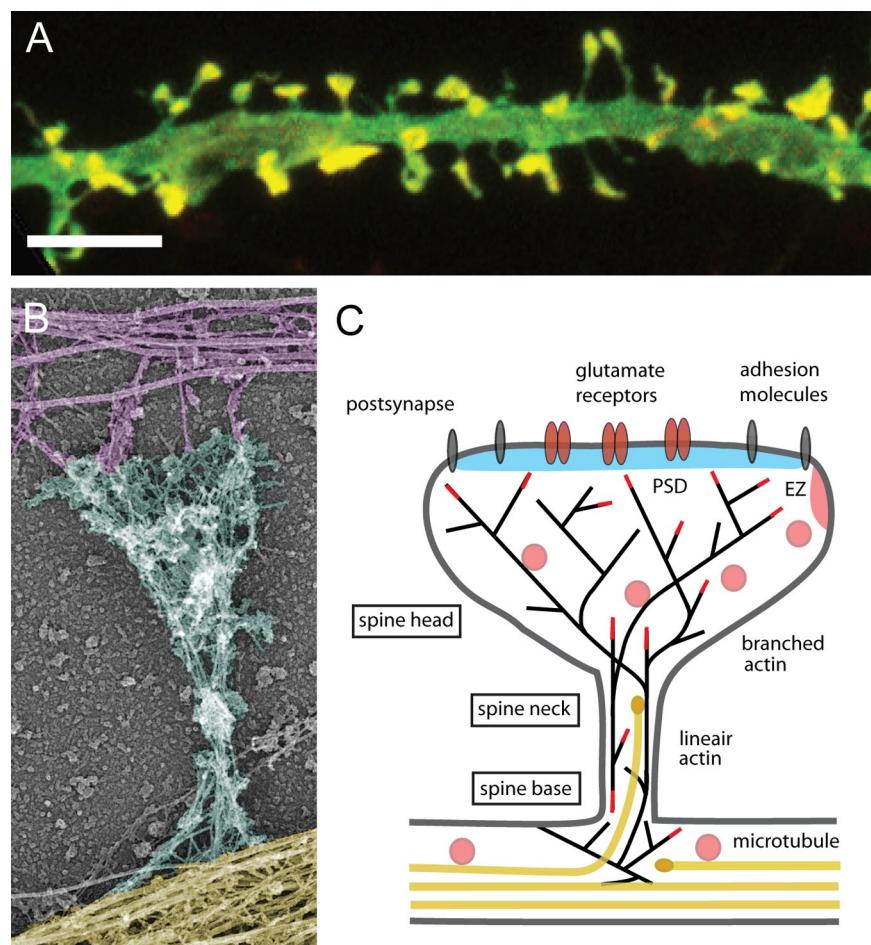
In this review, we discuss evidence for regulatory mechanisms of actin dynamics in dendritic spines. We will describe our current understanding of the organization of actin structures in spines and propose that specific actin signaling pathways regulate filopodia initiation, elongation, and spine head formation.

Dendritic spine structure and function

Dendritic spines are small protrusions that receive input from a single excitatory presynaptic terminal, allowing regulation of synaptic strength on a synapse-by-synapse basis. Spines occur at a density of 1–10 spines per micrometer of dendrite length,

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Figure 1. Cytoskeletal organization of dendritic spines. (A) Dendritic spine morphology (green) and localization of F-actin (red) in cultured hippocampal neurons. Merged red and green are shown in yellow. Bar, 5 μ m. (B) Actin and microtubule cytoskeleton organization in a mature dendritic spine from cultured hippocampal neurons visualized by platinum replica electron microscopy (EM). Axonal cytoskeleton, purple; dendritic shaft, yellow; dendritic spine, cyan. The spine head typically contains a dense network of short cross-linked branched actin filaments, whereas the spine neck contains loosely arranged longitudinal actin filaments, both branched and linear. The base of the spine also contains branched filaments, which frequently reside directly on the microtubule network in the dendritic shaft. Image courtesy of Drs. Farida Korobova and Tatyana Svitkina (University of Pennsylvania, Philadelphia, PA). (C) Schematic diagram of a mature mushroom-shaped spine showing the postsynaptic membrane containing the postsynaptic density (PSD; blue), adhesion molecules (gray) and glutamate receptors (reddish brown), the actin (black lines) and microtubule (yellow) cytoskeleton, and organelles. The endocytic zone (EZ) is located lateral of the PSD in extrasynaptic regions of the spine and recycling endosomes (pink) are found in the shaft and spines. Dendritic spines exhibit a continuous network of both straight and branched actin filaments (black lines). The actin network is spread in the spine base, gets constricted in the neck, undergoes extensive branching at the neck-head junction, and stays highly branched in the spine head. The actin-polymerizing barbed ends are indicated as red lines. Stable microtubule arrays are predominantly present in the dendritic shaft. A small fraction of the microtubules in mature dendrites are dynamic and depart from the dendritic shaft, curve, and transiently enter dendritic spines. The microtubule plus-ends are symbolized as yellow ovals.



