Cell. Mol. Life Sci. (2016) 73:3053–3073
DOI 10.1007/s00018-016-2214-1

Cellular and Molecular Life Sciences

REVIEW

Organization and dynamics of the actin cytoskeleton during dendritic spine morphological remodeling

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Received: 1 February 2016 / Revised: 31 March 2016 / Accepted: 31 March 2016 / Published online: 22 April 2016

Abstract In the central nervous system, most excitatory post-synapses are small subcellular structures called dendritic spines. Their structure and morphological remodeling are tightly coupled to changes in synaptic transmission. The F-actin cytoskeleton is the main driving force of dendritic spine remodeling and sustains synaptic plasticity. It is therefore essential to understand how changes in synaptic transmission can regulate the organization and dynamics of actin binding proteins (ABPs). In this review, we will provide a detailed description of the organization and dynamics of F-actin and ABPs in dendritic spines and will discuss the current models explaining how the actin cytoskeleton sustains both structural and functional synaptic plasticity.

Keywords Dendritic spines · Actin cytoskeleton · Actin binding proteins · PSD · CAMKII · Synaptic plasticity · Super-resolution microscopy

Abbreviations

ABP	Actin binding proteins
ASD	Autism spectrum disorders
EM	Electron microscopy
FRAP	Fluorescence recovery after photobleaching
FRET	Fluorescence resonance energy transfer
LTD	Long-term depression
LTP	Long-term potentiation
NPFs	Nucleation promoting factors
PSD	Post-synaptic density
SMLM	Single molecule localization microscopy
sptPALM	Single particle tracking photoactivation localization microscopy
STED	Simulated emission depletion microscopy

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Introduction

In 1888, Ramón y Cajal described for the first time that the surface of a neuron "appears bristling with thorns or short spines" [1, 2]. In subsequent work, he speculated that dendritic spines received axonal contacts and that their morphological changes were associated with neuronal function and learning processes [3–5]. Cajal's hypothesis that spines connect axons and dendrites was confirmed in 1959 by the first visualization of pre- and post-synaptic contacts by electron microscopy (EM) [6–8]. Although it was widely believed that long-lasting changes in synaptic function are the cellular basis of learning and memory, [9–11] it was only recently that direct links between synaptic activity, dendritic spine morphology and the formation of a memory trace in vivo were demonstrated [12, 13].

Dendritic spines are generally composed of a spine head (200 nm to 1 μm in diameter) that is connected to dendrites by a thin spine neck (100–200 nm thick) [14–17]. Spine heads contain the post-synaptic density (PSD), a macromolecular structure essential for synaptic transmission which mediates adhesion to the pre-synapse and anchoring of post-synaptic glutamate receptors. Despite their architectural role, dendritic spines are highly dynamic structures

that undergo distinct morphological changes on timescales of seconds to minutes, hours and years, both in vitro and in vivo [18–22]. The morphological remodeling of spines is correlated with changes in synaptic transmission. Synaptic plasticity-induced stimuli such as long-term potentiation (LTP) or long-term depression (LTD) cause not only the formation or removal of new spines [23–27] but also the enlargement or shrinkage of pre-existing spines [27–31]. However, the in vivo longitudinal measurement of a single spine experiencing morphological remodeling during LTP and LTD has not yet been observed [32–34]. In this review, this type of spine morphological remodeling will be referred to as structural plasticity, while functional plasticity is defined as increased or decreased synaptic transmission.

While the function of dendritic spines had been the major scope of pioneer studies, current studies are thoroughly focusing on their molecular composition. Biochemical and proteomic studies have shown that dendritic spines are composed of a plethora of proteins covering a wide range of functions including membrane receptors and channels, scaffolding proteins, adhesion proteins, molecular motors, GTPases, kinases/phosphatases and cytoskeleton proteins [35]. Actin and actin binding proteins (ABPs) are particularly enriched in PSD and accumulate in dendritic spine heads [35–39], with F-actin dynamics being the driving force of spine morphological remodeling [19, 40, 41]. Indeed, the actin cytoskeleton sustains the formation of dendritic spines during neuron development and their enlargement and shrinkage upon increase and decrease synaptic activity, respectively [28, 29, 42–44].

