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MICRODOMAINS IN FOREBRAIN SPINES: AN ULTRASTRUCTURAL PERSPECTIVE

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Abstract

Glutamatergic axons in the mammalian forebrain terminate predominantly onto dendritic spines. Long-term changes in the efficacy of these excitatory synapses are tightly coupled to changes in spine morphology. The reorganization of the actin cytoskeleton underlying this spine “morphing” involves numerous proteins that provide the machinery needed for adaptive cytoskeletal remodeling. Here we review recent literature addressing the chemical architecture of the spine, focusing mainly on actin-binding proteins (ABPs). Accumulating evidence suggests that ABPs are organized into functionally-distinct microdomains within the spine cytoplasm. This functional compartmentalization provides a structural basis for regulation of the spinoskeleton, offering a novel window into mechanisms underlying synaptic plasticity.

Excitatory signals in the mammalian forebrain are transmitted mainly by glutamatergic axons that terminate onto dendritic spines of pyramidal neurons. Most spines are tiny. A typical spine in CA1 hippocampus has a volume of ~0.02–0.03 fl [1], making them difficult to resolve with the limited resolution provided by the standard tools of live-cell imaging. Recent dramatic advances in super-resolution light microscopy [2, 3, 4, 5] can provide important insights into sub-spine dynamics. However, the outstanding spatial resolution provided by transmission electron microscopy (EM) makes it the primary tool of choice for studying the fine structure of the spine, though it is unsuitable for visualization of living cells.

Spine morphology

A variety of spine shapes can be distinguished, including thin, stuby, and mushroom-shaped (Fig. 1A, [6]). Mushroom-shaped spines are linked to their parent dendrite through a thin neck. It has been suggested that the spine neck might help to isolate the synapse from the shaft electrically (see review by [7]), though this idea remains controversial, and technical challenges make it difficult to address experimentally. In contrast, considerable evidence supports that the neck can provide a diffusion barrier for second messengers like Ca^{2+} [8, 9, 10, 11]. The biochemical compartmentalization thus achieved can isolate activity of synapses between neighboring spines, allowing independent modulation of single synapses [12, 13].

Spines proliferate rapidly in early postnatal life, but many of these early spines regress and disappear during maturation. Despite early suggestions that spines might be plastic or motile

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[14], by the latter part of the 20th century it was widely thought that spines in the mature brain were stable and fixed [see review by 15]. The introduction of new tools that allow direct visualization of spines in intact brain has led to the development of a more nuanced perspective. Large spines are generally stable [16, 17, 18]. In contrast, small spines are quite dynamic even in the adult; current evidence suggests that many of them are transient, and may either expand or disappear within a few days [16, 19, 20].

Extensive research in both hippocampus and neocortex documents a close relationship between spine size and synaptic efficacy [21, 22, 23, 24]. Spine volume itself predicts the size of the postsynaptic density (PSD), the number of AMPA receptors (AMPARs) at the synapse, and the magnitude of excitatory postsynaptic currents (EPSCs) in response to presynaptic glutamate release [25, 26, 27, 28, 29]. Moreover, stimuli that trigger long-term synaptic potentiation lead to spine growth and the insertion of additional AMPARs into the postsynaptic membrane [30, 31], whereas stimuli that trigger long-term depression lead to spine shrinkage and endocytosis of AMPARs [32, 33, 34].

The causal direction of this relationship between spine size and synaptic efficacy remains unclear, but the phenomenological evidence implies the presence of sophisticated biochemical machinery that links synaptic activity to the spine cytoskeleton (the “spinoskeleton”) [35, 36, 37, 38, 39, 40, 41]. Thus, a clearer understanding of the regulation of spine architecture may offer new insight into mechanisms underlying the control of synaptic processing. This line of inquiry may also have clinical implications, since several syndromes with prominent neuropsychiatric features are caused by genetic lesions directly linked to dysregulation of the actin cytoskeleton; these mutations can also lead to specific patterns of spine disruption [42, 43, 44, 45]. Considerable recent work suggests that the spatial organization of relevant proteins represents an important aspect of spinoskeletal regulation. Before reviewing these data, we will provide a brief overview of the functional organization of the spine.

Domains at the spine surface

When viewed with the electron microscope, the spine’s most prominent feature is the postsynaptic denisty (PSD), an electron-dense specialization extending beneath the plasma membrane, closely aligned with the presynaptic active zone across the 20–30 nm synaptic cleft (Fig. 1B). The PSD, which serves as the primary signaling domain of the spine, contains a complex matrix of receptors, scaffolding, and signal transduction molecules [46, 47]. Notwithstanding considerable variability, different synaptic proteins tend to segregate to distinct regions within the PSD. For example, the PSD exhibits a pronounced “laminar” organization: receptors are embedded in the plasma membrane, and adaptor proteins like PSD-95 concentrate near the exterior edge of the cytoplasm, whereas scaffolds like the Shank family lie deeper in the cytoplasm [2, 48]. Though less conspicuously, the PSD also seems to be organized in the tangential axis along the plasma membrane; for example, NMDA receptors generally lie in a central zone of the PSD, whereas AMPA receptors are more peripherally situated [49, 50, 51].

