

More than a mere supply of monomers: G-Actin pools regulate actin dynamics in dendritic spines

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Synaptic activity reshapes the morphology of dendritic spines via regulating F-actin arborization. In this issue, Lei et al. (2017. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201612042>) reports a novel, G-actin-dependent regulation of actin polymerization within spine heads. They show that actin monomer levels are elevated in spines upon activity, with G-actin immobilized by the local enrichment of phosphatidylinositol (3,4,5)-triphosphate (PIP₃) within the spine plasma membrane.

Dendritic spines are small, micrometer-sized actin-rich protrusions formed along the dendrites of excitatory and inhibitory neurons, which serve as the postsynaptic component for most of the excitatory inputs within the central nervous system. Dendritic spines are highly motile protrusions, which rapidly change their morphology and molecular composition according to synaptic activity. It is widely accepted that spine size and shape are intimately linked to synaptic plasticity. Thus, the strengthening or weakening of synaptic connections during long-term potentiation (LTP) or long-term depression are accompanied by the enlargement or shrinkage of dendritic spine heads, respectively (Okamoto et al., 2004).

The major cytoskeletal element of dendritic spines is actin, which serves both as a structural and dynamic framework and as the principal regulator of protein and vesicular trafficking. The organization of filamentous actin (F-actin) is determined upon the balance between (a) the addition of monomeric globular actin (G-actin) to the growing, barbed ends, (b) the generation of side branches, (c) the severing or (d) stabilization of the existing filaments, and (e) the depolymerization at the pointed ends (Bosch et al., 2014). So far, most studies have concentrated on the formation and maintenance of the F-actin network within dendritic spines and less emphasis was given on the local availability of G-actin, which provides the monomer supply needed for filament formation.

The intracellular concentration of G-actin exceeds with several orders of magnitude the critical concentration needed for rapid filament formation (Koestler et al., 2009), leading to a general assumption that the pool of G-actin provides a constant supply of monomers in a diffusible and excessive manner. In contrast, several evolutionary conserved actin-monomer binding proteins regulate the availability and subcellular localization as well as the nucleotide status of actin monomers (thus, ADP-G-actin or ATP-G-actin; Paavilainen et al., 2004). Therefore, it

is highly likely that these proteins provide additional means of spatial and temporal regulation of actin polymerization.

In this issue, Lei et al. report that the local enrichment of the G-actin pool plays an important role in regulating basal and activity-dependent actin polymerization within the dendritic spine. Working in primary dissociated and organotypic hippocampal cultures, they show that G-actin is enriched in dendritic spines under basal conditions as well as after chemically induced LTP (cLTP). To quantify the distribution of actin monomers between dendritic spines and the dendrite shaft, specific probes to endogenous G-actin (vitamin D-binding protein and an anti-G-actin antibody; Lee et al., 2013) and overexpression of EGFP-tagged, nonpolymerizable γ -actin mutants (R62D and G13R) were applied. In intact neurons, spine head-to-shaft ratios of fluorescent signals were elevated, suggesting a local enrichment of G-actin in spine heads. Signal intensity was reduced upon short-term permeabilization of living cells with the mild detergent saponin, confirming the specificity of detecting only nonpolymerized, monomer actin. The amount of EGFP-tagged actin monomers within the dendritic spine heads was increased rapidly upon TEA or glycine-induced cLTP, but the presence of nonpolymerizable γ -actin mutants inhibited spine head enlargement, known to occur during cLTP-induced structural plasticity (Bosch et al., 2014). Importantly, these data suggest that G-actin enrichment within the dendritic spines is regulated by synaptic activity but independently from actin polymerization.

Careful FRAP assays in dendritic spines expressing EGFP-tagged wild-type or the R62D and G13R actin monomers revealed recovery curves that were all fitted with double exponential functions but had significantly different time constants of the recovery curves. Both actin mutants and wild-type actin recovery curves exhibited similar rapid components, indicating that the diffusion of the three EGFP-tagged actin proteins into the spines happens with similar kinetics. In contrast, the slow recovery rate of the mutant actin monomers was significantly delayed, suggesting the possibility that these mutants might get trapped within the spine heads after their rapid delivery.