and some neurons, such as hippocampal neurons, contain thousands of spines throughout the dendritic arbors (Sorra and Harris, 2000) (Fig. 1 A). Spines consist of three distinct basic compartments: (1) a delta-shaped base at the junction with the dendritic shaft, (2) a constricted neck in the middle, and (3) a bulbous head contacting the axon (Fig. 1 B). They come in a wide range of sizes and shapes, their lengths varying from 0.2 to 2 μ m and volumes from 0.001 to 1 μ m³. Electron microscopy studies have identified roughly three categories of spines based on their morphology; thin, filopodia-like protrusions (“thin spines”), short spines without a well-defined spine neck (“stubby spines”) and spines with a large bulbous head (“mushroom spines”) (Bourne and Harris, 2008). The interesting feature of these spine structures is that they are not static, but change morphology continuously, even throughout adulthood, reflecting the plastic nature of synaptic connections (Grutzendler et al., 2002; Trachtenberg et al., 2002). Live imaging studies of spine dynamics reveal that the morphology of spines can be altered by neuronal activity in vitro and experience in vivo (Matsuzaki et al., 2004; Holtmaat et al., 2006; Roberts et al., 2010). Activity patterns that induce long-term potentiation (LTP), one of the major cellular mechanisms underlying learning and memory, causes enlargement of spine heads, suggesting that changes in dendritic spine morphology play an important role in memory formation (Yuste and Bonhoeffer, 2001; Kasai et al., 2003). Although de novo

formation of dendritic spines in adult mice has been described, most spines are thought to arise from dendritic filopodia during early postnatal life (Holtmaat and Svoboda, 2009; Yoshihara et al., 2009).

Dendritic spines contain the postsynaptic machinery, including glutamate receptors, postsynaptic density (PSD), and actin cytoskeleton, as well as a wide variety of membrane-bound organelles, such as smooth endoplasmic reticulum, mitochondria, and endosomes (Sheng and Hoogenraad, 2007; Fig. 1 C). The PSD is usually found at the tip of the dendritic spine head directly opposed to the presynaptic active zone. The PSD functions as a postsynaptic organizing structure where it clusters receptors, adhesion molecules, and channels and assembles a variety of signaling molecules at the postsynaptic membrane (Kennedy et al., 2005; Renner et al., 2008). Adjacent to the PSD is the endocytic zone, a stable membrane “hot spot” for clathrin-dependent endocytosis of postsynaptic receptors (Blanpied et al., 2002; Rácz et al., 2004). The main function of the endocytic zone is to capture and recycle the synaptic pool of mobile AMPA-type glutamate receptors required for synaptic potentiation (Lu et al., 2007; Petrini et al., 2009). One attractive mechanism for regulating changes in spine morphology is the local addition or removal of synaptic membrane and turnover of postsynaptic receptors. Indeed, synaptic stimulation mobilizes AMPA receptors in the recycling endosomal compartment into spines and leads to

subsequent spine enlargement (Park et al., 2004, 2006). However, most other signaling pathways controlling spine shape seem to converge more directly on the actin cytoskeleton.

Actin organization in dendritic spines

Early electron microscopy studies have shown that actin is the major cytoskeletal component of dendritic spines (Landis and Reese, 1983). A network of long and short branching filaments is present in the spine neck and short branched actin filaments are localized in the spine head just underneath the PSD (Fig. 1 B). The most likely role of actin in mature spines is to stabilize postsynaptic proteins (Allison et al., 1998; Kuriu et al., 2006; Renner et al., 2009) and modulate spine head structure in response to postsynaptic signaling (Fischer et al., 2000; Star et al., 2002; Okamoto et al., 2004). Mass spectrometry analysis of PSD fractions has uncovered large numbers of actin-binding and actin cross-linking proteins, including Ca^{2+} -calmodulin-dependent protein kinase II β (CaMKII β), cortactin, drebrin A, and neurabin I (Cheng et al., 2006). Down-regulation of these proteins reduces the formation and maturation of dendritic spines (Hering and Sheng, 2003; Terry-Lorenzo et al., 2005; Okamoto et al., 2007; Ivanov et al., 2009), which apparently makes them crucial for synaptic plasticity and memory formation (Wu et al., 2008; van Woerden et al., 2009; Kojima et al., 2010).

Several genetic and pharmacological experiments indicate that actin rearrangements drive the formation and loss of dendritic spines as well as their morphological plasticity, including shape, number, and motility (Halpain, 2000; Matus, 2000). Both the monomeric form of actin (G-actin) and filamentous polymers (F-actin) are present in spines and the degree of actin polymerization (and hence the G-actin/F-actin ratio) affects the various aspects of dendritic spine morphology (Cingolani and Goda, 2008). Measurements of fluorescence resonance energy transfer (FRET) between actin monomers revealed that synaptic stimulation rapidly changes the equilibrium between F-actin and G-actin (Okamoto et al., 2004). Long-term potentiation (LTP) induction shifts the G-actin/F-actin ratio toward F-actin (rise in spine actin filaments) and increases spine volume, whereas long-term depression (LTD) induction shifts the ratio toward G-actin (decrease in spine actin filaments) and results in spine shrinkage (Okamoto et al., 2004). To better understand the actin regulatory mechanisms that account for spine plasticity, we first need to consider what is known about the cytoskeleton organization in dendritic spines. Several recent studies present new insights in the organization and molecular composition of actin cytoskeleton in dendritic spines (Honkura et al., 2008; Hotulainen et al., 2009; Korobova and Svitkina, 2010). Using two-photon photoactivation of PAGFP (photoactivatable form of green fluorescent protein) fused to G-actin, the Kasai laboratory visualized the actin cytoskeleton within single dendritic spines at rest and during enlargement and found that three different actin pools dynamically regulate the structure and plasticity of the spines (Honkura et al., 2008). They also demonstrated that the actin pool at the tip of the spine treadmills to generate an expansive force in the spine head and is most likely the major determinant of spine volume. An elegant study by the Svitkina laboratory investigated the cytoskeletal organization of dendritic

spines by electron microscopy and showed that spines exhibit a continuous network of both branched and long, linear actin filaments (Korobova and Svitkina, 2010). Because similar actin structures have been found in other morphogenetic processes, we will compare the recent data in dendritic spines with the actin machinery in lamellipodia and conventional filopodia in fibroblasts (see Fig. 2, A and B).