Immunogold electron microscopy has shown that a perisynaptic zone of the postsynaptic membrane is enriched in several signal-related proteins, including mGluR1, diacylglycerol lipase-α (an essential enzyme in endocannabinoid signaling at central synapses) and the glutamate transporter EAAC1 [52, 53, 54], though this zone is not morphologically distinctive. Beyond the perisynaptic region is a specialized trafficking zone for entry and exit of protein cargoes. This microdomain (originally identified with functional live-cell imaging) is not readily visualized even with electron microscopy, but its location has been verified using immunogold EM to demonstrate the organization of proteins associated with

endo- and exocytosis [55, 56, 57]. It is generally assumed that the zone adjacent to the spine neck is also biochemically specialized, though little direct evidence is available. In summary, the presence of multiple functionally-distinct domains along the plasma membrane of the spine is now generally accepted [58], though the functional implications of this organization remain poorly-understood.

Compartments within the spine

Spines may also contain various endomembranous structures. Spines on cerebellar Purkinje cells are especially rich in smooth endoplasmic reticulum (SER) that contains high levels of the inositol trisphosphate receptor [59]. SER is less often associated with forebrain spines (the focus of this review), but large spines in the forebrain often contain a peculiar membrane-bounded structure, the spine apparatus (SA) [60, 61]. The SA, usually considered a specialized element of the SER [62], contains high levels of calcium [63], suggesting that it may help to regulate spinoplasmic free [Ca^{+2}] [63, 64, 65, 66].

Though endosomes appear to play an important role in synaptic plasticity, they are rather uncommon in spines of the adult forebrain; a serial-section EM study detected endosomes in less than half of spines in CA1 stratum radiatum of the rat [67]. However, LTP-associated spine growth is accompanied by an increase of intraspinous vesicles, pointing to the importance of membrane trafficking events during synaptic plasticity [68]. During LTP, recycling endosomes may provide AMPA receptors to the synapse [69], while also supplying lipid membrane to the growing spine surface [70], thus linking morphological changes with synaptic potentiation. Conversely, the reduction of synaptic strength during LTD is accompanied by both internalization of synaptic AMPA receptors and spine shrinkage [71, 72].

Aside from these vesicular structures, the spine cytoplasm has traditionally been viewed as nondescript or amorphous, but contemporary work challenges this view. As outlined below, recent studies suggest the presence of multiple distinct domains within the cytoplasm.

The actin spinoskeleton

Spines are rich in actin [73], a highly-conserved globular protein of ~43 KDa molecular weight with ATPase activity. Extensive work in model systems demonstrates that actin cycles dynamically between soluble monomeric G-actin and polymerized ~8–9 nm diameter filaments of F-actin. These filaments can assemble into complex networks, which may undergo rapid extension and rearrangement, producing force and deformation of the plasma membrane [74, 75, 76]. Actin filaments also undergo “treadmilling,” in which filament length remains constant, while actin monomers add at the “barbed” end, and dissociate from the “pointed” end. Actin is essential for basic cellular processes, ranging from establishment of cell polarity and directional migration, to organelle trafficking and exo- and endocytosis [77].

At EM, the actin spinoskeleton typically appears as loose clumps of filamentous material [73, 78, 79]. Recent studies using high-resolution techniques like electron tomography (Fig. 2A–C) and metal shadowing (Fig. 2D) have elucidated important aspects of the structure of these complex actin networks [80, 81], but the functional organization of actin filaments within the spine remains unclear. Live-cell imaging experiments led to a “two-pool” model of spinoskeletal organization, which postulates that filaments are organized into a relatively stable core and a dynamic periphery [82, 83, 84]. Direct visualization of actin treadmilling has been demonstrated within spines using super-resolution live-cell imaging [3, 85, 86, 87, 88]. A distinct “enlargement pool” of filaments has also been described [89], and an actin pool that directly interacts with synaptic glutamate receptors within the postsynaptic density

(PSD) has also been identified [36, 90]. Actin filaments can be seen to make direct contact with the PSD [81, 91, 92]. While actin has multiple functions at the PSD, perhaps its most fundamental role relates to stabilization of glutamate receptors [93]. NMDA receptors can be directly linked to actin filaments through the α-actinin (see below). The association of AMPA receptors to F-actin filaments is less direct, via a variety of scaffold proteins, including PSD-95 [94, 95], Abp1 [96], PICK1 [97], and neurabin [98].

