In: Dendritic Spines: Biochemistry, Modeling and Properties ISBN 978-1-60741-460-5
Editor: Louis R. Baylog © 2009 Nova Science Publishers, Inc.

Chapter II

The Actin Cytoskeleton: Central Regulator of Dendritic Spine Form and Function

Andreas Birbach
Medical University of Vienna, Wahringerstrasse, Vienna, Austria

Abstract

Dendritic spines, the postsynaptic specializations of excitatory synapses, are highly dynamic elements capable of undergoing profound changes of shape, protein composition and electrophysiological properties in response to synaptic activity. Shape changes are based on regulation of the actin cytoskeleton and depend on ion influx and a plethora of actin-regulating proteins. Dendritic spines can change their shape during development and in the adult, with shapes ranging from thin filopodial outgrowths to stable cup- or mushroom-shaped spines. Spine shape changes occur at different timescales, concomitant with short- and long-term electrophysiological changes. Likewise, changes in shape range from lamellipodia-like ruffling of the spine head to freezing of rapid motility and variation in postsynaptic size, and even comprise loss of spines and growth of new protrusions. These actin-based shape changes influence neuronal connectivity by regulating the number and strength of synaptic connections, affecting neurotransmitter receptor number and diffusion properties. The molecular mechanisms of how actin shapes dendritic spine changes during development and experience-dependent plasticity in the adult are beginning to emerge. In this chapter, I summarize how dendritic protrusions respond to plasticity signals by changing their shape and forming the basis for functional plasticity at different timescales. Morphological plasticity is sometimes described as being similar to processes occurring during development. However, the question whether the same mechanisms underlie developmental changes and experience-dependent changes in the adult is still unresolved. The actin cytoskeleton lies at the heart of all of these changes in the form of dendritic spines; understanding actin regulation should move us closer to understanding their function in cognitive processes.

Introduction

Actin

Actin is the most abundant protein in many eukaryotic cells. In mammalian cells, different actin genes exert distinct functions: skeletal muscle actins are responsible for actomyosin-based contractility of skeletal muscles, smooth muscle actins regulate the contractility of hollow organs, and cardiac muscle actins are main players in the rhythmic contractions of the heart muscle. These actins act in concert with myosins to control cycles of contraction and relaxation. Next to these actins, almost all cell types express two so-called cytoplasmic actins, β- and γ-Actin. These actins form the basis for the highly dynamic microfilament system, a meshwork of strands approximately 6–7 nm wide visible in electron microscopy [Svitkina et al., 1995]. Monomeric actin can be described as consisting of four distinct subunits forming a globular molecule, which already indicates actin's polarity [Kabsch et al., 1990]. Cytoplasmic actins are globular proteins with a molecular mass of ca. 42 kDa (G-actin), but can assemble into filaments (F-actin) under polymerization-favorable conditions. In the test tube, these conditions are buffers of certain ionic strength and an actin concentration above a critical threshold value. In cells, a plethora of actin-binding proteins with buffering, polymerization and depolymerization capacities influence the balance between filamentous and non-filamentous actin. Filament assembly is based on a head-to-tail arrangement of globular subunits, giving the filaments a molecular polarity. Polarity is essential for directed growth and transport processes. Actin monomers added to the growing end of the actin filament are preferentially bound to adenosine triphosphate (ATP), but during filament formation ATP is first hydrolyzed into adenosine diphosphate (ADP) and phosphate (Pi), with a following slower dissociation of the phosphate from ADP [Blanchoin and Pollard, 2002; Carlier and Pantaloni, 1986]. Thus, actin can also be regarded as an ATPase, with the ratio of ADP to ATP in the filament being an indicator of filament age. Since actin is preferentially added to one side of the filament and preferentially lost at the other, individual actin molecules seem to flow from through the filament while subunits are continuously exchanged, a process referred to as "treadmilling" (Fig. 1a).

Next to this long-known function of actin as the basic constituent of cytoplasmic microfilaments, putative functions for actin as a monomer or oligomer have been proposed recently. Nuclear actin has been established as a much smaller but significant fraction of the "cytoplasmic" actins, and functions such as chromatin remodeling, transcription cofactor or positional regulator of transcriptionally active genes have been reported. Monomeric or short oligomeric forms of actin have been proposed there, although possible structures comprise anything else than the classical cytoplasmic filament [Pederson, 2008].

Actin polymerization in vitro can be influenced by ion concentrations: under low-salt conditions, actin is found in the globular, monomeric form, while adding divalent cations or up to 100 mM potassium induces spontaneous self-assembly into F-actin [Carlier et al., 1986a, b; Strzelecka-Golaszewska et al., 1978]. The polymerization in the test tube proves that this polymerization is due to the direct influence of ionic strength on actin filament assembly, while in cells changes in the ion concentration may also work on one of the actin modulating proteins.

