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A network of networks: Cytoskeletal control of compartmentalized function within dendritic spines

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Introduction

Almost thirty years ago, actin was identified as the major cytoskeletal component of dendritic spines. Since then, its role in the remarkable dynamics of spine morphology have been detailed with live-cell views establishing that spine shape dynamics are an important requirement for synaptogenesis and synaptic plasticity. However, the actin cytoskeleton is critical to numerous and varied processes within the spine which contribute to the maintenance and plasticity of synaptic function. Here, we argue that the spatial and temporal distribution of actin-dependent processes within spines suggests that the spine cytoskeleton should not be considered a single entity, but an interacting network of nodes or hubs that are independently regulated and balanced to maintain synapse function. Disruptions of this balance within the spine are likely to lead to psychiatric and neurological dysfunction.

Common regulatory mechanisms of the spine actin cytoskeleton

Actin is the major cytoskeletal element of spines, and its organization there reflects an elaboration of highly conserved mechanisms of actin regulation. As in dynamic regions of other cell types, actin monomers within persistent filaments undergo a form of continuous, serial exchange known as treadmilling [1]. Force is generated by profilin-catalyzed addition of monomeric G-actin to the growing, barbed ends of filaments; cofilin-mediated severing and depolymerization occurs at the opposite, pointed end [2], which replenishes the cytosolic supply of free monomers. Most filaments in spines undergo complete turnover of their constituent monomers within 60 sec [1,3]. Only a small fraction of filaments in a subset of spines is more stable, taking perhaps 10s of minutes to exchange monomers [4]. Thus, though stable filaments may play important roles, continual growth and depolymerization of filaments are likely to mediate most actin functions in spines.

Treadmilling on each filament establishes a directional flow of monomers away from sites of polymerization. Targeted photoactivation to the tip of spines indicates that flow within spines proceeds generally from spine tip to base [4], suggesting that the spine tip—perhaps the synapse—is the major point of polymerization. However, the limited resolution of confocal microscopy

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is insufficient to distinguish whether a more complex organization exists. As we argue below, the overall flow within spines likely results from multiple, potentially independent filament networks with distinct sites of polymerization, rather than a single network centered at the synapse.

In most cell types, extension of unbranched filaments is accomplished by the formin family, and in neuronal filopodia, the formin mDia2 appears to play a large role [5**]. On the other hand, branching of filaments near their barbed end is catalyzed principally by the Arp2/3 complex, activated by the neural Wiskott Aldrych syndrome Protein (N-WASP). Because spines have a high degree of branched filaments [6,7], it is assumed that Arp2/3-mediated branching is particularly important there. Indeed, knockdown, pharmacological inhibition, or mutation of N-WASP drastically reduces the number of spines [8], and RNAi-mediated suppression of the Arp2/3 complex selectively reduces mature, mushroom spines [5**]. Mechanisms of disassembling filaments appear broadly conserved as well. Depletion of cofilin via siRNA slows actin turnover [5**], and altering cofilin activity results in misshapen spines [9], confirming the importance of this protein in spines. Thus, comparisons to other cell types reveal broadly conserved mechanisms of actin regulation that control spine morphology. However, rather than merely using these mechanisms to control spine shape, neurons spatially, temporally, and molecularly adapt them to coordinate a diverse array of actin-dependent processes within the microscopic confines of the spine. To emphasize this, we have divided the review into sections based on the location of actin function in spines.

Actin roles specifically at the PSD

At excitatory synapses, a collection of multi-domain scaffold proteins comprises the core of the postsynaptic density (PSD). The PSD positions glutamate receptors in close apposition to the presynaptic active zone and provides a scaffold for synaptic signaling molecules. Larger PSDs are generally more morphologically complex, include more glutamate receptors, and are closely correlated with larger spine heads. Thus, the size and shape of the PSD not only directly control synapse function, but may also seed organization of the remaining areas of the spine.