Filamentous actin (F-actin) consists of two-stranded helical polymers arising from the polymerization of globular actin (G-actin) (Fig. 1a) [45, 46]. Actin assembles in a head-to-tail manner giving rise to the structural polarity of F-actin. Therefore, polymerization and depolymerization occur preferentially at the barbed end and the pointed end, respectively. This creates a net flow of actin monomers within the filament called actin treadmilling. Fluorescence microscopy revealed that the nature of ABPs but also the spatiotemporal coordination of their activity determine the organization and dynamics of F-actin in motile structures such as the lamellipodium and filopodium (Fig. 1a) [45, 47]. In the past decade, seminal studies have established the central role of F-actin dynamics and ABPs in spine morphogenesis and in synaptic plasticity-induced morphological remodeling [43, 44, 48, 49]. The spatial resolution of conventional fluorescence microscopy is, however, limited by the diffraction of light (around 250 nm in the lateral direction), whereas many sub-synaptic structures are typically below this diffraction limit (synaptic cleft: 20 nm; PSD thickness: 25–50 nm; spine neck width: 100–200 nm). To date, EM provided most of our knowledge about synapse ultrastructure, including the synaptic cleft size, the PSD and F-actin organization in dendritic spines [15, 39, 50, 51]. In 2014, Eric Betzig, William Moerner and Stefan Hell were awarded the Nobel Prize in Chemistry for the recent development of single-molecule and super-resolution light microscopy. These techniques can circumvent the diffraction limit of light and have provided new insights into protein organization and dynamics in different cellular systems including neurons [52, 53]. We are therefore just starting to reach a deep understanding of the sequence of molecular events leading to dendritic spines functional and structural plasticity, such as neurotransmitter receptor dynamics and organization, PSD remodeling, and spine neck morphology [54–56]. In this review, we will focus on the organization and dynamics of the F-actin cytoskeleton in dendritic spines, the signaling cascades driving F-actin remodeling upon changes in synaptic activity, and the interplay between F-actin remodeling and spine structural and functional plasticity. We will also highlight how super-resolution microscopy and single-molecule tracking approaches provided new understanding on the architecture and the dynamics of actin and actin regulatory proteins in dendritic spines.

Actin dynamics in dendritic spines

Actin dynamics in dendritic filopodia

During neuronal development, highly motile structures called dendritic filopodia will emerge from dendrites [18]. Enlargement of dendritic filopodia occurs soon after their contact with axons leading to the formation of a dendritic spine [39, 57–60]. As opposed to the tight linear F-actin bundles present in filopodia from motile cells, dendritic filopodia are composed of unbundled F-actin of mixed orientations [39, 61]. However, dendritic filopodia display a fast retrograde actin flow powered by continuous polymerization of actin against the tip membrane protrusion, as observed for the lamellipodium and filopodium of motile cells and neuronal growth cones [62–67]. The molecular motor myosin II, accumulates at the base of dendritic filopodia and contributes to the retrograde F-actin flow [39, 66]. The initial contact between dendritic filopodia and the axon is driven by N-cadherin adhesion proteins and drastically reduces the retrograde F-actin flow [60]. This interplay between cell adhesion and actin dynamics is characteristic of a model used to explain cell migration called the ‘molecular clutch’ [68–72]. The molecular clutch consists of an assembly of ABPs that transmit forces generated by the F-actin flow to adhesive structures allowing cells to exert grip on the surrounding substrate or

