

Chapter 15

The Role of Drebrin-Binding Stable Actin Filaments in Dendritic Spine Morphogenesis

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Actin Cytoskeletons in Dendritic Spines

Actin Governs Dynamic Spine Motility

Dendritic spines are the postsynaptic receptive regions of most excitatory synapses in the brain [1]. They are small protrusions from the parent dendritic shaft and typically consist of a head (about $5.1 \times 10^{-20} \text{ m}^3$) and a neck (about $1.2 \times 10^{-20} \text{ m}^3$) [2]. They form various shapes and marked abnormalities in spine morphology in human children with mental retardation [3], suggesting that the differences between shapes reflect functional differences. Furthermore, high-frequency synaptic activity induces changes in the population of spine shapes [4] and the balance among various shapes is changed in a close correlation with learning and memory. Time lapse imaging analysis of hippocampal slices has shown that dendritic spines rapidly changed their shapes [5]. However, neither blockade nor induction of neuronal activity affect spine motility [6]. This suggests that the basal motility of dendritic spines is intrinsic to the neuron, but it does not directly link to the molecular mechanism of learning and memory.

Dendritic spines contain high concentrations of actin [7]. In general, high concentrations of actin plays a central role in supporting cell motility, suggesting that the morphological change of dendritic spines is governed by actin. In 1998, Fischer et al. showed using hippocampal neurons expressing actin labeled with green fluorescent protein that the motility of dendritic spines was completely inhibited when the neurons were treated by latrunculin A, a G-actin-sequestering agent [8]. This clearly indicates that actin plays a pivotal role in spine motility.

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Polymerization of F-actin (filamentous actin) against cellular membranes is thought to provide the force for cell membrane dynamics, such as the formation of plasma membrane protrusions of migrating cells, suggesting that the polymerization of dynamic F-actin in the spine head regulates spine motility. However, the molecular mechanism on how F-actin polymerization is regulated in the spine head has still not yet been clarified.

Studies of actin filaments in spines suggest the presence of two kinds of actin filaments. On the one hand, actin filaments appear to be stable over many hours and exhibit marked resistance to actin depolymerizing drugs such as cytochalasin D [9], which implies that actin filaments in spines are extremely stable and fulfill a purely structural role. On the other hand, individual spines undergo shape changes within a timespan of seconds or minutes as was described earlier. Actually the spines rapidly and continuously change shapes, but this motility seldom involves changes in spine size under basal conditions [8]. Halpain [10] addressed that dynamic and stable configurations of actin filaments fulfil their needs for motility versus structural integrity of dendritic spines. Two pools of actin filaments compose the spine head under normal circumstances. 'Core' of stable actin filaments form the structural foundation of the spine, while the peripheral population of actin filaments in the spine is dynamic.

Two Kinds of Actin Filaments in Mature Dendritic Spines

Dynamic and Stable Actins

In accordance with Halpain's prediction, Kasai and co-workers have reported the presence of dynamic and stable actins in dendritic spines using PAGFP-actin [11]. They photoactivated PAGFP-actin protomers in F-actin of dendritic spines. As the filaments treadmilled, activated PAGFP-actin protomers reached the end of the filament, depolymerized, and diffused away. The fluorescence from activated molecules decayed in two phases with time constants of 40 s and 17 min, indicating that there are two pools of F-actin in the spine: a dynamic one with a fast treadmilling rate and a stable one with a much slower treadmilling rate. Larger spines have a greater proportion of stable F-actin, although the proportion of stable F-actin is usually less than the dynamic one. While the dynamic F-actin is observed in the spine tip, the stable one is largely restricted to the base core of spine heads. The treadmilling of dynamic F-actin is observed from the apex to the base, but the actin protomer in the dynamic F-actin pool did not flow into the stable F-actin pool. This suggests that the two F-actin pools are differentially regulated.

It is known that polymerization of dynamic F-actin in spine heads regulates the spine motility; however, the spine size under basal conditions does not seem to be regulated by F-actin polymerization because, as stated previously, the spine size remains rather constant in spite of rapid spontaneous motility.