Actin filaments are closely associated with the PSD, apparently contacting it directly [6,10]. These filaments are involved in several distinct mechanisms of PSD regulation. Inhibition of polymerization with latrunculin destabilizes synapses in an age-dependent fashion [11], and prompts the dissociation of AMPA receptors [12-14] and PSD proteins [15]. Within minutes of latrunculin treatment, enough time for one or a few complete cycles of treadmilling on spine actin filaments, approximately half of the PSD-bound Shank, GKAP, and homer 1c (PSD-Zip45) is lost [16]. Surprisingly, this occurs without affecting the molecular exchange rate of the remaining 50% [16], and with only partial loss of AMPA receptors [12-14].

Whereas the loss of AMPA receptors could involve actin-dependent processes outside the synapse, the immediate influence of depolymerization on PSD protein copy number is strong evidence that actin filaments act directly at the PSD. However, the latrunculin-insensitive subset of PSD molecules is poorly understood. Interestingly, latrunculin does not affect the stability of PSD-95 [16,17**], presumably because of the protein's direct association with the plasma membrane or transmembrane proteins. Thus, the intermolecular binding of scaffold molecules that is the foundation of PSD architecture is apparently not sufficient to maintain GKAP, Shank, and Homer1C in the absence of polymerizing actin. So, while interconnected, the PSD is not entirely cohesive. Instead, it may be organized into functional subsets that are partially autonomous, allowing dispersal of molecules from PSD subcompartments. Alternatively, it may comprise interlinked lamina, with interfaces that require constant support from actin-driven processes. Measuring PSD internal organization and determining the role of

cytoskeleton in establishing this organization are important goals for understanding synapse architecture.

An important, additional mechanism for cytoskeletal regulation of the PSD is clear from high-resolution time-lapse imaging of PSD morphology. Surprisingly, even at synapses in mature neurons (i.e., synapses that on average persist days or weeks [18]), the morphology of the PSD undergoes continuous change on a time-scale of seconds to minutes [17**] (Fig 1A). Nevertheless, depolymerization of actin filaments rapidly halts ongoing morphology changes (Fig 1B), as does stabilization of filaments with jasplakinolide. Notably, these changes do not tightly correlate with changes in spine morphology, but are regulated by changes in synapse activity (Fig. 1C). Thus, the PSD behaves not as a rigid body but as a flexible matrix, the shape of which is continuously adjusted by dynamics or treadmilling of the actin cytoskeleton. Importantly, these actin-driven changes in the overall size of the synapse occur much more rapidly than fluctuations in scaffold protein copy number [16,17**]. Accordingly, it is likely that morphological distortion acutely regulates the density of PSD scaffold molecules, potentially within subdomains [17**].

Because the nm-scale local density of PSD molecules plays an important role in receptor confinement and mobility in the synapse [19,20], actin-driven change in the shape of the PSD may influence the alignment of presynaptic and postsynaptic elements.

Though NMDA receptors can attach to actin via single intermediate actin-binding proteins such as alpha-actinin [21], AMPARs in the synapse generally contact the cytoskeleton through PSD scaffold proteins (via PSD-95 to citron or SPAR [22], or via GKAP and Shank to cortactin [23], βpix, or oligophrenin [24]). Given that actin both regulates PSD scaffold composition and drives changes in overall PSD size and shape, how might these aspects of actin function control synaptic AMPA receptor number? One possibility is that this control stems from regulation of PSD composition and molecular positioning. Importantly, AMPARs reach the postsynaptic membrane through free diffusion from extrasynaptic sites[20], but once they reach the PSD, their motion is considerably slowed. Receptors are likely retained within the PSD both by direct interaction between binding partners such as PSD-95 and stargazin[25], but also by steric hindrance to free diffusion due to crowding within the molecularly dense postsynaptic membrane [26]. In fact, the motion of even exogenous proteins linked to the external leaflet of the plasma membrane is hindered by steric obstruction when these molecules enter the synapse [27]. However, this obstruction is reduced by latrunculin A [27], suggesting that actin-mediated changes in scaffold density control behavior of membrane proteins like receptors even in the absence of high affinity binding interactions. Thus, actin-mediated control of PSD scaffold density by component release [15,16] or by flexing [17**] may tune the ability of the synapse to retain AMPARs [12-14]..