The spine head contains branched actin filaments. Actin filaments are polar structures with one end growing more rapidly (plus or “barbed” end) than the other (minus or “pointed” end). In migrating cells, the barbed ends push the plasma membrane and induce cell shape changes in the form of sheet- and rod-like extensions, termed lamellipodia and filopodia, respectively (Pollard and Borisy, 2003). Lamellipodia contain short and highly branched actin filaments, whereas filopodia consist of long, unbranched, actin filaments arranged in tight, unipolar, parallel bundles (Le Clainche and Carlier, 2008). In mature spine, the base, neck, and head all consist of a mixture of branched and linear actin filaments; the neck contains different ratios of both linear and branched filaments, whereas most branched actin filaments localize to the distal regions of the spine head (Korobova and Svitkina, 2010). Actin filaments in the spine head are very dynamic and show a high turnover by continuous treadmilling (Star et al., 2002; Honkura et al., 2008). In this way the actin cytoskeleton of mature dendritic spines, especially of the spine head, resembles actin structures found in lamellipodia (Fig. 2 B).

Consistent with this idea, several lamellipodial actin-binding proteins described in fibroblasts are enriched in dendritic spines (Table I). (1) The Arp2/3 complex (a stable complex of seven conserved subunits including the two actin-related proteins Arp2 and Arp3 and as well as ARPC1, ARPC2, ARPC3, ARPC4, and ARPC5) is the main nucleator of actin filaments in lamellipodia and necessary for lamellipodia formation (Hotulainen and Lappalainen, 2006). The Arp2/3 complex binds to the sides of existing actin filaments to nucleate a new filament, which subsequently results in the formation of a branched network of actin filaments (Goley and Welch, 2006). Arp2/3 complex is concentrated in spines (Rácz and Weinberg, 2008) and its knockdown from hippocampal neurons revealed its importance in dendritic spine head formation (Wegner et al., 2008; Hotulainen et al., 2009). Depletion of the Arp2/3 complex activators, including cortactin, Abi2, WAVE-1 (WASP-family verprolin homology protein-1), N-WASP (neural Wiskott-Aldrich syndrome protein), and Abp1 (actin-binding protein 1) alters the morphology and number of spines (Hering and Sheng, 2003; Grove et al., 2004; Kim et al., 2006; Soderling et al., 2007; Haeckel et al., 2008; Wegner et al., 2008). Consistently, WAVE-1 and Abi2 knock-out mice show hippocampal-dependent learning and memory deficits (Grove et al., 2004; Soderling et al., 2007). (2) Profilin I and profilin II are important assistants in actin polymerization. Profilins change actin nucleotide from ADP to ATP to promote barbed-end polymerization (Pollard et al., 2000). Endothelial cells lacking profilin I exhibit defects in membrane protrusion and cell migration (Ding et al., 2006). Profilin II is the major brain isoform, although profilin I is also present (Witke et al., 2001). Interestingly, profilins are localized to only a subset of dendritic spines in resting hippocampal neurons,

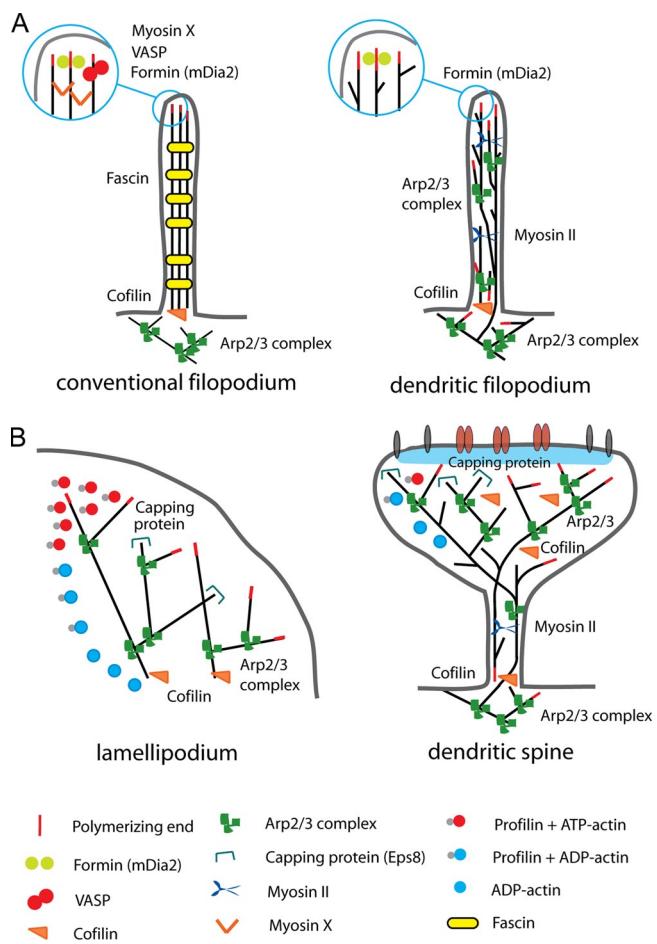


Figure 2. Comparison of actin organization in fibroblast cells and dendritic spines. (A) In conventional filopodia from fibroblast cells, actin filaments are elongated from the tip of filopodia by mDia2, Ena/VASP, and myosin X. Polymerized actin filaments are bundled by fascin. In dendritic filopodia (neurons), mDia2 elongates actin filaments from the tip of filopodia. The functions of Ena/VASP and myosin X have not yet been studied in dendritic spines. In addition to tip polymerization, actin filaments of dendritic filopodia elongate from base. Fascin is absent from dendritic filopodia. (B) In lamellipodia from fibroblast cells, actin filaments are nucleated by Arp2/3 complex. Actin barbed ends are capped by capping protein to maintain filaments short. ADF/cofilins depolymerize pointed ends of actin filaments to replenish the actin monomer pool. Profilins change the ADP to ATP and transport ATP-actin monomers to the free barbed ends. In dendritic spine heads (neurons), functions of Arp2/3 complex and cofilin resemble those in lamellipodia. The function of capping protein has not been investigated. Profilins localize to dendritic spines in an activity-dependent manner.