Controlling the spinoskeleton

An elaborate cascade of proteins modulates F-actin remodeling; this protein network is crucial for regulating shape and motility in eukaryotic cells. The Rho family of small GTPases links receptors at the plasma membrane to mediators of actin remodeling (see [37, 38, 90, 99, 100] for information on their role in spines). These and other upstream regulatory proteins operate on actin via a cohort of functionally-distinct families of actin-binding proteins (ABPs) that catalyze the construction and reorganization of actin networks. Numerous ABPs have been identified in dendritic spines (Table 1). Intriguingly, these ABPs are targets of many of the same signaling pathways involved in long-term synaptic plasticity [101]. Furthermore, agents that interfere with actin remodeling also impair synaptic plasticity [102], confirming a long-suspected functional link between actin and synaptic efficacy [92, 103]

Regulation of the cytoskeleton requires tightly restricted spatial control of filament dynamics. Considering their functional specificity and their direct linkage to actin, defining the spatial organization of ABPs within the spine makes it possible to apply the static information available via EM to deduce aspects of the dynamic control of actin, at resolution not yet possible with live-cell imaging. This possibility is of special interest for investigating the sub-femtoliter volume of the dendritic spine. Evidence from immunogold electron microscopy that functionally-distinct ABPs concentrate in spatially-distinct domains within the spine cytoplasm points to a complex arrangement of multiple functional actin microdomains. Based on these data, a more quantitative and geometrically realistic view of spinoskeletal architecture is emerging, complementing functional LM studies. We here review these ABP-defined microdomains, focusing on a limited set of ABPs for which high-resolution ultrastructural data are available, to illustrate general principles underlying spinoskeletal design.

Linking the cytoskeleton to the plasma membrane

The actin cytoskeleton interacts with the plasma membrane via a family of intermediary proteins [104, 105]. **α-actinin**, an ABP of ~100 KDa MW that can crosslink filamentous actin *in vitro* [106], links the cytoskeleton to transmembrane proteins at cell-cell contact sites in a wide range of eukaryotic cells [107]. In neurons, α-actinin has been linked to the PSD both by proteomic screens and immunogold EM. The presence of α-actinin-1 and -2 isoforms in the brain has been shown directly [108, 109], and α-actinin-3 was demonstrated in proteomic studies [110]; the α-actinin-4 isoform is also implicated in neural function [111]. Immunogold electron microscopy shows that α-actinin is associated with glutamatergic synapses; in hippocampus it concentrates in the PSD [109] (Fig. 3), and is also associated with the spine apparatus (see below).

α-actinin can bind directly to the GluN1 and GluN2B subunits of the N-methyl D-aspartate receptor (NMDAR) via its central spectrin repeats, providing a direct link between NMDARs and the actin spinoskeleton [112, 113, 114, 115]. The C-terminus of α-actinin can bind to the dodecameric holozyme **CaMKII**, and these two proteins may work cooperatively. LTP-inducing stimuli cause massive NMDAR-gated Ca^{2+} entry into the

spine, recruiting CaMKII to the PSD, as has been demonstrated by several methods, including quantitative immunogold EM [116] (Fig. 3). Concurrently, by binding to calmodulin, the Ca^{2+} displaces α-actinin from the GluN1 subunit, allowing CaMKII to bind to the NMDA receptor [112]. Interestingly, the β subunit of CaMKII has been shown to bundle F-actin [117]. Thus, α-actinin and CaMKII may function together at the PSD to modify synaptic efficacy, while also acting on the actin cytoskeleton [118, 119, 120].

α-actinin can also interact with **synaptopodin**, a 74 kDa ABP originally described in podocytes of the kidney which can elongate and bundle actin filaments [121]. Synaptopodin's α-actinin binding domain is crucial for its postsynaptic localization in neurons [60, 122, 123]. Both pre-embedding immunoperoxidase and postembedding immunogold methods demonstrate synaptopodin in a subset of spines of forebrain neurons (Fig. 3,[122]), concentrating in the spine neck and spine apparatus. This association with the SA is functionally significant: synaptopodin knock-out mice lack a typical SA [124]. Importantly, these knock-out mice also display impaired LTP [125]; moreover, synaptopodin has been implicated in memory formation in the behaving animal [124].

Turmoil under the surface: the shell domain

The spinoskeleton is continually changing in response to synaptic activity. A prerequisite for this remodeling is the destruction of preexisting architectural elements, to permit creation of a new network. This recycling task is performed by the **ADF (Actin Depolymerizing Factor)/cofilin** enzyme family, comprising three ~20 kDa proteins in vertebrates: ADF (also known as destrin), cofilin-1 (the major form in non-muscle tissue), and cofilin-2 (mainly in muscle). Proteins of the ADF/cofilin family enhance turnover of actin both by increasing the rate of depolymerization at filament ends, and by cutting long filaments to expose uncapped barbed ends, permitting new filament growth [126]. These proteins are enriched at sites of motility associated with rapid actin reorganization [127], as best documented at the leading edge and ruffling membranes of cultured fibroblasts [128]. An analogous enrichment is seen in corresponding regions of neuronal growth cones [129]. Here we focus on cofilin-1 (the major isoform in the brain), which we will refer to simply as "cofilin."