Segregation of actin function in spine membrane subdomains

Perisynaptic membrane

Single-particle tracking has made clear that receptors outside the synapse, even those very near it, for the most part diffuse nearly freely within the plasma membrane [28,20]. However, under specific circumstances, actin polymerization may directly restrict the motion of extrasynaptic AMPARs. The regulation of spine or spine neck morphology by actin may help to passively trap membrane proteins within the extrasynaptic spine membrane [29,30]. However, the cytoskeleton may more directly influence the distribution of AMPAR in the extrasynaptic membrane. For instance, the increase of receptors in the synapse during induction of long-term potentiation depends on delivery of AMPA receptors to a unique “pool” near but outside the synapse [31**]. Remarkably, whereas delivery of receptors to the pool was insensitive to latrunculin, latrunculin application after the induction stimulus disperses the receptors and

prevents synaptic potentiation. While the precise molecular mechanisms by which actin anchors this receptor pool near the synapse are unclear, it is not likely to be merely through control of spine morphology. This is clear because treatment with latrunculin two minutes following LTP induction resulted in a loss of these perisynaptic receptors and a decrease in potentiation, but did not block the LTP-associated increase in spine size [31**]. The site of this actin-dependent accumulation is unclear, as is the role of receptors themselves in establishing the accumulation. One interesting possibility is that GluA1 receptors themselves, through protein interactions with their C-termini that are triggered by LTP induction, may help stabilize actin filaments [32].

Non-synaptic spine membrane

The details of potential spine membrane specialization outside the PSD are largely uncharacterized. However, one clearly defined spine subregion is the endocytic zone (EZ), a point of persistent accumulation of clathrin several hundred nanometers outside the synapse, where AMPAR endocytosis takes place [33-35]. Molecular perturbations that displace EZs from their normal close association with the PSD result in synapses that contain fewer receptors, are smaller, and undergo less robust LTP [35]. Though strong roles of actin in endocytosis [36] might suggest that the cytoskeleton anchors or seeds the location of the EZ, latrunculin does not alter the perisynaptic positioning of the endocytic machinery within spines [33]. In fact, dynamin 3, an endocytic large GTPase, is required for PSD-EZ positioning, but its binding to the actin regulatory protein cortactin is dispensable for this function. Thus, this resistance to latrunculin may indicate that AMPAR endocytosis in spines requires actin polymerization only in sporadic bursts during internalization, in contrast to the need for ongoing polymerization to maintain the PSD.

If synaptic AMPA receptor stabilization and endocytosis are each regulated by actin polymerization, are there common molecules that coordinate these two processes? Recent evidence supports this idea. Rocca et al. [36] found that the BAR-domain protein PICK1, previously known to regulate AMPAR trafficking, binds to actin and inhibits the Arp2/3 complex, and that this negative regulation is important for AMPA receptor endocytosis. Importantly, the efficacy of PICK1 inhibition is strongly enhanced by concurrent binding of PICK1 to the C-terminus of GluA2. This causes a local inhibition of actin branching, and, potentially with contribution from the PICK1 BAR domain, facilitates progression of clathrin-mediated endocytosis. Consistent with this model, shRNA targeted against PICK1 blocked endocytosis of GluA2 in response to a chemical LTD stimulus [37**]. Thus, it may be that following LTD induction, PICK1's unique role can facilitate rapid and efficient synaptic removal and subsequent endocytosis of AMPA receptors.

An important question is where this inhibition of Arp2/3 by the GluA2-PICK1 complex takes place. One intriguing possibility is at the PSD directly, where inhibition of ongoing Arp2/3-mediated branching could transiently destabilize PSD components and help free receptors for migration to the endocytic zone during LTD. However, it is not clear whether mobilization of receptors between the synapse and the endocytic zone during LTD is influenced by non-synaptic actin. Thus, alternatively, transient inhibition of Arp2/3 may facilitate extrasynaptic receptor mobility. A third possibility is that actin branching may act as a constitutive negative regulator of endocytosis via establishment of a cortical actin barrier; transient disassembly of this barrier by inhibition of Arp2/3 at the endocytic zone could facilitate the maturation specifically of coated pits that contain GluA2. Testing these hypotheses will require refinement of techniques to discriminate actin molecular dynamics in spine subregions, and is ongoing.