whereas after chemical or electrical stimulation, profilins target to many more spines (Ackermann and Matus, 2003; Neuhoff et al., 2005). Consistently, fear conditioning in rats led to the relocalization of profilin from the dendritic shaft into dendritic spines (Lamprecht et al., 2006). A peptide competitor of profilin binding to polyproline-rich proteins prevented profilin targeting and destabilized spine structures (Ackermann and Matus, 2003), suggesting a role for profilin in activity-dependent actin regulation. However, detailed genetic studies are missing to clarify the roles of profilins in dendritic spine morphogenesis. (3) Another important factor for actin assembly in lamellipodia is a barbed end-binding protein called capping protein. Capping protein keeps the filaments short and maintains the actin monomer

pool by restricting the polymerization only to the areas where it is required (Pollard and Borisy, 2003). The actin monomer pool is pivotal for the fast reorganization of the actin cytoskeleton. Without capping protein, actin filaments in lamellipodia grow too long, resulting in filopodia formation and reduction of lamellipodia (Mejillano et al., 2004). Capping protein localizes throughout dendritic spines (Korobova and Svitkina, 2010) but no functional studies have been reported. Eps8 (EGF receptor pathway substrate 8), another protein with actin-capping function, has been studied in dendritic spine morphogenesis. Actin-capping activity of Eps8 was shown to be the relevant biochemical activity required to inhibit filopodia formation (Menna et al., 2009). (4) ADF (actin-depolymerizing factor)/cofilins, which induce depolymerization of actin filaments from their pointed ends, are essential for maintaining the cellular actin monomer pool (Hotulainen et al., 2005; Kiuchi et al., 2007). Cofilin depletion from motile fibroblasts induces stabilization of actin filaments, thus “freezing” cell dynamics (Hotulainen et al., 2005). In neurons, cofilin1 is required for proper actin turnover and morphology of the dendritic spines (Hotulainen et al., 2009). Consistently, inactivation of LIM kinase 1 (LIMK1), which inhibits the activity of ADF/cofilins, results in altered dendritic spine morphology and synaptic function (Meng et al., 2002). Consistent with the structural and electrophysiological deficits, LIMK1 knock-out mice exhibited abnormalities in behavioral responses, including altered fear responses and spatial learning.

The spine neck contains branched and linear actin filaments with mixed polarity. In both immature dendritic filopodia and the spine neck of mature spines, the actin filaments are arranged roughly longitudinally along the structure (Korobova and Svitkina, 2010). The straight actin filaments do not span the entire neck, but begin or end with unbound ends or branch off the side of another filament. Surprisingly, the actin filaments in dendritic filopodia (and the mature spine neck) differ from conventional filopodia in many aspects (Fig. 2 A). (1) Conventional filopodia consist of stiff bundles of straight actin filaments. Dendritic filopodia are made of a mixture of branched and straight actin filaments, but not to the extent of forming a tight bundle (Korobova and Svitkina, 2010). Consistently, the conventional actin filament bundling protein fascin is absent from the dendritic filopodia, whereas the Arp2/3 complex and capping protein are present (Korobova and Svitkina, 2010). (2) Actin filaments of conventional filopodia are polymerized on the tip of filopodia, whereas actin filaments in dendritic filopodia are polymerized from both the tip and the base (Hotulainen et al., 2009). (3) Conventional filopodia consist of parallel, unipolar arrays of actin filaments. Actin filaments of dendritic filopodia exhibit mixed polarity, although the predominant orientation is with the barbed end pointing toward the tip of filopodia (Hotulainen et al., 2009; Korobova and Svitkina, 2010). (4) The actin-dependent motor myosin II that binds and contracts actin filaments is absent from conventional filopodia but present in the dendritic filopodia (Korobova and Svitkina, 2010), consistent with the presence of anti-parallel actin filaments in dendritic filopodia. Despite these major differences, some similarities can be found between conventional filopodia and

Table I. Actin-binding proteins and signaling pathways in dendritic spine morphogenesis