Actin Assembly in Cells

Actin interacts with a plethora of actin binding proteins (ABPs) in mammalian cells. These proteins, together with other proteins and non-protein factors (e.g., lipids) that regulate their activity, are responsible for a markedly different picture of actin filament assembly in cells than for actin alone in the test tube. ABPs can facilitate nucleation of new filaments, growth or cessation of filament growth, branching of filaments, crosslinking or bundling of existing filaments, severing of filaments, buffering monomeric actin or actin nucleotide exchange. According to these demonstrated or proposed functions, ABPs are grouped as bundling, capping, monomer-binding, etc. proteins (Fig. 1b). However, certain ABPs may exhibit more than one function (e.g., the severing and monomer-binding protein cofilin and the capping and severing protein gelsolin) and some functions may be concentration-dependent. Actin assembly is dependent on a highly regulated interplay of these ABPs, and the binding proteins may show a different net effect on actin filament assembly based on the presence of other actin binding proteins. The overexpression or depletion of ABPs in different cell types may thus lead to seemingly contradictory results; the function most ascribed to certain ABPs is sometimes based on in vitro assays. The most prominent of these assays nowadays is the comet tail assay of bacterial motility (Fig. 1c). Here, adding purified proteins to a mixture containing either a specific bacterium or inorganic beads coupled to the bacterial protein attracting the actin-assembly machinery induces the formation of an actin tail and results in net movement of the bacterium/bead (see figure caption).

Other proteins not directly binding to actin but regulating nucleation, polymerization or branching by acting on ABPs provide a link to signaling cascades influencing actin assembly. Signals from the plasma membrane are conveyed to small GTPases of the Rho family as well as WASP/WAVE family proteins. These relay signals via adaptor proteins and kinases to various ABPs [Heasman and Ridley, 2008; Takenawa and Suetsugu, 2007] (Fig. 1d).

The concomitant activation of several proteins influencing actin filament assembly is necessary to induce growth of complex microfilament structures in cells.

Actin-based motility in mammalian cells occurs at specialized domains—wave-like protrusions called lamellipodia and membrane ruffles from which often emerge spike-like protrusions called filopodia [Small et al., 2002]. Lamellipodia are the leading-edge specializations of migrating cells and spread out in their search for contacts, which is mirrored by their branched and widespread actin cytoskeleton [Svitkina and Borisy, 1999]. They contain proteins like the Arp2/3 complex, filamin and many other actin-binding and -regulating proteins necessary to maintain a constantly dynamic filament system. Filopodia, in contrast, are characterized by a cytoskeleton of bundles of parallel actin fibers and, consequently, the presence of bundling proteins such as fascin [Vignjevic et al., 2006].

The different protein content of distinct actin-rich structures has been linked to activation of different small Rho-type GTPases during their development: In cultured fibroblasts, activation of RhoA leads to formation of stress fibers, while Rac signaling stimulates lamellipodia formation and Cdc42 induces filopodia [Kozma et al., 1995; Nobes and Hall, 1995; Ridley and Hall, 1992; Ridley et al., 1992]. However, more recent data have indicated that other, related small GTPases can account for functional redundancy [Heasman and Ridley, 2008].

In leading-edge motility, growing ends of the actin filament are located directly beneath the protruding plasma membrane, and hence individual subunits added there seem to flow retrogradely towards the cytosol [Pollard and Borisy, 2003].

The Actin Cytoskeleton

Neuronal Actin

Actin plays a major role in the differentiation of a neuron. Neuritogenesis, i.e., the outgrowth of neurites from a morphologically undifferentiated cell, is based on a local instability of the actin cortex in the neuronal sphere [da Silva and Dotti, 2002]. From there on, microtubule-filled neurites grow through the developing brain, guided by an actin-rich structure, the neuronal growth cone. Growth cones are perhaps the most drastic example of actin-based leading-edge growth in eukaryotic cells, embodying a huge lamellipodium at the tip of a cellular extension which senses the environment by filopodia emerging from its own tips. Comparable to cell migration in other contexts, growth cones can respond to chemotactic or cellular signals by turning towards an attractive or away from a repulsive stimulus. This guides the following neurite (axon or dendrite) to grow according to cues present in the developing tissue.

When dendritic trees and axonal arbors have been set, synaptogenesis between neurons is already going on. An active role for actin in this process involving outgrowth of protruding filopodia has been described, and is referred to in more detail in the later chapter on actin plasticity in developing dendritic spines. Here, emphasis is laid on the actin cytoskeleton in the mature excitatory synapse, particularly on the postsynaptic dendritic spines.

Actin is the major protein in dendritic spines, which are devoid of microtubules although emerging from the microtubule-rich dendritic shaft [Kaech et al., 2001; Matus et al., 1982]. In pyramidal neurons in the mature brain or fully differentiated in vitro, dendritic spines are by far the most actin-rich structures although some actin is also found presynaptically and very little in neurites and the soma [Cohen et al., 1985; Furuyashiki et al., 2002; Landis et al., 1988]. The function of actin in the presynapse may include presynaptic vesicle transport and endocytosis and is discussed elsewhere [Cingolani and Goda, 2008].