Mathematical Model for Lamellipodium Protrusion

It is suggested that protrusion formation is a consequence of the dynamics downstream from nucleation promoting factors (NPFs), with signaling setting the dynamic regime but not initiating the formation of individual protrusions [12]. Zimmermann and Falcke developed a mathematical model for lamellipodium protrusion. The model lamellipodium consists of an actin gel in the bulk and a highly dynamic range at the leading edge, called semiflexible region (SR). Signaling cascades that lead to the activation of NPFs, activate the actin related protein complex Arp2/3. Arp2/3 initiates the growth of a new filament branch from an existing filament in SR, pushing the lamellipodial membrane forward. Individual lamellipodia form due to random supercritical filament nucleation events amplified by autocatalytic branching. This model can be applied to the incessant lamellipodia formation in many cells with a constant state of the signaling pathways upstream from NPFs.

Model of Spontaneous Spine Motility

Applying Zimmermann and Falcke's model to the actin cytoskeleton in dendritic spine, spontaneous rapid spine motility (periodic lamellipodium formation) may be determined by the autocatalytic nature of branching of actin filaments of the dynamic F-actin pool, and by the length dependence of bundling, capping and severing of them. The stable F-actin core provides a stiff substrate for actin filaments in the lamellipodium to push back against to extend the postsynaptic membrane [13]. Although the nature of the stable F-actin core has not been well elucidated, a spine-resident side-binding protein of F-actin named drebrin, which is localized at the core region of dendritic spines [14], is thought to be an important element of the stable F-actin pool [15].

Although spine is too small to directly observe the two kinds of F-actins, we can observe fast and slow treadmilling of actin filaments in axonal growth cones. In the lamellipodia at the periphery of the growth cone, actin filaments are similar to the dynamic F-actin in dendritic spines in terms of the absence of drebrin. They flow retrogradely at rates of approximately 4 $\mu\text{m}/\text{min}$ [16]. F-actin in the actin arc at the base of the lamellipodia contains drebrin, which flows transversely rather than longitudinally. This retrograde flow of drebrin-binding F-actin occurs more slowly (approximately 1 $\mu\text{m}/\text{min}$) [17]. This indicates that peripheral F-actin that does not bind to drebrin shows more rapid treadmilling than drebrin-binding actin filaments. In addition, the drebrin-binding F-actin is resistant to the actin depolymerizing agent cytochalasin D, suggesting that they are stable actin filaments.

Together it is suggested that dendritic spines consist of dynamic and stable F-actin pools. Basal motility of dendritic spines occurs due to random supercritical filament nucleation events amplified by autocatalytic branching in the dynamic F-actin pool. Drebrin-binding actin filaments in the stable F-actin pool form a cross-linked gel, serving as the structural element for treadmilling of dynamic F-actin to push back against the spine membrane.

Drebrin Governs the Formation of Stable F-Actin

Modulation of Helical Structure of F-Actin by Drebrin

F-actin consists of a double helix of actin protomers decorated with its binding proteins. The helical structure plays an important role in modifying the relationship (binding activity) between F-actin and actin-regulating proteins [18]. Variations in the helical structure of F-actin are modulated by several side-binding proteins of F-actin (the double helix of actin protomers).

Tropomyosin is a typical side-binding protein of F-actin found in virtually all eukaryotic cells. Brain tropomyosin binds to F-actin with a stoichiometry of 1:7 (tropomyosin: actin protomer) with a dissociation constant (K_d) of 2.2×10^{-7} M [19]. Similarly drebrin binds to F-actin with a stoichiometry of 1:5 (drebrin : actin protomer) with a dissociation constant (K_d) of 1.2×10^{-7} M [20]. In spite of their similarity in the biochemical actin-binding property, atomic force microscopy analysis shows the significant differences in the helical structure. Tropomyosin forms a helix pitch of 36.5 nm, which is similar to the pitch of bared double helix of actin protomers. In contrast, drebrin forms the 40.0 nm pitch of actin filaments [21]. This difference makes it possible that the drebrin-binding F-actin and the other F-actins respond differently to the same signal within small dendritic spines.