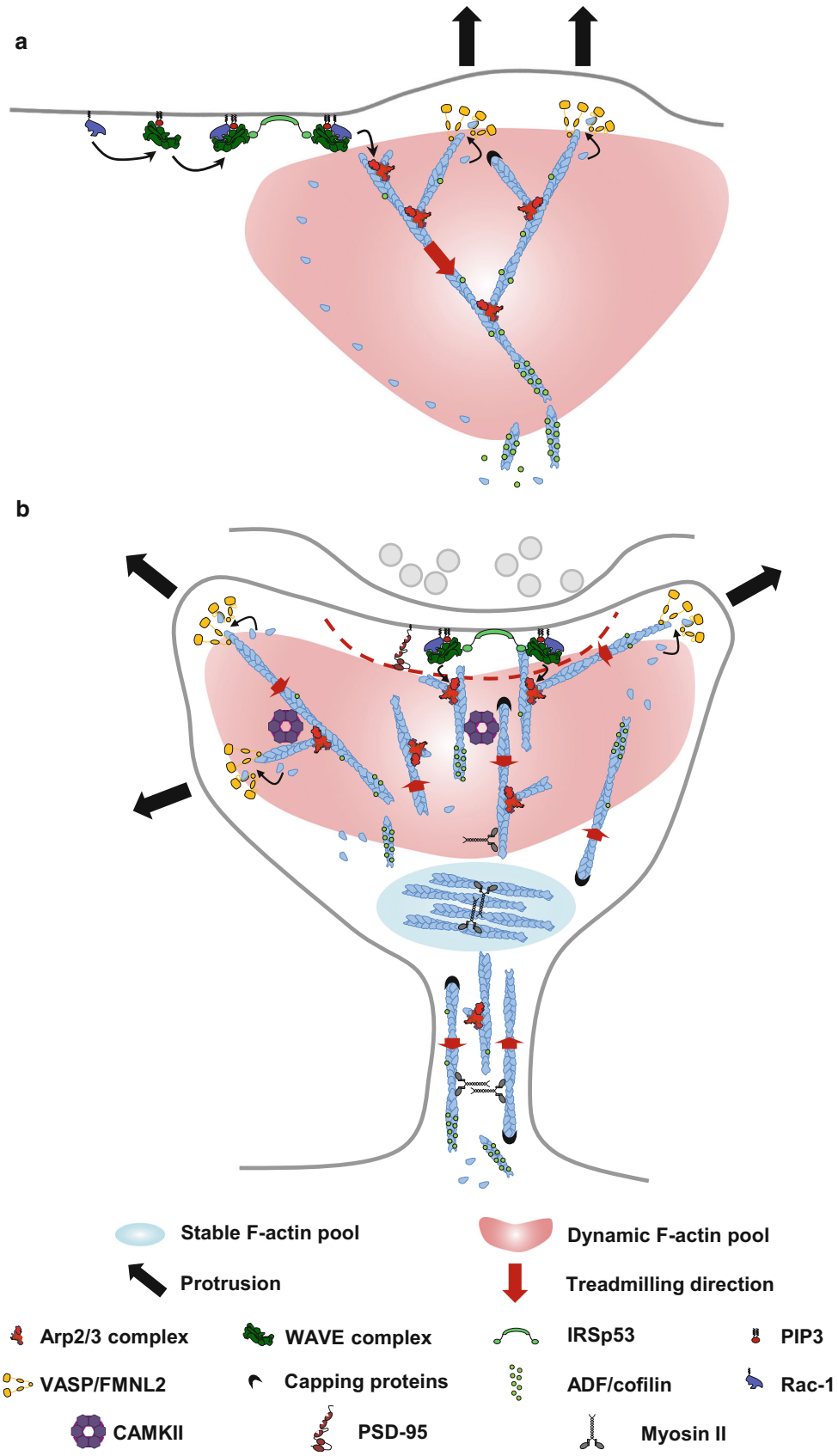

Fig. 1 Comparison of F-actin and ABPs organization in the lamellipodium and in dendritic spines. **a** In lamellipodia of motile cells, nucleation and elongation of F-actin are co-localized at the tip of membrane protrusion triggering a fast and concerted rearward flow of the branched F-actin network. Activation of the Arp2/3 complex is mediated by the synchronized convergence of prenylated Rac-GTP, PIP3, IRSp53 and the WAVE complex, while elongation of F-actin filaments are powered by VASP and FMNL2, all these proteins being localized at the lamellipodium tip. Capping proteins are also enriched at the lamellipodium tip, while cortactin associates with the entire F-actin network. ADF/cofilin associates with the entire F-actin network and induces F-actin severing. **b** Dendritic spine heads are characterized by slow and non-polarized motions of the branched F-actin pool. This distinct F-actin dynamics in spines could be sustained by spatial segregation between stationary nucleation zones and delocalizing elongation zones. Indeed, since the PSD is a persistent confinement zone for the WAVE complex and IRSp53, branched F-actin nucleation occurs with a higher probability at the PSD vicinity, while elongation of F-actin filaments by VASP and FMNL2 occur at the tip of finger-like protrusions. Dendritic spines are also composed of a stable F-actin pool localizing at the base of the spine head and stabilized by the crosslinking activity of myosin II. Dendritic spine necks are composed of both branched and non-branched long F-actin filaments that exhibit slow and non-polarized motions

cells and to generate membrane protrusions [70]. According to this model, dendritic filopodia that are not contacting axons are in the slipping mode and display fast rearward F-actin motion driven by F-actin polymerization and myosin II activity. Upon filopodia/axon contact, clutch engagement between N-cadherin and F-actin, possibly mediated by α-catenin, will immobilize actin filaments counteracting myosin II activity and leading to a reduction of the F-actin flow, which causes F-actin growth to push against the plasma membrane. The resulting F-actin accumulation, decreased motility and dendritic filopodia enlargement might therefore be required for the initiation of dendritic spines [60].

Actin dynamics in mature dendritic spines