Within the dendritic spine, actin is concentrated at the postsynaptic density (PSD), but is also present in subsynaptic regions and the spine apparatus [Cohen et al., 1985; Landis and Reese, 1983]. Actin isoforms in the spine are beta and gamma cytoplasmic actins, with protein coding regions sufficient to induce targeting to postsynapses, while other actin isoforms do not enter the spine when expressed ectopically [Kaech et al., 1997]. More recently, studies using fluorescent microscopy techniques with different variants of the green fluorescent protein (GFP) showed that actin in the spine consists of at least 2 pools, as already proposed several years ago: A stable actin pool at the base of the spine and another pool near the tip turning over rapidly [Halpain, 2000; Honkura et al., 2008]. Thus dendritic spine actin can be described as similar to actin in huge lamellipodia or in growth cones, consisting of a stable actin core and a dynamic actin periphery (Fig. 2). This is consistent with early electron microscopy data showing at least two distinct pools of microfilaments of different diameter and arrangement [Landis and Reese, 1983].

A growing number of actin-regulating proteins have been identified in dendritic spines, starting from ABPs to signaling molecules linking actin dynamics to surface receptors. The variety of actin binding proteins found there makes another strong case for the view of dendritic spines as lamellipodia-like protrusions with the ability to form filopodia sensors. An overview of actin-binding proteins found in dendritic spines is shown in Table 1. Some electron microscopy studies have described the distribution of ABPs within the spine. With more data accumulating, it will not only be possible to distinguish the distinct actin pools morphologically and according to turnover times, but also define them molecularly.

The small GTPases responsible for distinct actin organizations observed in cultured mammalian cells have been thoroughly studied with respect to the dendritic spine cytoskeleton. It has been shown already several years ago that ectopic expression of lamellipodia-forming Rac induces ectopic formation of spines in transgenic mice, while dominant negative versions of this molecule interfere with spine formation [Luo et al., 1996; Nakayama et al., 2000].

Actin-Based Plasticity during Development and in the Adult

Actin-based functions are vital throughout the lifetime of a neuron, as briefly mentioned in the previous chapter. Here, the focus is exclusively on postsynaptic spine formation and plasticity in the young animal brain and in the adult. As postsynaptic parts of excitatory synapses, spines emerge from dendrites during postnatal development. Their development has been linked to the appearance of dendritic filopodia.

Dendritic filopodia appear early in dendrite development and are suggested to be involved in formation of the dendritic tree as well as synaptogenesis [Hua and Smith, 2004]. In fact, filopodia are much more numerous during early dendrite development and are also very abundant in non-spiny neurons, indicating a main role in dendrite development. However, it is still maintained that filopodia protruding from more mature dendrites are likely precursors of mature spines [Jontes and Smith, 2000]. Thus two pools of dendritic filopodia can be distinguished, those residing on dendritic growth cones and those extending from established dendrites [Portera-Cailliau et al., 2003]. According to this view, the latter dendritic filopodia could sample the environment for axons and initiate the synapse in case of successful contact with the axon, possibly in combination with other signals like afferent activity. Dendritic filopodia as precursors of spines are also suggested by temperature-induced plasticity of brain tissue: When cooled down, spines disappear in organotypic slice cultures of the hippocampus, but re-warming induces filopodial outgrowth from actin-rich protrusions followed by re-formation of mature spines [Roelandse and Matus, 2004].

The formation of a mature spine is far more complex than a simple transformation from one actin-rich structure to another. Spines contain neurotransmitter receptors, the postsynaptic density (PSD), signaling molecules, scaffolding molecules associated with the PSD and possibly a spine apparatus. Signaling to recruit these factors should also include the formation of the spine cytoskeleton with a stable core and a more dynamic periphery. It was shown that lack of a major PSD protein does not interfere with spine morphogenesis in the murine brain, while disruption of the actin cytoskeleton during spine formation leads to dispersal of various elements of the postsynapse [Allison et al., 1998; Migaud et al., 1998]. Thus the assembly of

the actin cytoskeleton can be regarded as a crucial event in spine morphogenesis. In accordance with this view , ectopic epression of a truncated form of the actin binding protein NrbI (neurabin) is sufficient to induce spine growth in hippocampal slice cultures [Zito et al., 2004].

One key signaling factor in this respect is signaling via Ephrins, a family of signaling molecules involved in neuronal development at multiple levels [Aoto and Chen, 2007; Egea and Klein, 2007]. In the absence of all EphB receptors from the murine brain, spines were abnormal and did not form at all in an in vitro culture system of hippocampal neurons [Henkemeyer et al., 2003]. EphB receptors signal to the actin cytoskeleton by activating a guanin nucleotide exchange factor (GEF) of the small GTPase Rac called Kalirin-7 [Penzes et al., 2001; Penzes and Jones, 2008]. Kalirin-7 can activate Rac by catalyzing the exchange of GDP for GTP and prompting it to activate downstream signaling molecules such as p21-activated kinases (Pak). In agreement with Rac implicated in lamellipodia formation, expression of Kalirin-7 in fibroblasts induces lamellipodia formation and membrane ruffling [Penzes et al., 2000]. Another RacGEF, Tiam1, also contributes to spine development. Both GEFs can be activated by Calcium/calmodulin –dependent kinase II (CamKII), a key molecule in synaptic plasticity which is activated by calcium influx through synaptic neurotransmitter receptors [Penzes et al., 2008]. Generally, the role of activity in spine outgrowth is complex. While synaptic connections including spines form in the absence of any synaptic activity [Verhage et al., 2000], in a normal brain activity has a modulatory role on synapse growth and maintenance (for review, see [Matus, 2005]).