Stimuli that trigger long-term depression (LTD) in hippocampal neurons also cause spine shrinkage. Considerable evidence implicates cofilin as an essential agent linking hippocampal LTD to structural plasticity [33, 130]. Cofilin's enzymatic activity is tightly regulated by an interplay between (Lin-11/Isl-1/Mec-3)-domain-containing protein kinase (LIMK) and the phosphatase Slingshot [131, 132, 133]. The LIMK gene is deleted in Williams-Beuren syndrome, a disorder associated with severe mental retardation and visuo-spatial cognitive deficits [134]; thus, dysregulation of neuronal cofilin has clinical implications. Interestingly, cofilin-actin "rods," which form under conditions of cellular stress, have been reported in postmortem studies of patients with Alzheimer's disease [135, 136].

Immunogold EM labeling reveals that cofilin in hippocampal spines concentrates in the immediate vicinity of the plasma membrane (the "shell"), and is sparse in the central "core" of the spine (Fig. 3) [137]. Since cofilin is a prerequisite for rapid actin reorganization [138], these immunogold data point to the shell domain as the most dynamic part of the spinoskeleton. Cofilin concentrates near the extrasynaptic plasma membrane, so it is not surprising that it also lies in the PSD, where it may help to regulate the postsynaptic signaling scaffold. Cofilin has been reported to play an active role in LTP consolidation via modifications of both receptor number and spine size [30, 133, 139]. Phosphorylation inactivates cofilin, and spines enriched in phosphocofilin were reported to have unusually large synapses [35, 139]. Conversely, cofilin is dephosphorylated and activated by LTD

inducing-stimuli, which lead to spine shrinkage. These observations are especially intriguing because spine volume is closely linked to synapse size, which in turn is closely linked to the number of AMPA receptors [26]. In summary, current evidence suggests a distinct shell microdomain that can remodel quickly in response to external signals.

Branches below the surface

Cytoskeletal remodeling requires extension and branching of actin filaments, and stabilization of these branches. A number of ABPs that serve these functions have been identified in model systems. The **Arp2/3 complex**, a multi-protein assembly of net molecular weight ~225 kDa, plays a central role in filament branching and membrane protrusion [140, 141]. The purified complex contains one copy each of seven subunits: two **a**ctin-**r**elated **p**roteins (Arp**2** and Arp**3**) whose tertiary structure resembles that of actin, and five additional subunits, ARPC1–5. Several upstream modulators, including the nucleation-promoting N-WASP (Neural-Wiskott-Aldrich syndrome protein), WAVE/Scar [142], cortactin [143], and the inhibitor PICK1, regulate the activity of the Arp2/3 complex [97]. Upon activation, Arp2/3 acts to initiate a new “daughter” actin filament, branching at ~70° from the side of an existing “mother” filament [144].

Activation of Arp2/3 in neurons promotes spine head expansion [145, 146, 147]. Inhibition of its activity by PICK1, a PDZ and BAR-domain containing protein that can bind to AMPA receptors, leads to defective neuronal architecture associated with a reduction in the number of mature dendritic spines [148]. The PICK1-Arp2/3 interaction is also necessary for spine shrinkage during LTD [97]. ImmunoEM indicates that the Arp2/3 complex concentrates in a restricted donut-shaped domain in the spine head: label is found away from the plasma membrane, but also avoids the center of the spine, lying mainly in a zone 20 – 100 nm from the plasma membrane on the “side” of the spine, away from the PSD (Fig. 3)[149]. In model systems, the Arp2/3 complex is spatially restricted within the extending lamellopodium during polarized extension [150]. The restricted Arp2/3 zone in the spinoskeleton may represent an analogous microdomain specialized for actin branching and nucleation [80, 144, 145, 147].

Deep in the spine: a stable but dynamic core

The central zone of the spine head is relatively stable, suggesting that the structure of the actin network in the core may be maintained for prolonged periods. Consistent with this notion, recent *in vivo* and *vitro* super-resolution imaging of spines [4, 85] indicates that the density of actin is higher in the center compared to the periphery. Since these studies imaged actin itself, the actin-related biochemical machinery could only be deduced indirectly. Using quantitative immunoelectron microscopy, three ABPs, **cortactin**, **profilin** and **drebrin**, have been found to localize to the central core, where they seem to play related but distinct roles in maintaining structural integrity.