Spine-localized endocytosis is well established, but the role of AMPAR exocytosis into the spine plasma membrane is under intense consideration [38-42]. However, AMPAR and transferrin receptors undergo activity-triggered exocytosis from large organelles directly in

spines of cultured neurons at zones of clustered syntaxin 4 lateral to the PSD [43*]. Actin directly binds syntaxin 4 [44] and regulates its plasma membrane clustering [45]. Syntaxin 4 is also found in Rab11-positive endosomes [46], though whether this facilitates or retards exocytosis of these organelles is not known. Thus, whether actin plays a role at sites of exocytosis near synapses in neurons remains to be determined, and it will be important to distinguish whether actin independently or coordinately regulates both exocytosis and endocytosis at distinct zones of the spine membrane.

Distributed sites of actin function during receptor trafficking

Intracellular mobilization of organelles and vesicles to, from, and within the spine requires myosin-based motors that move cargo along actin filaments. Myosin transport is directionally specific along the filament, and so the distribution within spines, the length, and the orientation of filaments dictate the molecular requirements of transport. Myosin VI is required for efficient endocytosis in many cells [47], and for clathrin-mediated AMPAR endocytosis in neurons [48]. Because it walks towards the pointed end of actin filaments, myosin VI could draw vesicles inward from the membrane on filaments with barbed ends polymerizing at the membrane, a likely orientation. Because latrunculin does not obviate AMPAR endocytosis, it may be that filaments required by myosin VI are more stable than those involved in securing receptors at the synapse [49]. In fact, generation of shorter, branched filaments may compete with long filaments ideal for myosin-based transport [49]. If so, then the localized inhibition of Arp2/3 that facilitates endocytosis [37**] may do so by permitting the assembly of longer filaments that more easily transport myosin VI-driven endocytic vesicles away from the membrane.

A number of potential myosins have been proposed to mediate the trafficking of endosomes in the spine [50], including myosin Vb [40] which walks toward the barbed end of actin filaments. Thus, a potential regulator of endosome traffic in spines is not merely the density but the orientation of polymerizing actin filaments within the spine head. In addition to myosin-dependent traffic, exocytosis may be regulated by other actin-dependent steps. Exocytosis of GluA1 AMPARs requires the actin-binding protein 4.1N, which binds to GluA1 at phosphorylated, membrane-proximal residues [41]. Because 4.1N is member of the large FERM domain actin/spectrin-binding family, this suggests a further role of cytoskeleton in exocytic AMPAR trafficking. It will be important to determine whether this regulation occurs at the membrane or during prior organelle mobilization.

Other organelles important for spine function, including mitochondria [51] and mRNA granules [52], are also mobilized within spines, often via myosin-dependent transport. Regulation of actin filament assembly, branching, and length at distributed locations within the spine can thus actively control spine organelle trafficking.

Actin in other regions of the spine

Ongoing actin polymerization has been implicated in the maintenance of spine function at other locations, including the spine neck and spine apparatus. Spine necks, like the filopodia they develop from, have traditionally been thought to contain linear bundles of actin filaments. Consistent with this, actin-bundling proteins such as neurabin/spinophilin affect both filopodia outgrowth and spine morphological maturation [53]. However, recent work has highlighted the presence of anti-parallel [5**] or even branching [54*] networks in the spine neck, suggesting a more complex neck cytoskeleton. This spine neck actin network is likely to play several roles. First, because the orientation of actin filaments determines the direction of travel of specific molecular motors carrying cargo during plasticity [50,40], heterogeneous filament orientation may facilitate motion through the neck based on diverse myosins. However, locations in the neck where pointed ends of antiparallel filaments abut one another may serve

as filters, since only organelles carrying more than one kind of motor could transit through. Second, neck morphology, presumably actin-dependent [9], controls the diffusion of both receptors [30] and cytosolic molecules or Ca^{2+} ions [55,29].