In conclusion, mature spines exhibiting a lamellipodia-like cytoskeleton can form after initial filopodial contact induced signaling pathways converging on the activation of small GTPases.

When spiny neurons such as pyramidal neurons of the rodent hippocampus are grown in culture to maturity, spines can be seen as moving rapidly. This movement is seen in neurons expressing GFP-tagged actin, plasma membrane markers or simple GFP as a space filler. Matus and colleagues first described this phenomenon as actin-based plasticity of mature spines and noted its rapidness, with visible changes occurring within seconds and possibly on a sub-second timescale [Fischer et al., 1998]. Different authors have tabbed the phenomenon as spine dancing or morphing, and due to the rapidness of the phenomenon and small size of spine together with the limited resolution of light microscopy, an exact description is still outstanding. However, a case can be made for this sub-second shape changes to be most closely related to lamellipodial activity of migrating cells or neuronal growth cones: The actin-based plasticity is located at the spine tip with its fine meshwork of microfilaments similar to lamellipodial actin, and the movement can be best described as miniature growth cones extending and contracting randomly [Halpain, 2000]. In some cases, even filopodial outgrowth from the spine head can be resolved [Dunaevsky et al., 1999].

The dependence on actin dynamics can be shown by drugs interfering with actin polymerization such as Cytochalasin D or Latrunculin B [Fischer et al., 1998]. Overall, the activity of the spine head resembles the search of a growth cone for an attractive or repulsive signal to grow towards or away from. In the case of the spine head, this signal is synaptic activity. Neurotransmitter reaching the postsynaptic spine stops morphological dynamics and freezes the rapid actin turnover [Fischer et al., 2000]. In excitatory postsynaptic spines, two classes of glutamate receptors can be distinguished: α-Amino-3-hydroxy-5-Methyl-4-isoxalone Propionic Acid (AMPA) and N-Methyl-D-Aspartate (NMDA) type receptors,

named after chemical agonists of the respective receptor classes. Interestingly, activation of both types of receptors can block spine shape changes, albeit on a different timescale: While AMPA activation immediately freezes spine actin dynamics (within seconds, as soon as can be recorded), NMDA receptor activation takes minutes for the spine to stabilize. The other important difference in receptor-specific spine actin freezing is the reversibility of the effect for AMPA receptor activation, while spine motility does not return at least for several hours after removing the NMDA stimulus [Brunig et al., 2004].

Since the AMPA-mediated block of actin dynamics is very rapid and AMPA receptors mediate influx of small cations (particularly sodium), it could be speculated that the effect is due to a direct impact of ionic strength on actin assembly as in test-tube experiments (discussed in the first chapter). However, it has been demonstrated that subsequent calcium (Ca2+) influx through voltage-gated calcium channels is necessary for blocking spine dynamics [Fischer et al., 2000]. The molecular mechanism beyond Ca2+ remains elusive. Intuitively, candidate molecules for mediating this rapid effect are calcium-binding ABPs, in particular gelsolin and α-actinin. Neurons from mice lacking gelsolin show impaired activity-dependent stabilization of actin [Star et al., 2002]. However, the stabilization paradigm in that study was dependent on NMDA receptors. Alpha-actinin has been found to be physically coupled with the NMDA receptor [Wyszynski et al., 1997]. Thus the exact molecular mechanisms of rapid spine actin freezing by AMPA receptors and Ca2+ is still elusive, with a direct effect of calcium entering through voltage-gated channels not being excluded. Intriguingly, movement of axonal filopodia can be manipulated in the same way by AMPA treatment, essentially freezing motility [Chang and De Camilli, 2001]. This further argues for the rapid motility in dendritic spines to be filopodial protrusions emerging from a lamellipodial network.

NMDA receptor activation leads to a long-lasting and irreversible block of actin dynamics, at least on a timescale of several hours. Activation of NMDA receptors takes a few minutes to completely freeze spine actin dynamics, and is accompanied by changes in the protein content in the spine including redistribution of actin-binding proteins. The first ABP to be identified to redistribute to spine heads in an activity dependent manner and to be involved in stabilization of spine actin was profilin [Ackermann and Matus, 2003]. Profilin targets to spine heads in the same time frame as spine motility disappears, and interfering with profilin redistribution by overexpressing the profilin-binding domain of VASP destabilizes spines. Although profilin is often regarded as an inducer of actin polymerization and positive regulator of actin dynamics, it can also exert the opposite effect in a concentration-dependent manner [Birbach, 2008]. NMDA receptor activation leads to influx of extracellular Ca2+ by the calcium channel properties of the receptor. As discussed above, calcium-binding ABPs have been implicated in the changes downstream of Ca2+. Gelsolin and alpha-actinin are candidate molecules to mediate at least parts of the reorganization of the actin-cytoskeleton, but further signaling cascades activated by calcium and converging on actin seem likely. Along these lines, calcium/calmodulin dependent protein kinase II (CaMKII) has been shown to be not only a signal transuction mediator and scaffolding protein, but also acting as an F-actin bundling protein [Okamoto et al., 2007].