Protein	Effect on actin cytoskeleton ^a	Function in dendritic spine morphogenesis	Signaling pathway ^a
Nucleating			
Arp2/3 complex	Nucleates branched actin filaments	Required for spine head growth (Wegner et al., 2008; Hotulainen et al., 2009)	Rac and Cdc42 signaling cascades activate Arp2/3 complex
DRF3/ mDia2	Induces elongation of straight actin filaments	Required for proper dendritic filopodia and spine neck formation (Hotulainen et al., 2009)	Rif activates DRF3/mDia2
Proteins regulating Arp2/3 complex			
Cortactin	Activates Arp2/3 complex	Regulates spine density; binds Shank (Hering and Sheng, 2003)	Src family kinases activate cortactin
N-WASP	Activates Arp2/3 complex	Regulates spine density (Wegner et al., 2008)	Cdc42, Rac, and PIP ₂ activate N-WASP
WAVE-1	Activates Arp2/3 complex	Regulates spine density (Soderling et al., 2007)	Rac and PIP ₃ binding activate WAVE-1
Abp1	Controls Arp2/3 via N-WASP	Abp1 expression increases mushroom spine; binds Shank (Haeckel et al., 2008)	
ADP/ATP exchanger			
Profilin	Enhances exchange of ADP to ATP and actin treadmilling rate	Stabilizes spine morphology and moves to spines upon activity (Ackermann and Matus, 2003)	
Depolymerizing			
ADF, Cofilin	Depolymerizes and severs actin filaments	Required for spine head morphology and stabilization during LTP formation (Chen et al., 2007; Hotulainen et al., 2009)	PAK3 phosphorylates LIM kinase, which inactivates ADF/cofilins
Capping			
Eps8	Caps plus-ends of actin filaments	Inhibits BDNF-induced neuronal filopodia formation (Menna et al., 2009)	MAPK phosphorylation inhibits Eps8
Cross-linking			
α -Actinin	Bundles actin filaments	Expression induces spine elongation and thinning (Hoe et al., 2009)	FAK reduces binding of α -actinin to actin
Calponin	Bundles and stabilizes actin filaments	Expression induces spine elongation and increase in density (Rami et al., 2006)	
CaMKII β	Bundles and stabilizes actin filaments	Required for spine maturation and LTP-induced stabilization (Okamoto et al., 2007)	CaMKII β autoinhibition is released by NMDA receptor activation
Neurabin I	Bundles actin filaments	Required for spine maturation (Terry-Lorenzo et al., 2005)	Cdk5 phosphorylation inhibits Neurabin I
Drebrin	Bundles and stabilizes actin filaments	Expression induces spine elongation (Ivanov et al., 2009)	
Motor proteins			
Myosin II	ATP-driven, actin-based motor	Required for proper spine head and neck morphology (Ryu et al., 2006)	RhoA activates myosin II
Myosin VI	ATP-driven, actin-based motor	Required for spine formation (Osterweil et al., 2005)	

^aFor review see Le Clainche and Carlier, 2008.

dendritic filopodia. The formin DRF3/mDia2 (human Diaphanous-related formin 3/mouse Diaphanous 2) is one of the main factors inducing elongation of actin filaments in conventional filopodia. The formin family proteins are known to polymerize straight actin filaments (Paul and Pollard, 2009). Depletion of DRF3/mDia2 from neurons resulted in reduced number of filopodia and abnormal spine neck morphology (Hotulainen et al., 2009). Moreover, Enabled/vasodilator-stimulated phosphoprotein (Ena/VASP) family proteins play an important role in filopodia formation in motile cells. Ena/VASP proteins anti-cap the polymerizing plus-ends of branched actin filaments, resulting in elongation of straight

actin filaments. In the developing cortex, Ena/VASP proteins are required for filopodia formation and neuritogenesis (Dent et al., 2007; Kwiatkowski et al., 2007). Although recent evidence suggests that the transmembrane heparan sulfate proteoglycan Syndecan-2 promotes the formation of filopodia via the Ena/VASP pathway (Lin et al., 2007), the role of Ena/VASP family proteins in dendritic spines has been poorly investigated. In addition to DRF3/mDia2 and Ena/VASP proteins, myosin X has a key role in formation of filopodia in fibroblasts (Bohil et al., 2006). However, there is presently no study published on the role of myosin X in dendritic filopodia formation.

Signaling to the actin cytoskeleton in dendritic spines

The actin-signaling pathways in spines are regulated by many synaptic receptors such as the excitatory NMDA and AMPA-type glutamate receptors (Fischer et al., 2000). NMDA receptor regulates actin cytoskeleton in at least two ways: (1) by mediating the influx of Ca^{2+} ions into postsynaptic neurons, which modulate the activity of many actin binding proteins, e.g., CaMKII β (Lisman et al., 2002) and gelsolin (Nag et al., 2009); and (2) by binding directly to actin-binding or -regulating proteins, e.g., CaMKII (Raveendran et al., 2009), α -actinin (Wyszynski et al., 1997), and myosin regulatory light chain (Bajaj et al., 2009). Also, various receptor tyrosine kinases, such as members of the Trk (BDNF receptor; Menna et al., 2009) and Eph/ephrin families (Schubert and Dotti, 2007), as well as synaptic adhesion molecules (Yoshihara et al., 2009), have been shown to be important in regulating actin in spines. The major signaling hot spots in actin cytoskeleton regulation in fibroblasts are small Rho and Ras GTPases (Ethell and Pasquale, 2005; Tada and Sheng, 2006). Rho GTPases, including RhoA, Rac, and Cdc42 have been extensively studied in neurons and have profound influence on dendritic spine morphogenesis. In dendritic spines, RhoA activation has been shown to be necessary for expression of LTP via cofilin inactivation (Rex et al., 2009). In fibroblasts, Cdc42 induces filopodia formation, but in dendritic spines it induced spine head enlargement (Irie and Yamaguchi, 2002; Wegner et al., 2008; Hotulainen et al., 2009). In a simplified view, RhoA is important to inhibit cofilin activity, which will result in actin filament and spine stabilization. Rac and Cdc42 regulate spine head formation, mainly by activating Arp2/3 complex-induced nucleation and inhibiting actin depolymerization via cofilin. The Rho family GTPase Rif (Rho in filopodia) is an important regulator of filopodia formation (Hotulainen et al., 2009).