Cortactin, an 80–85 kDa ABP implicated in nucleation, branching, and stabilization of actin filaments, can activate the Arp2/3 complex when phosphorylated [151, 152]. Studies in fibroblasts show that cortactin localizes to lamellopodia, filopodia, and membrane ruffles, where it colocalizes with F-actin and the Arp2/3 complex [153, 154]. In neurons, cortactin concentrates in spines [155]. Cortactin can directly bind to NMDA receptors through its SH3 domain [143, 156], and quantitative immunogold electron microscopic data show that cortactin is indeed found — though at modest levels — at the PSD. However, cortactin is at much higher levels within the spine core, 100–150 nm away from the PSD (Fig. 3) [157]. Multiple lines of evidence suggest that activation of NMDA receptors causes cortactin to move from the spine to the dendritic shaft [143, 155, 158]. Interestingly, this activity-driven

translocation of synaptic cortactin is lost in the Fmr1 knock-out model of fragile X syndrome, in which stabilization of both actin filaments and LTP is impaired [44]. While this and other evidence suggests an important role for cortactin in both electrophysiologically-defined synaptic plasticity and behavioral models of learning, it remains unclear exactly how the loss of functional cortactin causes these deficiencies.

Profilins, a family of 14–16 kDa proteins, are essential for actin polymerization. By attaching G-actin to the barbed end of an F-actin filament, profilin mediates filament elongation [159]. Four isoforms of profilin have been identified, the products of four genes (*Pfn1-Pfn4*). The most ubiquitous isoform is profilin-1 [160]. Profilin-2 has two splice variants, 2a being primarily expressed in neurons [161], whereas expression of profilins 3 and 4 is largely restricted to kidney and testes [159]. In dendritic spines, profilin plays a role complimentary to cortactin [162]. Profilin-1 is present in most spiny neurons; immunogold analysis reveals that profilin-1 localizes to the spine core, though labeling is also seen in the PSD (Fig. 3) [163]. An elegant study in the lateral amygdala shows that fear conditioning drives profilin to the spine head, resulting in enlarged spines [164]. Profilin-2 has been demonstrated in spines of cultured hippocampal neurons expressing GFP-tagged protein. In contrast to cortactin, LTP-inducing activation of postsynaptic NMDA receptors recruits profilin-2 from the dendritic shaft into the spine core [162]. This recruitment stabilizes spine morphology; moreover, blocking its translocation from shaft to spine prevents formation of LTP. Nevertheless, profilin-1 and profilin-2 knock-out mice have normal LTP and LTD, and exhibit normal learning [165, 166], perhaps reflecting compensatory mechanisms. Interestingly, immunolabel for profilin is often visible in the spine neck [163], consistent with its ability to shuttle between the spine head and the dendritic shaft. The available evidence suggests that profilins are highly mobile, and can enter and leave the spine head in an activity-dependent manner.

Drebrins (***d***evelopmentally ***re***gulated ***br***ain prote***ins***) are a family of 95–100 kDa actin-binding proteins. Drebrin can bind to F-actin and inhibit its interactions with tropomyosin and α-actinin, leading to thick, curving bundles of actin filaments [167, 168, 169]. Two isoforms have been identified, products of alternative splicing from a single gene: the embryonic (drebrin E), which is found primarily in developing spines of the rat brain at postnatal day (PND) 7 [170, 171], and adult (drebrin A), which becomes common by PND21 [172]. While both isoforms are expressed in neurons, drebrin A is neuron-specific, localizing to the postsynaptic side of excitatory synapses [173]. Early in postnatal development, drebrin A and E localize to the spine shell [170, 173], suggesting that both isoforms play a role in spine maturation. However, drebrin A has been shown by immunoelectron microscopy to concentrate in the core region of mature spines (Fig. 3). Spines with large heads and PSDs contain higher levels of drebrin A than found in small spines [174]. This is particularly interesting in the view of a recent study in neurons showing that drebrin A changes the mechanical properties of actin filaments, rendering them resistant to depolymerization [175]. Thus, modulation of filament assemblies by drebrin A likely plays an important role in the activity-dependent spine expansion and stabilization associated with long-term synaptic potentiation [176, 177].

In summary, although the spine core is considered the stablest zone of the spinoskeleton [89], actin filaments in the core are nevertheless regulated by the coordinated interplay of cortactin, profilin, and drebrin, in an activity-dependent manner.