It remains to be discovered how the same second messenger (Ca2+) can be responsible for both the rapid, reversible freezing of spine motility following AMPA receptor activation and the slower developing, irreversible block in actin dynamics induced by NMDA receptors. One possibility is that different actin pools are involved: While calcium entering through

voltage gated channels could mainly act on the dynamic actin at the periphery, a stronger calcium wave entering through NMDA receptors could induce polymerization of a more stable pool at the spine head base [Matus, 2000].

Spines do not only stop dancing following synaptic activity. Recent evidence demonstrates that different forms of synaptic stimulation are coupled with enlargement or decrease in spine size. Both phenomena are dependent on spine actin, and experimental evidence has been obtained that the actin state shifts from G-actin to F-actin during spine enlargement. The increase in filamentous actin is apparent in less than a minute, while increase in spine size takes several minutes to fully develop [Okamoto et al., 2004]. Interestingly, this is the same time frame for full profilin redistribution to the spine head and NMDA receptor-dependent actin stabilization. The size increase is prominent in smaller spines, while large mushroom spines do not further enhance their size. Thus size increase could be coupled to plasticity events during learning and memory formation, while larger spines in the adult brain could represent traces of memory built earlier [Matsuzaki et al., 2004]. Mechanistically, new F-actin polymerization at the expense of G-Actin can be achieved by reducing depolymerization of continuously treadmilling filaments. Indeed, the depolymerizing agent cofilin can be phosphorylated by LIM kinase and thereby inactivated. Increased levels of phospho-cofilin have been measured after induction of long-term potentiation concomitant with actin polymerization in hippocampal spines [Fukazawa et al., 2003]. Consistent with this finding, spines in LIM kinase knockout mice are depleted in F-Actin and smaller [Meng et al., 2002]. The p21-activated kinases are molecules phosphorylating and activating LIM kinase. Increased Pak phosphorylation has been found concomitant with cofilin phosphorylation following stimulation protocols leading to actin polymerization [Chen et al., 2007]. In Pak1 deficient mice, the F-actin content in spines is decreased [Meng et al., 2002].

Signaling via presynaptically released brain derived neurotrophic factor (BDNF) can enhance the phenomenon of spine enlargement: BDNF spikes together with repetitive uncaging of glutamate in hippocampal slices led to a long-lasting steady increase in spine size over a period of up to one hour, increasing the effect by glutamate seen after minutes [Tanaka et al., 2008]. This effect is dependent on protein synthesis and actin polymerization and demonstrates an early and a late phase in spine enlargement in response to strong patterns of synaptic activity.

Fluorescent microscopy using a photoactivatable version of the green fluorescent protein demonstrated that actin polymerization from the dynamic actin pool underlies spine enlargement. The newly formed actin is intermediate in its turnover times between the dynamic pool at the tip of the spine head and the more stable pool at its base. Hence, the interpretation is that the protocols leading to spine enlargement form a third actin pool, called enlargement pool [Honkura et al., 2008].

The different phenomena referred to as actin-based plasticity are summarized in Table II.

All these plastic changes of spines are mediated by the actin cytoskeleton and thus by actin-binding proteins. In accordance with this, ectopic expression of ABPs or mutants thereof has been found to selectively modify actin shape, size or number. A mutant neurabin can induce the formation of dendritic spines [Zito et al., 2004].Reduction of cortactin levels in cultured neurons reduces spine density, while overexpression leads to spine elongation [Hering and Sheng, 2003]. This phenotype is also found upon overexpression of drebrin A or its actin-binding domain [Biou et al., 2008; Hayashi and Shirao, 1999]. Drebrin

overexpression leads to loss of synaptic contact and a filopodia-like phenotype, resembling spine precursor structures [Biou et al., 2008]. Alpha-actinin overexpression can also lead to spine elongation, as well as to an increase in the number of dendritic protrusions [Nakagawa et al., 2004].

Electrophysiological and Morphological Plasticity at the Synapse

The synapse has been hypothesized to be a main player in experience-dependent plasticity since the days of Ramon y Cajal. Rules for synaptic plasticity as the basic unit of information coding have been formulated by Donald Hebb and are still relevant to modern theories of learning and memory [Hebb, 1949]. Since information in neural circuits is somehow encoded by electrical impulses, electrophysiological recordings have been the main focus of studies of synaptic plasticity. Indeed, several phenomena have been described comprising changes of postsynaptic electrical responses following certain stimulation paradigms of the circuitry. Among these, long-term potentiation and depression are the most prominent and have been proposed as synaptic correlates of learning and memory. Experiments aimed at elucidating the molecular mechanisms of the electrophysiological plasticity have identified changes in neurotransmitter expression and activity at the synapse [Malinow and Malenka, 2002]. On the other hand, structural changes in neuronal connectivity have been suggested by experiments showing outgrowth of postsynaptic spines in young neuronal slice cultures upon induction of LTP [Engert and Bonhoeffer, 1999]. Another group showed that developing hippocampal slice cultures respond by growing out filopodial extensions [Maletic-Savatic et al., 1999].