Superresolution imaging of actin flow and the distribution of proteins regulating actin polymerization within the spine already indicated distinct nanoscale domains for slow and nonpolarized actin nucleation in the close vicinity of the postsynaptic density or promoting finger-like protrusions at the perisynaptic sites via fast actin polymerization (Frost et al., 2010; Chazeau et al., 2014). This spatially and functionally distinct actin turnover

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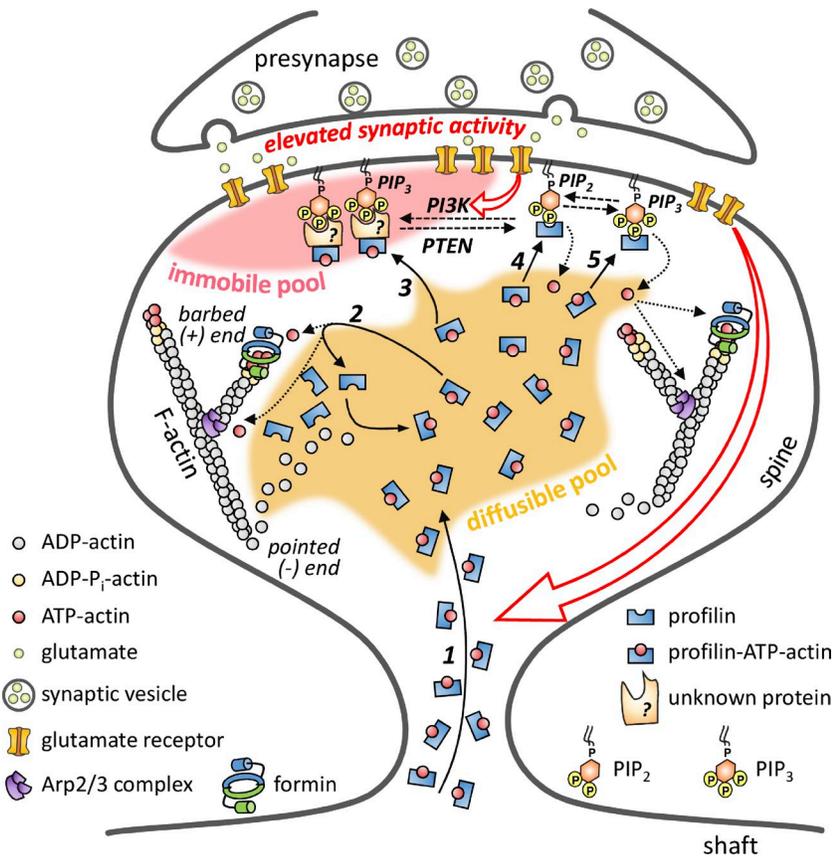


Figure 1. **Proposed role of profilin in G-actin enrichment within dendritic spines.** Actin monomers are continuously transported from the dendritic shaft to the spine head, mainly by profilin (1). Profilin facilitates the delivery of ATP-actin to the barbed ends of F-actin, providing the monomer supply for Arp2/3-mediated nucleation or formin-dependent filament elongation (2). ADP-actin dissociating from the pointed end of F-actin is bound by profilin and converted to ATP-actin, providing a diffusible pool of actin monomers (orange shading). G-actin, possibly via profilin or other, yet unknown proteins, can be immobilized by PIP₃ in the plasma membrane (3), which is generated from PIP₂ by PI3K. PIP₃ is hydrolyzed by PTEN back to PIP₂. Profilin can bind directly to PIP₂ (4) and PIP₃ (5), as well, but phosphoinositide binding competes with actin binding and leads to the release of G-actin. Elevated synaptic activity (depicted by red arrows) facilitates profilin transport to the spine and activates PI3K, leading to an increase in the immobile pool of G-actin (pink shading).

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can be regulated by local interactions with the plasma membrane either via membrane-associated small GTPases and their interactors or via anchoring actin binding proteins to different membrane lipids (Bezanilla et al., 2015).

In their work, Lei et al. (2017) investigated the molecular machinery regulating the G-actin pool within the spine heads by concentrating on the involvement of phosphatidylinositol (4,5)-bisphosphate (PIP₂)– and phosphatidylinositol (3,4,5)-triphosphate (PIP₃)–dependent signaling. To start with, they overexpressed the EGFP-tagged pleckstrin homology (PH) domains of phospholipase Cδ (PLCδ) and Akt kinase, known to preferentially bind to PIP₂ and PIP₃, respectively. Using quantitative measurement of these probes, they verified that PIP₂ and PIP₃ are enriched in dendritic spines. Importantly, high levels of EGFP-tagged PH domains (a) decreased the level of G-actin in the spines, (b) induced the formation of deformed spines with spine head protrusions, and (c) transformed mushroom-like spines to filopodial-like protrusions with less expanded spine heads. These changes were very similar to that induced by high levels of R62D and G13R nonpolymerizable γ -actin mutants, indicating dominant-negative effects over the normal regulation of endogenous G-actin pool and F-actin assembly in both cases.