Conclusion and perspective

Actin filaments play a crucial role in regulating both spine morphology and synaptic plasticity. The architecture of the actin-based spinoskeleton is controlled by multiple

families of ABPs. The data reviewed here suggest that these ABPs concentrate in distinct spatial domains, notwithstanding the rapid turnover of actin (Fig. 4). Since immunogold quantification relies on averaged values, it may fail to detect heterogeneity among spines [178]. However, this averaging attenuates underlying nonrandom distributions, so the true *in vivo* distribution of specific ABPs is likely to be even more restricted. These distinct ABP-defined microdomains imply a highly compartmentalized regulation of the spinoskeleton [179]. This conclusion is supported by observations from live-cell imaging, which also point to functionally discrete sub-spine actin domains [3, 89, 145, 180]. Taken together, these data suggest that the spinoskeleton is regulated by spatially-restricted ABP domains, which control the activity-dependent actin remodeling observed during synaptic plasticity.

While suggesting hitherto-unrecognized functional domains within the spine, this ABP-based view also raises a number of questions. For example, the mechanisms that target and hold ABPs in position within the spine are unclear: how can the spinoskeleton maintain a stable architecture in the face of rapid turnover of actin filaments? How dynamic are these cytoskeletal domains, and how much variability is there among different spines? It would also be useful to know how ABP domains are localized during neural development. Since spine morphology is closely linked to synaptic plasticity, and abnormalities in spine morphology have been linked to a variety of clinical disorders [42, 45, 181], it will be important to determine whether specific disease states are associated with specific pathological changes in ABP-defined microdomains. Gaining further insight into the organization of proteins that regulate actin in dendritic spines may provide important new clues to basic mechanisms underlying mental retardation and neuropsychiatric disease. This will require new tools and approaches, but these new tools cannot yet replace the resolution provided by the electron microscope.

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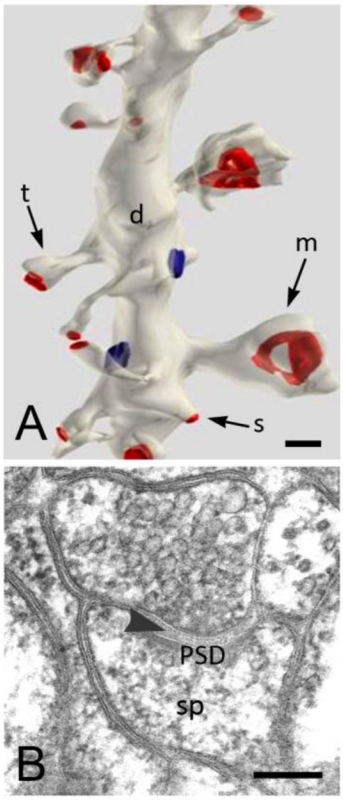

Figure 1. Appearance of dendritic spines in the electron microscope

A. 3D reconstruction of serial thin sections (from stratum radiatum of rat CA1 shows stubby (s) spines on the same segment of a dendritic shaft as spines with thin (t) and mushroom (m) shapes. Synaptic contacts have been colorized in red. Image is from http://synapses.clm.utexas.edu/anatomy/compare/compare.stm, reprinted with kind permission from J. Spacek. **B.** Micrograph of a thin (70 nm) section shows a typical mushroom-shaped spine from the rat hippocampus. Note the presynaptic terminal with synaptic vesicles, the synaptic cleft (arrowhead) and the postsynaptic density (PSD). The filamentous material in the spine head (sp) represents the actin cytoskeleton. Scale bars: 200 nm

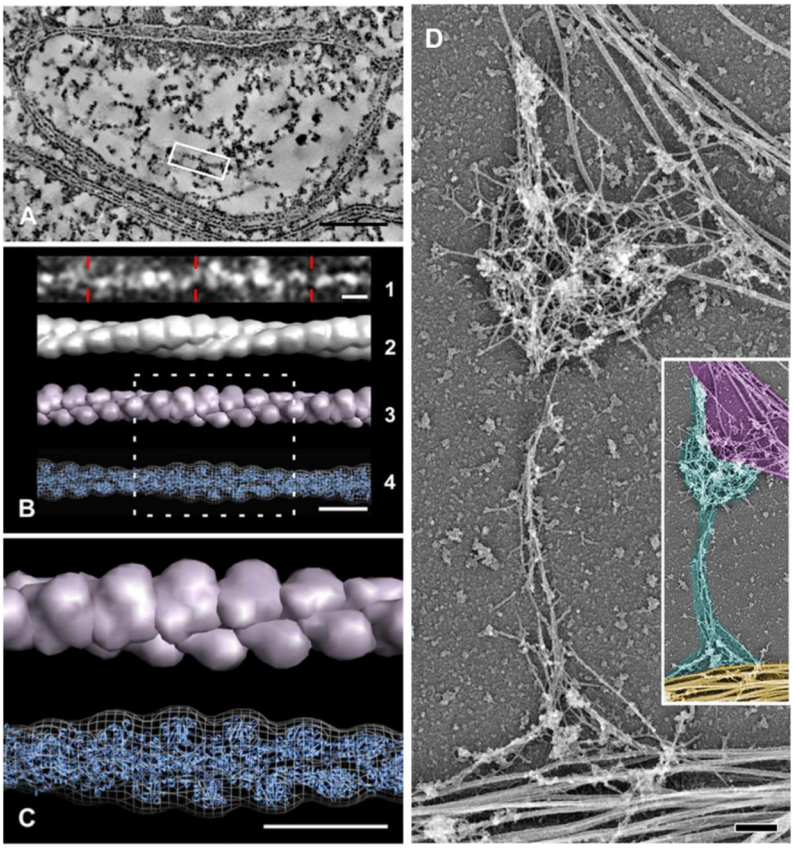

Figure 2. Appearance of F-actin in spines, demonstrated by high-resolution EM techniques