As the major structural element at the postsynapse, it is not surprising that the actin cytoskeleton plays a role in mediating molecular effects involved in synaptic plasticity. A dynamic actin cytoskeleton in the postsynaptic neuron has been found to be necessary for the maintenance of LTP [Kim and Lisman, 1999; Krucker et al., 2000]. However, it is still a conflicting topic whether actin's main role is in modulating the neural circuitry by growing new connections or by more subtle internal modification of the existing ones, or both.

A major rearrangement within the synapse laying the foundation for changes in the postsynaptic potential observed in LTP and LTD is the change in AMPA receptor expression at the postsynaptic membrane. While long-term potentiation drives AMPA receptors into the synapse, long-term depression leads to internalization of AMPA receptors. Actin is an active player in this process, as blocking actin polymerization leads to AMPA receptor internalization, whereas stabilizing actin filaments prevents internalization [Zhou et al., 2001]. This underlines the actin-dependent anchorage of glutamate receptors in the postsynaptic membrane [Allison et al., 1998] and the involvement of actin in intraspinal modifications during long-term plasticity processes.

In recent years, the focus of actin in LTP and LTD has shifted to the aforementioned shape changes associated with these phenomena. As described earlier in the text, induction of LTP in various systems leads to an increase in spine size best described as a swelling of the spine head [Matsuzaki et al., 2004]. LTD on the other hand leads to a reduction in spine size [Zhou et al., 2004].

It has been known for a long time that spine size and synapse size are highly correlated (Lisman and Harris, 1993). Moreover, AMPA receptor content correlates with synapse size

[Matsuzaki et al., 2001; Nusser et al., 1998]. Thus spine size expansion and reduction could be sufficient to explain the effects of LTP/LTD on actin-dependent changes in receptor expression.

Given the different plasticity phenomena shown by the spine actin cytoskeleton, which ones are more relevant during in vivo plasticity? Several groups have imaged spines in different brain regions over periods of hours to days to even months [Grutzendler et al., 2002; Trachtenberg et al., 2002]. The main conclusion drawn from these experiments is that a subset of spines turns over, i.e. these spines disappear and reappear over a time course of days or weeks. This is in line with the fact that actin-regulating molecules in spine outgrowth continue to be expressed during adulthood [Penzes et al., 2008]. However, the subset of spines turning over varies greatly with brain regions. It is unknown whether this reflects differences in the molecular machinery of actin filament assembly or distinct afferent stimuli regulating the turnover or both.

Interestingly, in the hippocampus, the brain region mostly used for in vitro experiments, imaging dendritic spines over hours did not reveal any significant changes in spine number or in spine size, not even after induction of epileptic seizures [Mizrahi et al., 2004]. These studies demonstrate the importance for selection of the right model system for experiments and indicate that the cytoskeleton may behave differently across brain regions.

Conclusion

Actin has been known as the major cytoskeletal protein in dendritic spines for more than 25 years. Early pioneering work using electron microscopy has revealed the basic structure of filaments and, decades later, technological advances in light microscopy have confirmed what was hypothesized based on the static EM pictures and the background knowledge of dynamic behavior of actin in cells: dendritic spine actin is highly dynamic, especially at the tip of the spine head, with a more stable component at its base. Additionally, experiments coupling electrical stimulation of neural circuits with imaging of postsynaptic spines have led to insights potentially explaining the heterogeneity of spines seen on dendrites. Spines can increase or decrease in size by means of enhancing or reducing their F-actin/G-actin ratio, leading to distinct spine shapes seen in vivo. Molecularly, a number of actin binding proteins have been identified that are involved in the stabilization of spine actin, spine neck length and overall spine size regulation. Key signaling molecules involved in spinogenesis from filopodial precursors to mature, lamellipodia-like protrusions are known and couple receptor signaling to small GTPases and the actin cytoskeleton. These molecules continue to be expressed throughout adulthood, consistent with spine turnover in the adult brain.

Within the spine, actin is coupled to signaling platforms and neurotransmitter receptors: it is involved in receptor surface expression and removal of receptors and has an impact on signaling cascades. Changes in shape or size of the spine modulate its electrophysiological properties, thereby impacting neural circuits.

Morphological plasticity of spines is seen at different levels: regression of spines, outgrowth of new spines, spine shape changes and increase or decrease in size. These phenomena are regulated by synaptic activity, implicating them in experience-dependent plasticity such as learning and memory processes. However, it remains unclear which

morphological changes represent which learning or plasticity paradigm. Outgrowth of new spines, regression of old ones or change in presynaptic partner of a given spine can have a profound impact on neural circuitry and explain why synapses are made on dendritic protrusions rather than on the dendritic shaft. Researchers have identified stimuli leading to profound changes in spine shape, spine turnover or intraspine protein composition. The question regarding which stimuli play a more important role during the in vivo phenomena of cognition, learning and memory is to be resolved by novel approaches that have to face the difficulties of (1) which cognitive paradigm to use, (2) where to look for changes, and (3) examining changes both on a very short and long timescale. The answers will be difficult to reveal and exciting to watch.