According to the literature, PIP₂ and PIP₃ both play an important role in synaptic plasticity and control the spatial and temporal assembly of diverse signaling complexes at the plasma membrane (Dotti et al., 2014). PIP₃ is generated from PIP₂ by phosphoinositide 3-kinase (PI3K), whereas its hydrolysis by phosphatase and tensin homologue (PTEN) produces PIP₂. Endogenous as well as nonpolymerizable mutant G-actin levels in the spines were reduced upon the pharmacological inhibition

of PI3K and, conversely, were elevated upon blocking PTEN activity. PIP₃-dependent enrichment of G-actin within the spines was evident during cLTP, as well. FRAP analysis also confirmed that reduced PIP₃ levels speed up the slow component of mutant actin recovery. Thus, the nondiffusible G-actin pool seems to depend primarily on PIP₃-mediated effects. This was also confirmed by the findings that overexpression of the PIP₃-interacting PH domain of AKT kinase more profoundly decreased G-actin in spines than when expressing the PH domain of phospholipase Cδ, which exerts a dominant-negative effect over PIP₂. Based on these experimental data and competition models to test interactions between G-actin, PH domains, PIP₂, and PIP₃, it is proposed that G-actin accumulation in the spines is dependent primarily on PIP₃ interactions.

Lei et al. (2017) suggest that profilin is a plausible mediator between membrane lipids and actin monomers. Profilin is a G-actin binding protein that facilitates the exchange of ADP for ATP on G-actin and promotes the addition of actin monomers to the growing end of filaments (Paaivilainen et al., 2004). Profilin is rapidly recruited to dendritic spines in an activity-dependent manner, and preventing its binding to G-actin destabilizes spines (Ackermann and Matus, 2003; Lamprecht et al., 2006). Knocking down both profilin1 and 2 isoforms in cultured neurons decreased G-actin levels in the spine and evoked similar morphological effects as observed by overexpressing the PH domains or the mutant actin monomers. FRAP experiments executed in combination with profilin knockdown confirmed that the slow component of the actin recovery curve was profoundly reduced. These data unambiguously indicate that profilin is needed for the maintenance of the slowly recovering G-actin pool, which is maintained in a PIP₃-dependent manner.

The exact molecular mechanisms underlying the regulation and function of G-actin spine enrichment have not yet been revealed. Nevertheless, in accordance with previous studies in axonal growth cones (Lee et al., 2013), data from Lei et al. (2017) confirm that the G-actin pool within the spine heads is not uniform and should not be regarded as being responsible only for the constant supply of monomers needed for F-actin formation. Indeed, a part of the actin monomers belongs to a pool that is bound to profilin (or to other actin-monomer binding proteins) and regulates activity-induced F-actin polymerization in dendritic spines.

According to the proposed model and available literature data (Fig. 1), G-actin is continuously delivered into the spine head from the shaft by profilin, leading to a local enrichment of actin monomers. The fast diffusible fraction of G-actin likely provides the monomer supply for actin polymerization and is renewed by ADP-bound actin dissociated at the pointed ends of F-actin. Profilin enhances the ADP to ATP exchange of G-actin and facilitates actin-ATP delivery to the barbed ends. In contrast, G-actin can associate, probably via profilin or other proteins, with membrane phosphoinositides (especially with PIP₃), forming a relatively stable, immobile pool of G-actin. Upon increased synaptic activity, profilin-dependent delivery of actin monomers to the spine head and local PIP₃ level in the spine plasma membrane are both increased, leading to an increase in the immobile G-actin pool caused by the sequestration of actin-bound profilin to PIP₃-rich membrane domains.

Several steps in this model await further clarification. For example, profilin can bind to both PIP₂ and PIP₃, and it is also known that phosphoinositide binding disrupts the actin–profilin interaction in a competitive manner (Paavilainen et al., 2004). Additionally, it should be clarified which other proteins play a role in the association and release of the actin–profilin–plasma membrane phosphoinositide complex. It is also likely that PIP₂ and PIP₃ might convey different signaling via profilin because association of profilin with different membrane phosphoinositides has been proposed to regulate profilin's interaction with formin and Ena/VASP or with the Arp2/3 complex, promoting the elongation of unbranched actin filaments or the nucleation of actin side branches, respectively (Bezanilla et al., 2015). Interestingly, an increase in the amount of deformed spines with spine head protrusions was observed upon overexpressing the PH domains of PIP₂- and PIP₃-interacting proteins. As PIP₃ has been reported to play a critical role in the formation of filopodial-like protrusions (spinules) from the spine heads (Ueda and Hayashi, 2013), an intriguing question is whether the observed spine head protrusions are similar structures to spinules and whether they are induced by the decrease of the local G-actin pool and/or by a dominant-negative effect on the recruitment of other lipid binding proteins. In the future, superresolution imaging will hopefully resolve how the different lipid compartments in

the plasma membrane as well as local protein interactions regulate actin polymerization within dendritic spines.

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