Left panel shows electron tomography of a thin section from rat neocortex (images adapted with permission from [81]); right panel is from a platinum-shadowed replica of cultured hippocampal neurons ((image adapted with permission from [80]). **A** shows a virtual slice (~4 nm thick) through a spine (the PSD is at top). A volume containing a long, straight filament (highlighted by the white box) is shown in $\mathbf{B_1}$ (image inverted to highlight features). This filament displays helical periodicity corresponding closely to that predicted for F-actin. $\mathbf{B_2}$ shows a helically-averaged surface representation of the extracted density. $\mathbf{B_3}$ is a low-resolution representation of an atomic model of a canonical actin filament obtained from high-resolution electron microscopy analysis. $\mathbf{B_4}$ shows the fit of this atomic model (blue cartoon representation) into the symmetrized, extracted filament density (chicken wire representation). **C** is an enlargement of the boxed region in B. Scale bars: 100 nm for A, 10nm for B and C. **D** illustrates the cytoskeleton of a spine after membranes have been removed. The organization of filaments in the head and neck of a mushroom-shaped spine associated with dendrite (yellow pseudocolor, at bottom of inset) and with axon running along the head (magenta, top of inset) from 14 DIV neurons, treated with Triton X-100 to reveal internal features. Thick fibers in dendritic shaft represent microtubules. Actin filaments (cyan pseudocolor in inset) are the main cytoskeletal elements in the spine head and neck. Inset in D is a shrunk and pseudocolored version of panel D. Scale bar 200 nm.

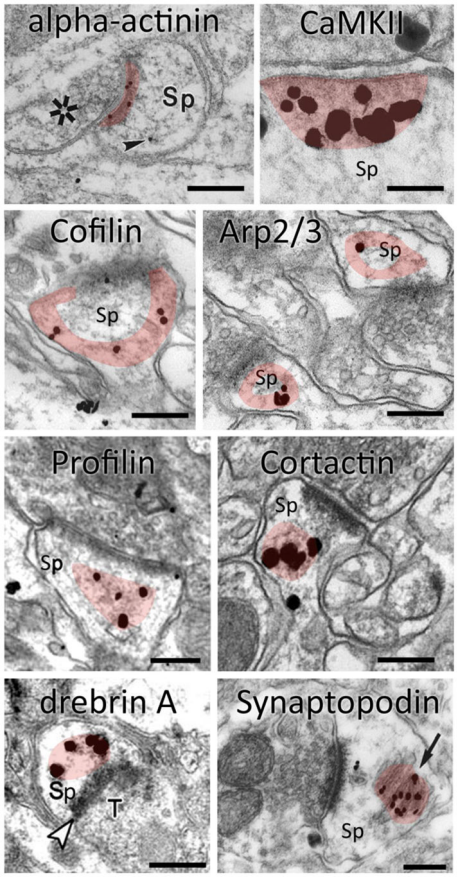

Figure 3. ABP content of spines, revealed by immunogold

Hippocampal spines (Sp) labeled with immunogold for eight different actin-binding proteins. (Images adapted with permission from [109] (α-actinin), [116] CaMKII, [137] (cofilin), [149] (Arp2/3 complex),[163] (profilin), [157] (cortactin), [174] (drebrin) and [125] (synaptopodin). Shaded area represents the zone of concentration, for each protein. Scale bar: 200 nm.