The Ras family of GTPases and their downstream signaling pathways as well as many of the Rho and Ras GTPase activators (GTP exchange factors [GEFs]) and inhibitors (GTPase-activating proteins [GAPs]) have been shown to be fundamental for dendritic spine morphology and neuronal functioning (Kennedy et al., 2005). A major postsynaptic inhibitor of Ras signaling is synaptic GAP (SynGAP), which is abundantly enriched in the PSD. Interestingly, heterozygous deletion of SynGAP in mice has been shown to cause an increase in the number of mushroom spines on hippocampal neurons (Vazquez et al., 2004). The spine phenotype is most likely explained by the cross talk of multiple signaling pathways where increased activation of Ras due to the absence of SynGAP leads to the downstream activation of Rac, which subsequently decreases cofilin function (Carlisle et al., 2008). Interestingly, mutations in the several Rho and Ras family GEFs and GAPs have been shown to cause mental retardation (Newey et al., 2005).

Actin dynamics modulate dendritic spine formation during development

During neuronal development upon synaptic contact with the axon, thin and highly motile dendritic filopodia can transform into more stable mushroom spines (Craig et al., 2006; Arikath and Reichardt, 2008; Yoshihara et al., 2009). We will now discuss

specific mechanisms of actin regulation controlling filopodia initiation, elongation, and spine head formation (Fig. 3).

Initiation and elongation of dendritic filopodia.

The molecular mechanisms involved in the initiation of dendritic filopodia are currently unknown. Dendritic filopodia initiate directly from the dendritic shaft, often from preexisting patches of branched actin or from small lamellipodia (Andersen et al., 2005; Korobova and Svitkina, 2010). The actin-rich sites of initiation subsequently become the base of the filopodia. Current literature supports a model of both random initiation and signal-induced initiation. It has been shown that glutamate released from presynaptic sites influences filopodia initiation and elongation (Tashiro et al., 2003; Andersen et al., 2005). Here, we list some possible mechanisms involved in the initiation and elongation process (see Fig. 3, A and B). (1) Ena/VASP-induced anti-capping of filaments generated by the Arp2/3 complex could initiate filament elongation. (2) DRF3/mDia2 formin is important for proper filopodia formation (Hotulainen et al., 2009). It is plausible that also other proteins of the formin family are involved in polymerization of straight actin filaments for filopodia initiation and elongation. (3) Myosin X could initiate filopodia formation. It has been proposed that myosin X moves barbed ends laterally along the leading edge. This lateral movement converges the barbed ends of the actin filaments, thus producing the base of filopodia (Tokuo et al., 2007). (4) Actin filaments frequently reside on and branch off microtubules in the dendritic shaft (Korobova and Svitkina, 2010). In this way, microtubule-associated actin filament nucleators or actin–microtubule cross-linking factors could initiate filopodia formation. (5) Microtubules and actin filament–binding protein septin 7 localizes to the base of dendritic protrusions (Tada et al., 2007). Septins polymerize into heterotrimeric filaments and form small ring structures, which could initiate filopodia formation and work as a “diffusion barrier” in dendritic spine maturation. (6) Filopodia initiation could occur through membrane-deforming proteins that contain I-BAR (inverse Bin/Amphiphysin/Rvs) domains (Saarikangas et al., 2009; Yang et al., 2009). Electrostatic interactions between the positively charged poles of I-BAR domains and the negatively charged PI(4,5)P₂ head groups induce clustering of PI(4,5)P₂ and generation of membrane curvature due to the convex geometry of the lipid-binding interface of the domain (Saarikangas et al., 2009). In cultured neurons, acute depletion of I-BAR-containing protein IRS53 has been shown to affect dendritic spine morphogenesis (Choi et al., 2005).

Formation of the spine head. Once a dendritic filopodia is formed and axonal contact is made, spine motility gradually decreases and the spine structure is stabilized, which requires the assembly of pre- and postsynaptic components (Craig et al., 2006; Arikath and Reichardt, 2008; Yoshihara et al., 2009). Newly formed spines are usually thin and elongated and in general have a small spine head. The Arp2/3 complex and its proper regulation are necessary for spine head growth (Grove et al., 2004; Kim et al., 2006; Soderling et al., 2007; Wegner et al., 2008; Hotulainen et al., 2009) (Fig. 3 C). A recent study showed that a complex with WAVE and Arp2/3 inhibits DRF3/mDia2-induced filopodia formation in fibroblast cells (Beli et al., 2008). This is an attractive mechanism to induce a switch from