Motility of dendritic spines is characterized by the formation and retraction of membrane protrusions [22, 73, 74]. However, the precise role of those spine protrusions is still unknown but might be needed to continuously adjust the position of the post-synaptic structure to the pre-synaptic one. Dendritic spines are F-actin-rich protrusions with a dynamic equilibrium between G-actin and F-actin [75]. As for dendritic filopodia, F-actin dynamics was demonstrated to be the driving force of dendritic spine motility. Indeed, pharmacological treatments preventing F-actin polymerization (cytochalasin-D) or promoting F-actin depolymerization (latrunculin-A) stop spine dynamics [19, 41]. Fluorescence recovery after photobleaching (FRAP) and photoactivation experiments provided more insights into actin dynamics in dendritic spines [76, 77]. These studies revealed that F-actin was composed of a large dynamic pool (80–95 %; 40 s turnover time constant) and a small stable pool (5–20 %; 17 min turnover time constant) (Fig. 1b); the size of the stable pool being positively correlated with spine volume. While the stable pool remains stationary at the base of the spine heads, the dynamic pool exhibits a slow retrograde flow (5–20 nm/s) from the tip to the base of the spine head [77, 78]. The recent development of single-particle tracking photoactivation localization microscopy (sptPALM) [79, 80] has enabled F-actin tracking in dendritic spines [67, 78, 81]. Those studies did not reveal a concerted flow of F-actin from the tip to the base of the spine head but rather an overall non-polarized motion. A large fraction of actin molecules had no detectable motion, while the remaining ones displayed retrograde and anterograde motions with heterogeneous speeds (8–40 nm/s). These dynamic properties are different from those found in other protrusive structures such as the lamellipodium of motile cells, axonal growth cones or dendritic filopodia where F-actin movements are faster, highly polarized rearward and driven by F-actin growth against the tip of membrane protrusions [66, 67, 82, 83]. Therefore, slow actin movements found in dendritic spines might result from a complex pattern of force generation, involving polymerization of new filaments pushing older filaments, F-actin severing and recycling, but also protrusion ruffling [22, 77, 81, 84–86]. Interestingly, F-actin motions in dendritic spine necks are also slow and non-polarized [39, 66]. This complex F-actin dynamics could also arise from the distinct spatial distribution and dynamics characterizing F-actin pools and from a specific spatiotemporal organization of ABPs in spines.