Inhibition of F-Actin Depolymerization by Drebrin

Mikati et al. [22] reported that drebrin significantly decreased the depolymerization rates of uncapped filaments, reaching 88 % inhibition at full saturation, and 50 % inhibition is achieved at a low binding density of drebrin (~18 %). Drebrin causes stronger inhibition of barbed-end depolymerization compared to pointed-end depolymerization at the same binding density. Even in the presence of latrunculin A, drebrin inhibits the full depolymerization of actin filaments. Furthermore, differential scanning calorimetry (DSC) study shows that the T_m of F-actin was increased by 0.5 °C in the presence of saturating amounts of drebrin. Taken together, it is indicated that drebrin forms stable actin filaments.

Spine Morphogenesis

Drebrin Clustering in Dendritic Filopodia Mediates Spine Morphogenesis

Dendritic spines have two major structural elements, the postsynaptic density (PSD) and the actin cytoskeleton. Although PSD scaffold proteins such as PSD-95, Shank, and Homer are known to play pivotal roles in spine morphogenesis [23–25],

the initiation of spine morphogenesis precedes synaptic assembly of PSD-95 [26]. Moreover mutant mice which lack PSD-95 expression exhibit standard spine morphology [27], suggesting that molecules other than PSD scaffold proteins govern spine morphogenesis.

There are two models for the formation of dendritic spines. One model is that dendritic filopodia serve as the precursor of dendritic spines, and the other is that dendritic spines emerge from shaft synapses. The former model predominates during neuronal development. Developmental changes of the actin cytoskeleton within filopodia during spine morphogenesis have been intensely studied because the actin cytoskeleton regulates the morphology of both filopodia and spines.

In vitro study shows that filopodia from the dendrites are classified into two types in terms of the presence of drebrin clusters: diffuse-type filopodia and cluster-type filopodia [28]. Most cluster-type filopodia appose presynaptic terminals, but diffuse-type filopodia do not. This indicates that cluster-type filopodia are more matured than diffuse-type. On the other hand, the half of cluster-type filopodia do not contain PSD-95, while most mature spines contain PSD-95 [28], indicating that cluster-type filopodia are not mature spines but their precursors. Similarly, drebrin has been already observed at the nascent contact site of the dendrite by the axon in vivo [29]. Thus it is indicated that dendritic spines develop via cluster-type filopodia that have been transformed from diffuse-type filopodia.

Drebrin-binding stable F-actin seems to play a pivotal role for the establishment of postsynaptic structures. Drebrin content in dendritic spines correlates with spine head size, suggesting that the proportion of stable F-actin in the spine head seems to regulate the spine size [14]. During development, clustering of drebrin with F-actin occurs at postsynaptic sites in dendritic filopodia. In parallel with this change, drebrin changes its isoform from embryonic-type (drebrin E) to adult-type (drebrin A) [29, 30]. Interestingly, synaptic clustering of PSD-95 and NMDARs partially depend on drebrin [28, 31]. Additionally, drebrin is involved in the regulation of AMPAR trafficking to the postsynaptic site [32].

AMPA Receptor Facilitates the Drebrin Clustering in Dendritic Spines

How is drebrin clustered at postsynaptic sites? Although the aforementioned studies suggest that the conversion of drebrin isoform expression from drebrin E to drebrin A is involved in the drebrin clustering, the premature expression of drebrin A induces abnormally large headless protrusions with the unrestricted accumulation of F-actin, PSD-95 and drebrin [33], indicating that the conversion of drebrin isoform plays a role for the targeting mechanism of postsynaptic molecules, but is not a sufficient condition for postsynaptic formation.