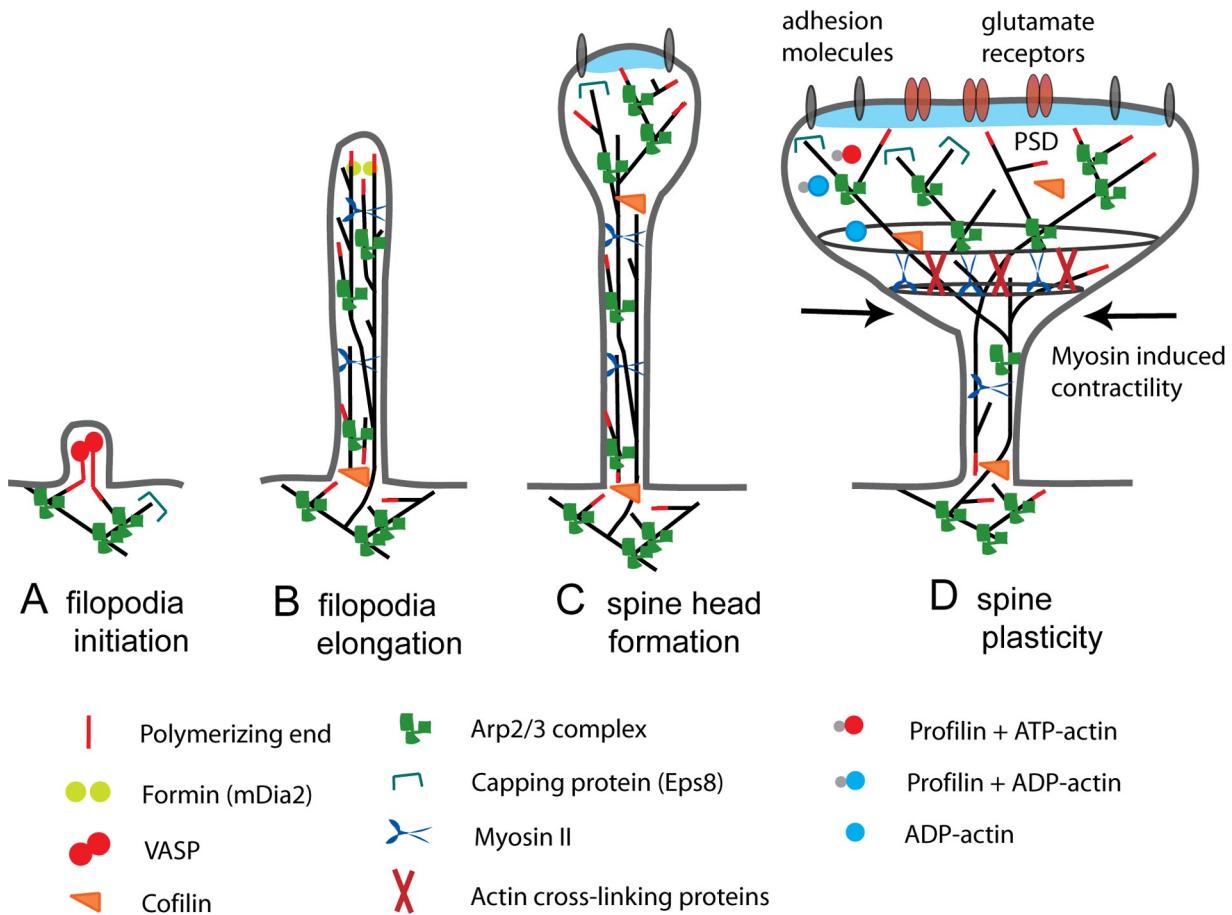


Figure 3. Actin regulatory mechanisms during spine development and plasticity. (A) Spine development starts with the initiation of the dendritic filopodium and its elongation. Eps8 inhibits filopodia initiation by its capping activity. We propose that Ena/VASP proteins could induce filopodia elongation from Arp2/3 complex-generated branched filaments by anti-capping the actin barbed ends. (B) mDia2 promotes actin filament polymerization in the filopodium tip. We propose that Ena/VASP and myosin X take part in filopodia elongation. At this stage, the elongation of dendritic filopodia protrusions is mechanistically more similar to the promotion of lamellipodia protrusions. The factors driving actin filament polymerization in the base of filopodia remain to be identified. (C) Extensive actin branching occurs at the filopodium tip and the spine head begins to form. The mechanism of actin assembly is now increased and the large Arp2/3-nucleated branched actin filament network leads to enlargement of the spine head. The function of ADF/cofilins, in addition to replenishing the cytoplasmic actin monomer pool in neurons, is to control the proper length of actin filaments and thus to prevent formation of abnormal protrusions from spine heads. (D) Mature spines are still dynamic but maintain their overall morphology. Dynamics occur as small Arp2/3 complex-induced protrusions on the surface of the spine head (morphing). Myosin II-dependent contractility and cross-linking of actin filaments further modulate the shape of the spine head. We propose that during LTP, the activities of Arp2/3, profilin, actin cross-linking proteins, myosin II, and actin filament capping proteins are increased whereas activity of cofilin is reduced. The actin-ring structure is oversimplified to highlight the possible dynamic changes in the spine head morphology.

DRF3/mDia2-based actin polymerization to Arp2/3 complex-based actin polymerization, leading to the initiation of spine head growth (Hotulainen et al., 2009). Alternatively, transient microtubule entry into dendritic spines may induce Arp2/3 complex activation. We recently demonstrated that microtubule invasion into spines is associated with transient morphological changes, such as the formation of spine head protrusions and spine growth (Jaworski et al., 2009). Dynamic microtubules may induce a signaling cascade affecting actin dynamics through the microtubule plus-end tracking protein EB3 (Jaworski et al., 2009; Fig. 1 C). A proteomics screen for EB-binding partners in hippocampal neurons uncovered p140Cap (also known as SNAP-25 interacting protein) as an abundant PSD protein in spines (Jaworski et al., 2009) and regulator of Src tyrosine kinase (Di Stefano et al., 2007). p140Cap also interacts with cortactin (Jaworski et al., 2009), a Src substrate that can activate the Arp2/3 complex, stabilize branched actin filament network

in spines, and promote formation of mushroom-like spines (Hering and Sheng, 2003). It is likely that the association between EB3-bound microtubule ends and p140Cap controls Src kinase activity and regulates cortactin function, which could lead to Arp2/3 complex activation and spine head growth. In this way, dynamic microtubules in mature neurons can serve as signaling devices to locally reorganize the actin cytoskeleton and regulate spine head size.

In addition to actin filament polymerization, three-dimensional organization of actin filaments is required in spine head formation. Both activation and inhibition of myosin II activity modifies the size and shape of spines, indicating that myosin II-induced contractility is an important process in dendritic spine morphogenesis (Zhang et al., 2005; Ryu et al., 2006; Fig. 3 C). Moreover, the actin cross-linking proteins CaMKII β , neurabin I, and drebrin A could also play roles in spine head modification and stabilization (Terry-Lorenzo et al., 2005; Okamoto et al., 2007;

Ivanov et al., 2009). Regulation of cofilin activity is important for the proper morphology and stabilization of spines (Hotulainen et al., 2009) (Fig. 3 C). It is plausible that capping activity is required to establish the normal shape of the spine head and inhibit the formation of extra protrusions.