Actin organization in dendritic spines

Formation and organization of the branched F-actin network in dendritic spines

Essential F-actin regulators are located in spines, and have been found to critically control spinogenesis, spine morphology and synaptic plasticity [44]. Despite having different F-actin dynamics as compared to the lamellipodium, dendritic spine heads are also composed of a dense branched F-actin network, while the spine neck is composed of both branched and non-branched long F-actin filaments [39, 61, 87, 88]. The actin-related protein 2/3 (Arp2/3) complex is the only known actin nucleator forming side branches on a "mother" actin filament [89–92]. Knockout of a single Arp2/3 subunit decreases F-actin turnover and spine head size, induces a progressive spine loss, alters spine structural plasticity and leads to behavioral abnormalities [93]. However, the Arp2/3 complex possesses low intrinsic activity and requires nucleation promoting factors (NPFs) for an efficient activation [92]. NPFs such as the WAVE complex and N-WASP can activate Arp2/3 to induce branched actin nucleation; while cortactin stabilizes Arp2/3 during and after nucleation [92, 94, 95]. The Arp2/3 complex can also be inhibited by PICK1 [96]. Importantly, these proteins are all present in dendritic spines, interact directly or indirectly with PSD components and are involved in the regulation of spine density, morphology and plasticity (WAVE [97–101]; cortactin [102, 103]; N-WASP [104, 105]; PICK1 [96, 106]).

Filament length is also an important factor determining F-actin network dynamics [107]. Profilin binds with a one-to-one ratio to G-actin favoring F-actin polymerization at the barbed end [108, 109]. Profilin-II is enriched in spines upon increased synaptic activity and preventing its binding to G-actin destabilizes dendritic spines [110]. Vasodilator-stimulated phosphoprotein (VASP) and formins promote barbed end F-actin polymerization while capping proteins (CP or CapZ) and EGFR substrate pathway #8 (Eps8) cap

the barbed end to prevent F-actin polymerization [111–115]. Interestingly, knockout of Eps8 and knockdown of CP lead to the formation of thin long spines [116, 117], whereas decreasing the activity of the formin mouse diaphanous 2 (mDia2) increases the proportion of large spines [61]. Actin filaments decoration by sub-stoichiometric densities of actin depolymerization factor/cofilin (ADF/cofilin), in coordination with coronin and actin interacting protein 1 (AIP1) recruitment, will induce severing and stochastic disassembly of F-actin [118–121]. Numerous studies have shown that ADF/cofilin and its upstream regulators are tightly regulating dendritic spine shape [29, 61, 122, 123].