The synchronous development of drebrin clustering and functional turnover of synaptic vesicles indicates that synaptic activity is involved in drebrin clustering at

postsynaptic sites. Inhibition of action potentials with TTX decreases drebrin cluster density, while inhibition of GABAA-receptor with picrotoxin, which enhances the excitatory component of synaptic transmission, increases drebrin clustering [34]. Thus spontaneous synaptic activity is involved in the drebrin clustering. Moreover, the study using subtype-specific blockers of glutamate receptors has shown that AMPA receptor, but neither NMDA receptor nor metabotropic glutamate receptor, regulates the clustering of drebrin at the postsynaptic site [34].

Then how does AMPAR regulate drebrin clustering? Using the fluorescence recovery after photobleaching (FRAP) analysis we have explored a cellular basis for activity-dependent drebrin clustering and have demonstrated that AMPARs specifically regulates drebrin dynamics within dendritic spines. Neurons were transfected with vectors that encoded drebrin A fused to enhanced green fluorescent protein (eGFP). Individual eGFP molecules can be rendered nonfluorescent, or 'bleached', with high-intensity laser pulses. Such pulses darken the target area until new, unbleached eGFP-drebrin replaces the bleached molecules during normal protein turnover. Under normal physiological conditions that allow spontaneous neuronal activity, about a quarter of total drebrin within a single spine is stabilized. Applications of CNQX or AP5 show that the activity of AMPARs, but not that of NMDARs, significantly decreases the level of stable drebrin in spines. Together it is indicated that activated AMPAR accumulates the stable F-actin bound by drebrin at the postsynaptic site, facilitating the recruitment of PSD-95, NMDAR and other postsynaptic proteins, including AMPARs themselves as suggested in the above section, into dendritic spines during development.

Spikar Is Involved in the Drebrin-Mediated Spine Formation

Drebrin initiates spine formation and the decrease of drebrin results in the decrease of spine density [31, 35]. However, an increased amount of drebrin does not raise the number of normal spines, but forms the large number of small protrusions from the dendritic shaft [36, 37]. These facts suggest that there is an unidentified protein which mediates the drebrin-dependent spine formation.

To explore a drebrin-binding molecule mediating spine formation, we performed a yeast two-hybrid screen using drebrin as bait and found a novel drebrin binding protein [38]. This protein localizes in neuronal nuclei as well as in dendritic spines, and this is why we named it spikar (localizes in *spine* and *karyoplasm*). Unlike drebrin, the up-regulation and down-regulation of spikar expression results in the increase and decrease of the spine density, respectively. Interestingly spikar does not affect the spine morphology different from drebrin [38]. The localization of spikar depends on drebrin whereas that of drebrin does not depend on spikar. In addition, spine formation activity of spikar depends on drebrin. Together it is suggested that drebrin might function to include spikar to the stable F-actin complex at postsynaptic sites, resulting in the spine formation.

Conclusion and Perspective

Dendritic spines are formed from dendritic filopodia in parallel with the appearance of stable F-actin instead of dynamic F-actin at postsynaptic sites. Stable F-actin consists of drebrin-binding actin double helix polymers, which shows the slow treadmilling and the increase of heat stability as well as the elongation of the helix pitch. Although the developmental conversion of drebrin isoforms, drebrin E to drebrin A, is involved in the accumulation of stable F-actin, which facilitates further accumulation of postsynaptic scaffold proteins and neurotransmitter receptors, AMPA receptor activation seem to be needed for more precise accumulation of stable F-actin at postsynaptic sites.

It is believed that the motility of actin filaments is of importance for synaptic plasticity but further investigation, particularly focusing on the stable and dynamic F-actin, is needed to reveal the actual role of the actin filaments. Furthermore, since the appearance of the stable actin pool is a good marker of synaptic maturation, we suggest drebrin as an appropriate surrogate marker of synaptic function. Recently, it has been recognized that mislocalization and dysregulation of postsynaptic cytoskeletons are crucial events regarding pathophysiology of so-called “synaptopathies” such as Alzheimer disease. In addition, human induced pluripotent stem cells (hiPSCs) provide new possibilities for drug discoveries because human specific side effects could be tested easily using those cells. Thus, drebrin can be used as the surrogate marker in hiPSCs-derived neurons as well. For this reason we expect drebrin be widely used in drug discovery and developmental fields.

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