Table 1. Actin-binding proteins described in dendritic spines. A growing number of actin binding proteins have been found in dendritic spines, and subspine localization of these molecules confirm distinct pools of actin in the spine core and at the periphery.

Molecule	**Comments**	**References**
Gelsolin	Calcium-dependent capping and severing protein, necessary for NMDA receptor dependent stabilization of actin dynamics.	[Star et al., 2002]
Profilin	Activity-dependent redistribution is necessary for NMDA receptor dependent stabilization of spine morphology. Fear-conditioning in animals drives profilin into spines which appear enlarged.	[Ackermann and Matus, 2003; Lamprecht et al., 2006; Neuhoff et al., 2005]
Cofilin	Localizes to dynamic actin pool. Inactivation following strong synaptic stimulation patterns leads to actin polymerization and spine swelling. Other patterns lead to activation and spine shrinkage.	[Fukazawa et al., 2003; Racz and Weinberg, 2006; Zhou et al., 2004]
Arp2/3	Localizes to intermediate doamin between stable and dynamic actin pool.	[Racz and Weinberg, 2008]
Cortactin	Mainly localizes to stable pool at the spine core, with small fraction in dynamic pool. Overexpression results in more and elongated dendritic protrusions.	[Hering and Sheng, 2003; Racz and Weinberg, 2004]
Drebrin	Localizes to stable core region; overexpression elongates protrusions	[Biou et al., 2008; Hayashi and Shirao, 1999; Kobayashi et al., 2007]
a-actinin	Bundling protein, coupled to NMDA receptor; implicated in AMPA receptor transport; overexpression leads to elongation of spines and ectopic protrusions	[Nakagawa et al., 2004; Schulz et al., 2004; Wyszynski et al., 1998; Wyszynski et al., 1997]
Neurabin	Localizes to dynamic pool around PSD; expression of actin-binding domain induces ectopic spine growth	[Muly et al., 2004a; Zito et al., 2004]
Spinophilin (Neurabin II)	Localizes to dynamic pool around PSD. Interacts with protein phosphatase 1 to regulate glutamate receptor activity	[Feng et al., 2000; Muly et al., 2004b]
CaMKII	Signaling and scaffolding protein that can act as F-actin bundling protein. Activity-dependent translocation to spines.	[Okamoto et al., 2007; Shen et al., 1998; Zhang et al., 2008]
Synaptopodin	Localizes to spine neck and spine apparatus, necessary for spine apparatus formation	[Deller et al., 2003; Deller et al., 2000]
Myosin IIb	Motor protein; inhibition or knockdown leads to loss of mature spines in favor of filopodial protrusions	[Ryu et al., 2006]
Myosin Va	Motor protein, necessary for AMPA receptor trafficking	[Correia et al., 2008]
Myosin VI	Motor protein, loss leads to shorter spines and deficit in AMPA receptor internalization	[Osterweil et al., 2005]
Espin	Ca2+ independent bundling protein specific to Purkinje cell spines	[Sekerkova et al., 2003]