The spine apparatus, a specialization of smooth endoplasmic reticulum found in a subset of mushroom spines [56,57], is associated with dense, filamentous actin [10] and the actin/α-actinin binding protein synaptopodin [58]. Deletion of synaptopodin eliminates the spine apparatus and reduces hippocampal LTP [57], though the precise role of the spine apparatus remains a mystery. Actin filaments at the spine apparatus are a candidate for what may constitute the small fraction of rather stable spine actin that is regulated during LTP [4], and it will be important to determine whether the bulky spine apparatus and associated actin filaments may regulate transit of organelles through the spine neck.

Spine actin as an interacting network of networks

We believe the variety and spatial distribution of documented functions for actin within spines, as well as their different modes of regulation, turnover rates, and bundling leads inevitably to the idea that actin within spines is regulated as a series of interdependent filament networks, not as a single assembly. This is illustrated in Figure 1D, which highlights some of the principle sites of actin function in spines. Such a view is in many respects simply an elaboration of well-characterized processes in other cells, but diverse roles within the tight confines of the spine suggests interplay among these different networks with potentially complex functional implications that may be unique to spines.

The postulated local regulation of cytoskeleton at subdomains within spines that are on the order of only 1 μm across would require very tightly restricted spatial control of filament dynamics. Several potential mechanisms to generate such control are illustrated in Figure 2. These include localization of molecules that control extension or branching of filaments, mechanisms to regulate the stability and conformation of existing filaments in precise regions, and direct control of filament lifetime via severing and depolymerization. In addition, signaling cascades initiating at the cell membrane that impact cytoskeletal dynamics are likely to be spatially restricted by molecular anchorage of key components.

Some of these possible means of local regulation have been demonstrated recently, most notably mechanisms to control actin dynamics specifically at the PSD; similar mechanisms may operate at other subdomains. A number of molecular links provide a spatially precise mechanism for recruitment of actin regulatory machinery to the PSD. The Arp2/3 complex, the regulator of actin filament branching, has been shown to be enriched within 200 nm of the PSD [59], and even appears in biochemical fractions of the PSD [37**]. This positioning may be facilitated by the filamentous actin binding protein Abp1. Abp1interacts with F-actin via two N-terminal domains and simultaneously with Shank PSD scaffold protein family members via its C-terminal SH3 domain [60]. Disrupting the Abp1 link between the PSD and F-actin disrupts normal spine morphological development [61]. Importantly, Abp1 stimulates actin branching by binding and activating N-WASP, the Arp2/3 regulator. Thus, filaments binding Abp1 at the PSD are likely to undergo Arp2/3-dependent polymerization and branching. These filaments, in turn, may provide additional binding sites for Abp1 near the PSD. Abp1 may not be the only such molecule to facilitate Arp2/3 function at the PSD. Cortactin, which has similar ability to bind Shank, actin, and N-WASP [23] and to activate Arp2/3-mediated polymerization [62], is enriched in spines and near the PSD [63]. Cortactin regulates spine morphology[64], but its effects on the PSD itself are unclear. Thus, it will be important to determine how this mechanism contributes to control of the morphology or internal density of the PSD, and to receptor traffic at the synapse. Further regulation of actin filaments at the synapse could be

mediated by subspine targeting of molecules that regulate branching dynamics, such as coronins, which compete with cortactin at branch points to remodel actin branches [65*].