Synaptic plasticity is associated with a rapid and persistent reorganization of the spine actin cytoskeleton (Cingolani and Goda, 2008). Sequestering free actin monomers by latrunculin A blocks LTP expression, suggesting that actin polymerization is required for LTP (Fukazawa et al., 2003; Okamoto et al., 2004; Chen et al., 2007; Ramachandran and Frey, 2009). It seems evident that the Arp2/3 complex, helped by relocalized profilin, induces spine head enlargement shortly after LTP induction. During LTP expression, the actin cytoskeleton is stabilized and is resistant to latrunculin A (Ramachandran and Frey, 2009), which has been shown to occur through cofilin inactivation (Fukazawa et al., 2003; Chen et al., 2007; Fedulov et al., 2007). In addition, actin cross-linking by CaMKII and other actin cross-linking proteins may assist to stabilize actin filaments and spine heads during LTP formation (Okamoto et al., 2007). Myosin II-induced contractility could further regulate spine morphology and synaptic strength by controlling the geometry of the spine neck (Fig. 3 D), which is important for the diffusional coupling between the spine head and dendritic shaft (Bloodgood and Sabatini, 2005). Additional plus- and minus-end actin-capping proteins could further stabilize the actin filaments (Fig. 3 D).

Future perspectives: role of actin dynamics in dendritic spines in learning and memory

Over the past decades the function of the cytoskeleton has been extensively studied in developing and mature neurons *in vitro* and *in vivo* (Cingolani and Goda, 2008; Conde and Cáceres, 2009; Hoogenraad and Bradke, 2009), and there has been tremendous progress in understanding the molecular mechanisms that underlie dendritic spine changes and synaptic plasticity (Sheng and Hoogenraad, 2007; Bourne and Harris, 2008; Holtmaat and Svoboda, 2009; Newpher and Ehlers, 2009). Future studies will continue to visualize the localization and trafficking of synaptic and spine components and test the influence of synaptic activity on these processes (Fischer et al., 1998; Blanpied et al., 2002; Wang et al., 2008; Jaworski et al., 2009; Kapitein et al., 2010). Monitoring the spatiotemporal dynamics of postsynaptic signals (Lee et al., 2009) and photo-activation localization microscopy (PALM)-based single-molecule tracking to analyze cytoskeletal structures in single dendritic spines (Tatavarty et al., 2009) will be of great additional value.

There is substantial evidence that structural changes in spines, driven by remodeling of the actin cytoskeleton, underlie the stabilization of memories after learning (Lamprecht and LeDoux, 2004). For instance, drugs that block actin polymerization suppress LTP (Krucker et al., 2000; Fukazawa et al., 2003; Okamoto et al., 2004; Chen et al., 2007; Ramachandran and Frey, 2009); LIMK1 knock-out mice, which are unable to regulate cofilin activity, have enhanced hippocampal LTP (Meng et al., 2002); and WAVE-1 and Abi2 knock-out mice exhibit deficits in learning and memory (Grove et al., 2004; Soderling et al., 2007). The precise biological functions of many other actin regulatory

proteins, however, remain largely unknown and are subjects for future studies. Recent data from *Drosophila* provide a strong basis to genetically dissect the role of the actin cytoskeleton in learning and memory formation in flies. Dendrites of visual system interneurons have recently been demonstrated to exhibit protrusions closely resembling vertebrate spines and consist of actin filaments and Rac1 (Leiss et al., 2009). Moreover, a recent report showed that Rac in mushroom body neurons contributes to both passive memory decay and interference-induced forgetting, suggesting that actin reorganization may contribute to memory erasure in *Drosophila* (Shuai et al., 2010). It has become clear that the actin cytoskeleton is engaged in multiple aspects of spine morphology and synaptic function and that local actin rearrangements and synaptic activity have a close cooperative feedback relationship. Synaptic activity regulates the integrity of the spine actin cytoskeleton; in its turn, actin dynamics affect spine morphology and thereby synaptic plasticity. However, it should be noted that actin polymerization in spines is required but not sufficient for functional LTP. Actin depolymerization by pharmacological approaches prevents spine enlargement and LTP, but actin-polymerizing agents do not potentiate synaptic transmission or induce LTP on their own (Okamoto et al., 2004).

In recent years it has become evident that many psychiatric and neurological disorders ranging from mental retardation and autism to Alzheimer's disease and addiction are accompanied by alterations in spine morphology and synapse number (Blanpied and Ehlers, 2004). For instance, mutations in synaptic scaffolding protein Shank3 and adhesion molecules neuroligin-3 and neuroligin-4 have been linked to autism spectrum disorder (Südhof, 2008). Further evidence suggests that various memory disorders involve defects in the regulation of actin cytoskeleton (Newey et al., 2005). For example, the Schizophrenia risk factor DISC1 regulates dendritic spine morphology via Rac1 (Hayashi-Takagi et al., 2010) and mutations in the cofilin kinase PAK3 (p21-activated kinase) gene lead to X-linked mental retardation (Allen et al., 1998). Moreover, decreased levels of PAK3 relate to synaptic dysfunction in Alzheimer's disease (Kreis and Barnier, 2009). PAK3 inhibition in mice causes cofilin pathology and memory impairment, consistent with a potential causal role of PAK defects in cognitive deficits in Alzheimer's disease (Zhao et al., 2006). Future genetic studies will most likely identify new mutations that impact spine actin regulation in association with synapse dysfunction. Moreover, investigating basic cell biological mechanisms underlying spine development and plasticity will lead to a better understanding of synaptic plasticity, brain function, and neurological diseases.

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