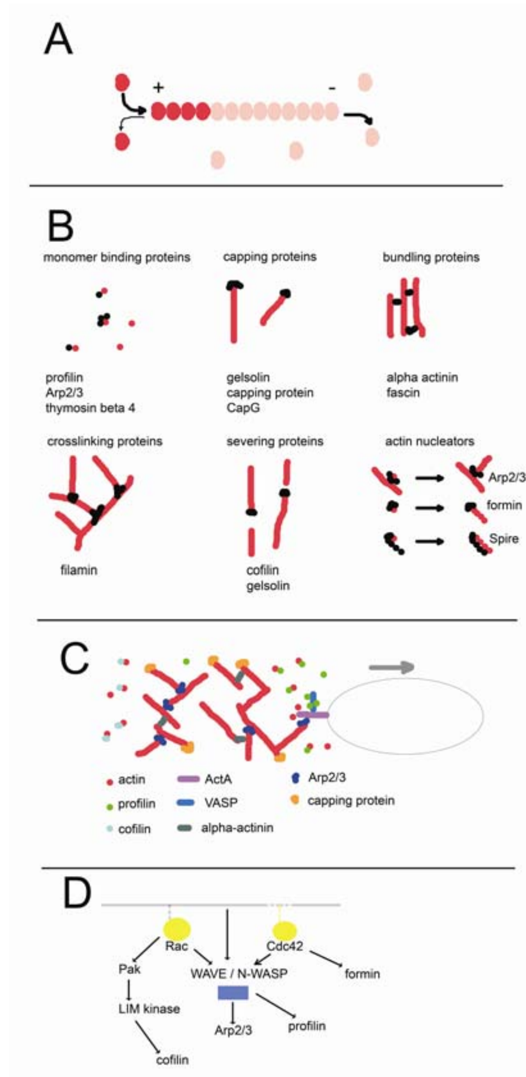

Figure 1. The actin filament assembly machinery. A, treadmilling of actin subunits: actin subunits coupled to ATP (red) are preferentially added to one side of the actin filament, hence called the growing ("+") end of the filament or barbed end. ATP-actin is hydrolyzed to ADP-actin and phosphate with subsequent dissociation of the phosphate, leaving ADP-Actin (pink). On the other end of the filament, actin is preferentially lost, thus called the shrinking ("-") or pointed end. In polarized migrating cells, the + end is directly beneath the protruding membrane. B, actin binding proteins are often classified according to their main functions. Molecular actions of these subclasses on the actin filament are shown, together with main family members mentioned in the text. C, bacteria traversing through the gut such as Listeria monocytogenes are used to unravel the actin assembly machinery: Surface proteins such as Listeria's ActA attract actin regulatory proteins such as VASP or Arp2/3 and lead to assembly of actin filaments. When ActA is coupled asymmetrically to an inorganic bead and purified ABPs together with actin and energy (ATP) are added, a net movement (arrow) can be observed. For Listeria motility, Arp2/3 complex, cofilin and capping protein are required for motility, while inclusion of α-actinin, VASP and low concentrations of profilin enhance motility. D, signals from the plasma membrane are transduced to actin binding proteins via actin regulatory proteins such as small GTPases, the WAVE/N-WASP/WASP family members and downstream kinases or binding partners. These are just a few examples for pathways converging on actin assembly.

Table 2. Different forms of actin-based plasticity in dendritic spines. The described phenomena, although distinct and happening on different timescales, are sometimes collectively described as actin-based motility or morphological plasticity. Molecules involved in the regulation of the processes are likely many more than the ones clearly shown to be involved to date, since regulation of the actin cytoskeleton needs the concerted action of many proteins. See text for details and references.

Phenomenon	**Timescale**	**Molecules involved in regulation of the process**
Outgrowth of spines	Hours	Ephrins, Kalirin-7, Rac
Spine tip motility (“dancing”)	Seconds (sub-seconds)	Gelsolin, profilin
Volume increase (“swelling”)	Minutes	Cofilin, LIM kinase, Pak, BDNF/TrkB
Volume decrease (shrinkage)	Minutes	Cofilin, LIM kinase, Pak

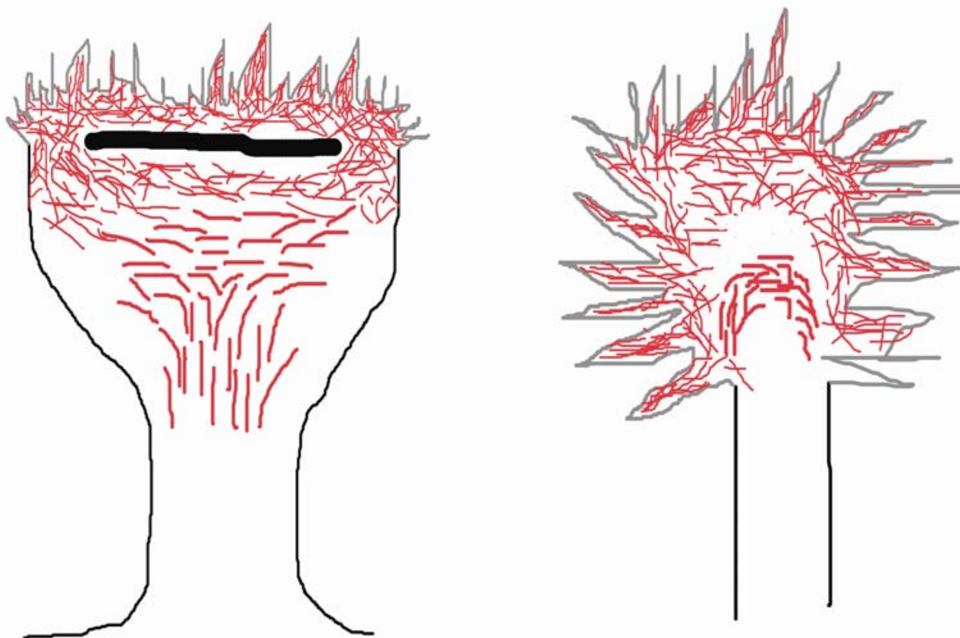

Figure 2. The basic appearance of the actin cytoskeleton in dendritic spines (left) compared to the cytoskeleton in neuronal growth cones (right). Both actin assemblies are defined by two distinct pools of actin, a stable pool with somewhat thicker, bundled filaments at the core, and a dynamic meshwork at the periphery. The dynamic actin pool accounts for shape changes by protruding lamellipodia and filopodia emerging from its tips. The dynamic actin in the spine is localized around the postsynaptic density (PSD, black bar in scheme), an electron-dense accumulation of postsynaptic proteins close to the synaptic cleft. Some, but not all spines also contain a stack of membrane cisternae at the core region extending into the neck, called the spine apparatus.

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