Positioning of assembled filaments may provide an additional level of regulation. Though many mechanisms likely exist, the interplay between CaMKII and actin is a particularly important one that has capability of providing highly localized regulation of each of these molecules. Translocation of CaMKII to the PSD following NMDA receptor activation is thought to be critical for phosphorylation of appropriate synaptic targets during long-term synaptic plasticity. CaMKIIβ binds actin [66], but is displaced by Ca^{2+}/CaM [67,68]. Thus, CaMKII holoenzymes are likely actin-bound when Ca^{2+} levels are low, meaning that the distribution of filaments within spines may dictate positioning of CaMKII. For instance, filaments near NMDA receptors in the PSD or near other Ca^{2+}channels may help CaMKII respond to Ca^{2+} influxes. Conversely, CaMKIIβ increases filament stability [69,68]. Intriguingly, binding of CaMKII to targets in the PSD might then regulate actin specifically at the synapse through filament bundling or by phosphorylating RacGEFs directly at the synapse [70]. Moreover, it has been suggested that when CaMKII dissociates from the PSD, its β subunits may bind again with reorganized actin bundles and thereby help to maintain enlarged spine structures after long-term synaptic plasticity [69,71]. Thus, CaMKII provides an example of how, in addition to local control of filament polymerization or depolymerization, localized regulation of filament positioning or stability within spine subdomains adds important functional complexity to synaptic signaling in spines.

Spine actin in disease: Spine morphology or localized control of specific functions?

Perturbing the balance of spine and synapse actin dynamics has a powerful effect on synapse function, effects which manifest themselves in disorders ranging from schizophrenia to mental retardation [72]. A critical question is whether this imbalance is functionally important because of changes in spine morphology or because of disruption of the many distributed roles played by actin within spines—independent of spine morphology. Assessing this directly in disease models will be important. Until that can be accomplished, mechanisms of long-term potentiation and long-term depression (thought to be key points of functional disruption during disease progression) provide one means to assess this question. It has grown increasingly clear that changes in spine morphology can be both temporally and mechanistically dissociated from plasticity of synapse function [73-75]. For instance, long-term depression depends on the phosphatases calcineurin and PP1, whereas spine shrinkage induced by the same protocols depends only on calcineurin, which dephosphorylates cofilin. Cofilin activity is dispensable for LTD of AMPAR-mediated transmission in hippocampus [76] but required for LTD-associated spine shrinkage [75]. Interestingly, phosphorylation-mediated inhibition of cofilin may be required for LTP induction [77,78]. These examples thus provide functional evidence for independent regulation of subspine actin networks.

Accumulating examples suggest that disease frequently impacts spine actin function by aberrant modulation of small GTPases. This is because receptor-mediated signaling via small GTPases of the Rho family mediate important, spine-specific control over cytoskeletal dynamics during neuron growth and plasticity [79]. One unexplored key question is whether the function of these proteins and their downstream effectors is spatially restricted within spines. Anchoring components of these signaling cascades within spine subdomains may help compartmentalize this regulation. An important example of this is anchorage of the Rho GTP/GDP exchange factor (RhoGEF) Kalirin-7 to the PSD. Kalirin-7 activates Rac1 and Rap1 to catalyze NMDAR-activated spine growth and AMPAR addition [70], but when bound to PSD-95 via a complex including the protein Disrupted-in-Schizophrenia1 (DISC1), its access to Rac1 is limited [80**]. This provides an potentially general mechanism to

subcompartmentalize such regulatory signaling cascades within single spines. It will be important to determine whether this serves only as a means of sequestering kalirin-7, or whether dissociation of DISC1 permits kalirin-7 activity directly at the PSD.

Open questions and emerging methods

A better understanding of the organization of spine cytoskeletal dynamics and compartmentalization is likely to emerge soon, as several new methodologies pass from development phases to application. Nanometer-scale fluorescence microscopy via STED, PALM, and single-molecule tracking via sptPALM offer ability to visualize dynamics of even single molecules in living spines [80,81,82*]. Platinum-replica EM [54*] and tomographic EM [84] offer more highly detailed snapshots of spines at critical phases of plasticity. Importantly, new methods to control actin regulatory proteins with light [85*,86*] offer hope of dissecting mechanisms with both temporal and spatial precision.

We anticipate that these new methods will help answer several key questions regarding the spine actin network of networks. Perhaps most fundamentally, which aspects of cytoskeletal regulation are truly independent of one another within spines, or does, for instance, enhanced polymerization in one spine nanodomain inevitably influence cytoskeletal dynamics in “distant” regions of the spine? Which actin regulatory molecules are available in rate-limiting supplies? By which of many potential direct or indirect means does actin control receptor density at the synapse? What is the dynamic organization of spine neck filaments, and does this organization play a passive or active role in controlling spine organelle mobility? What functional roles of actin can be altered in the absence of overt changes in spine morphology or morphological dynamics? And most importantly, which of these are associated with disease?