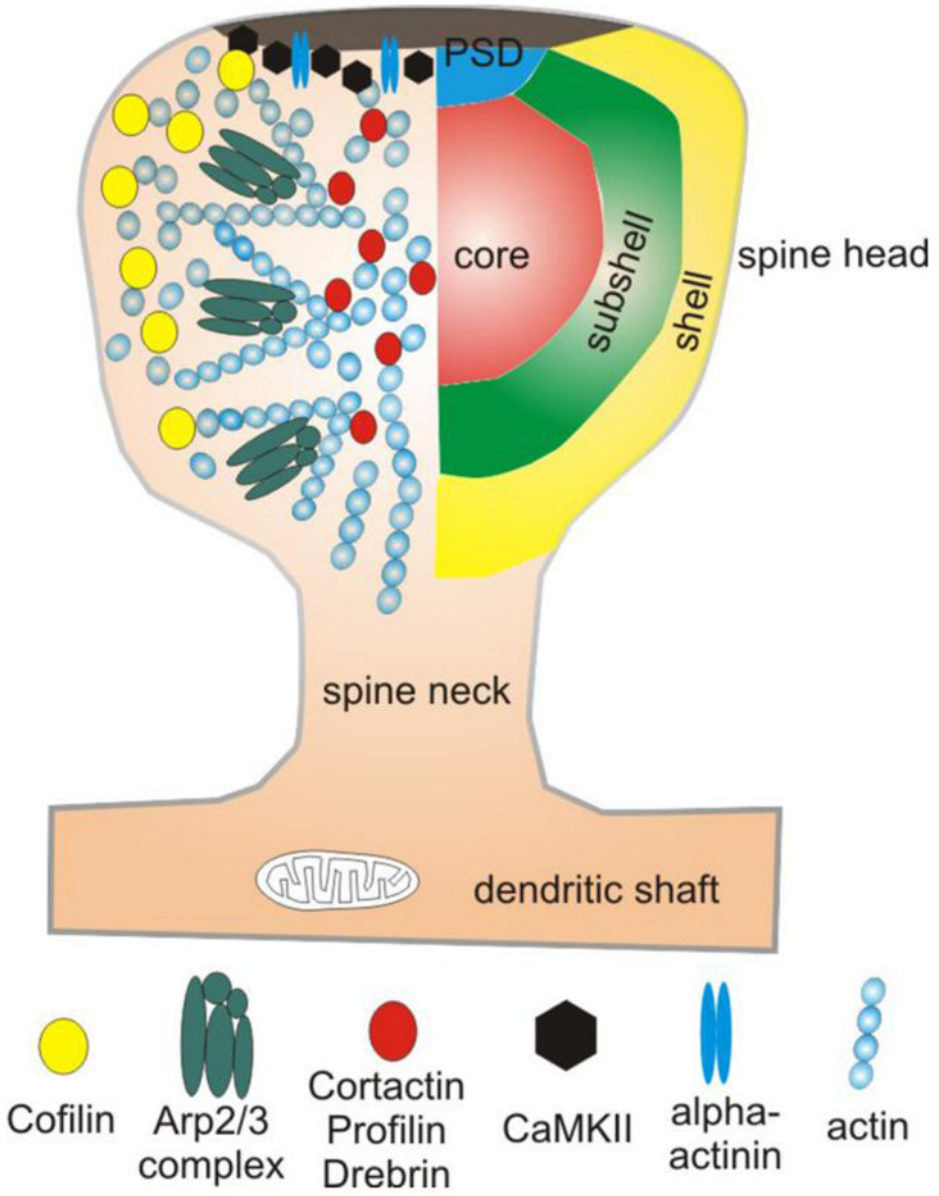

Figure 4. ABP microdomains in the spinoskeleton

Proteomic studies of forebrain synapses have identified a sizable number of ABPs in the biochemically-defined PSD [110, 182, 183]. Some of these may be contaminants, but several, including **α-actinin** (blue double ovals) and **CaMKII** (black hexagons), have been demonstrated with immuno-EM to lie within the morphologically-defined PSD. Imaging studies reveal that actin (small blue circles) is more dynamic in the shell of the spine than in the center, suggesting a functional gradient of activity, from shell to core. Consistent with these data, **cofilin** (yellow circles) — a protein responsible for depolymerization of filaments — is heavily concentrated in this shell domain. The presence of a distinct "subshell" microdomain within the spinoplasm is suggested by the accumulation of the **Arp2/3 complex** (green composites), which mediates filament branching. The center ("core") of the spine contains a relatively stable pool of actin. A heterogeneous pool of ABPs, including **cortactin, profilin** and **drebrin** (red circles), concentrate in this core microdomain.

Table 1

Functional families of actin-binding proteins in spines

	Function	Name (references)
ABPs regulating F-actin assembly	Monomer sequestration	***profilin*** [162, 164, 165, 184]
	Depolymerization, severing	***ADF/cofilin*** [30, 33, 137]
	Nucleation, branching	***Arp2/3 complex*** [97, 149, 185]; ***WASP*** and ***SCAR/WAVE***[142, 146]; ***formins*** [140]; ***cortactin*** [143]
	Capping	***Capping protein*** (CapZ)[186]
ABPs regulating network superstructure	Bundling	***drebrin*** [174, 176, 177]; ***synaptopodin*** [60, 124, 125]; ***CaMKII*** [117, 118, 119, 187]
	Cross-linking	***α-actinin*** [108, 109, 112, 114, 188]; ***filamin*** [189]; ***spectrin*** [190]