It has long been recognized that actin is the major cytoskeletal element in dendritic spines. Just why spines require such enrichment of actin has been unclear. Recent work illuminating the spatially distributed set of spine functional domains that actin regulates offers great promise for understanding synapse dynamics and their modulation in disease etiology, progression, and therapy.

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* of special interest

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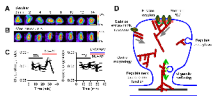

Figure 1. Actin regulates the PSD scaffold, and acts at other distinct locations throughout spines

A. Individual PSDs undergo morphological changes accompanied by internal changes in scaffold protein density. Hippocampal neuron 4 weeks in culture, transfected with PSD-95-GFP, and imaged by confocal microscopy. Color scale shows the proportion of total PSD-95 molecules per 1,000 nm^2 in the image. (Spatial scale bar: 1 μm). Panels A-C adapted from Blanpied et al. [17**], copyright 2008 National Academy of Sciences, U.S.A.

B. Actin drives changes in shape and spatial fluctuations of PSD-95 molecular density of individual PSDs. Same PSD as in A, after application of latrunculin A to prompt depolymerization of actin filaments.

C. Increasing neuronal activity increases the rate of actin-driven PSD morphological change, as measured with a shape change index based on the variance of PSD shape over time. This increase is blocked by glutamate receptor antagonists.

D. Summary of several known sites of actin regulation throughout individual spines, illustrating that the spine cytoskeleton is a network of networks that coordinately control synapse function via numerous distributed mechanisms. Gray arrows indicate direction of actin flow; green arrows indicate potential for cargo transport along filaments oriented in either direction.

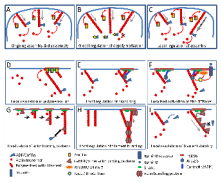

Figure 2. Mechanisms of spatially restricting actin regulation

A. Treadmilling of actin mediated by addition of monomers at filament barbed ends (red arrow) and depolymerization by cofilin (blue wedge) at or near filament pointed ends. Polymerized actin molecules and binding proteins flow along the active filament as monomers are added. Gray arrows indicate direction and rate of flow.

B. The pointed end of many filaments probably lie deep in the spine interior, and depolymerizing molecules are free to diffuse in the cytoplasm. Thus, even localized activation of signalling pathways near the spine membrane is likely to result in a widespread alteration of filament structure. For example, regulation of cofilin activity (by phosphorylation - yellow circle).

C. Conversely, sites of ongoing monomer incorporation near the spine membrane are likely to be important sites for localized regulation of filament structure, because rates of monomer incorporation may be controlled by anchored or membrane-associated factors.

D. Sites of assembly require profilin (yellow boxes), which is enriched near the PSD. This represents one known example of a potentially larger strategy for spatial regulation.

E. Spatial pattern of branch formation is determined by localization of the Arp2/3 complex (blue complex), which has binding partners Abp1 and cortactin that bind the PSD scaffold Shank.

F. Localization of signaling molecules including Kalirin-7, a Rho-GEF which binds PSD-95, may be responsible for the spatial segregation of downstream actin regulatory pathways.

G. A number of actin binding proteins such as CaMKII are localized at the PSD through specific binding to PSD scaffolds. their ability to bind to and be transported away from the membrane by a growing actin filament may be a mechanism for controlling the number and composition of signalling molecules at the PSD. In addition, these molecules compete with other actin binding proteins for sites on the growing filament.

H. A number of molecules, including CaMKII and alpha actinin, are localized at the synapse, and through association of multiple actin binding domains, have the ability to bundle actin filaments.

I. Actin binding proteins compete for binding sites on growing filaments. Thus, the preferential decoration of filaments near the synapse with molecules such as CaMKII or cortactin may represent a protective mechanism which allows growing filaments to penetrate more deeply into the spine interior before they are severed by cofilin.