Electron microscopy provided the first insights into F-actin organization in dendritic spines [36, 39, 51]. By performing single-molecule localization microscopy (SMLM) and single-particle tracking, our recent study has now revealed the specific nanoscale organization of ABPs in dendritic spines [67] (Fig. 1b). Incorporation of Arp2/3 complexes into the F-actin network occurred in close vicinity to the PSD, and the WAVE complex was found to form a single stable domain overlapping with PSD-95 [67]. The insulin receptor substrate p53 (IRSp53), an I-BAR protein binding and regulating the WAVE complex, was also shown to form a stable domain overlapping with PSD-95 [67, 124, 125]. PSD-95, IRSp53 and the WAVE complex share similar diffusion properties suggesting that branched F-actin nucleation occurs at the membrane apposed to the PSD [67]. Consistently, IRSp53 and subunits of the WAVE and Arp2/3 complexes can directly and/or indirectly bind to components of the PSD, including PSD-95, Shank1, Shank3 and CaMKII [101, 126–129]. In addition, the post-synaptic cell adhesion protein neuroligin-1 bears an interacting sequence binding directly to the WAVE complex [130]. Thus, sequestration of the WAVE complex and IRSp53 at the PSD might provide an efficient mechanism for spatiotemporal control of the branched F-actin network during changes in synaptic transmission. SMLM has also shown that VASP and the formin-like proteins2 (FMNL2), but not the WAVE complex, accumulate at the tip of finger-like protrusions growing away from the PSD [67]. Thus, in contrast to the lamellipodium, zones of branched F-actin nucleation and elongation do not colocalize at protrusion tips [47, 82, 83, 131] (Fig. 1). This demonstrates that it is the respective nanoscale organization of F-actin regulators and not their nature that determines the shape and dynamics of protrusive structures. Those finger-like protrusions might arise from the branched F-actin network but are unlikely to be formed by elongation of tight F-actin bundles, as demonstrated for filopodia emerging from the lamellipodium [39, 132]. The densely packed PSD could represent a physical barrier forcing F-actin barbed ends to grow away and generate finger-like protrusions in dendritic spines. Consistent with this hypothesis, in an in vitro reconstituted system, branched F-actin networks can elongate their barbed ends away for a nucleating surface [84]. Because of the inverse relationship between the rate of F-actin flow and membrane protrusion, the nanoscale segregation between stationary nucleation zones and the delocalizing elongation zones could also explain the slow and non-polarized motion of branched F-actin networks in dendritic spines [67, 133] (Fig. 1b). Despite these recent findings, the current models cannot yet assign a specific F-actin nanoscale organization to the previously described stable and dynamic F-actin pools.

Heterogeneity of F-actin crosslinker organization in dendritic spines

F-actin organization is also controlled by crosslinking proteins. Dendritic spines are for instance enriched in F-actin crosslinkers including α-actinin, neurabins and the neuron-specific F-actin parallel bundler drebrin (α-actinin [134–136]; neurabins [137–141]; drebrin [142–145]). Fascin, however, which stabilizes parallel F-actin bundles in conventional filopodia, appears absent from dendritic filopodia and spines [39, 146, 147]. While α-actinin knockdown delays spine maturation [136] and neurabin knockdown decreases surface glutamate receptor surface expression [148], drebrin knockdown was suggested to promote spine maturation [149]. This discrepancy might arise from specific binding capabilities to different fractions of the F-actin networks. On the one hand, α-actinin was shown to be enriched at the PSD where it could bind subunits of the GluN receptor (NMDA receptor), and therefore link and stabilize GluN receptors with the branched F-actin network [150]. On the other hand, drebrin is located at the spine center where it can associate with the stable F-actin pool and prevent the F-actin remodeling required for spine maturation [51, 151, 152].

The Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), an essential protein involved in post-synaptic plasticity [153–155], can directly bundle and stabilize F-actin in its inactive state via its CaMKIIβ subunit [156, 157]. Under basal conditions, the binding of CaMKII to F-actin prevents other ABPs, such as the Arp2/3 complex and ADF/cofilin, to interact with F-actin, but upon GluN receptor activation, autophosphorylation of CaMKII prompts its release from F-actin allowing these ABPs to bind and regulate F-actin organization and dynamics [158]. Accordingly, recent sptPALM experiments showed that CaMKIIβ shares similar dynamics to F-actin and that depolymerization of the F-actin network (latrunculin-A) or

dissociation of the CaMKIIβ subunit from F-actin increase CaMKII diffusion [158, 159]. The formation of F-actin bundles by CaMKIIβ could suggest an association with the stable F-actin pool [156]. However, CaMKIIβ displays inward and forward movements in dendritic spines similar to actin and Arp2/3, rather suggesting an association with the dynamic F-actin pool [158]. In the following sections, we will see how the activation status of CaMKII during synaptic transmission can act as a gating mechanism to trigger the nanoscale reorganization of the F-actin network [49, 128, 158].

The molecular motor myosin II can bind and generate forces on F-actin, leading to F-actin networks' contraction and/or disassembly. Myosin II is therefore a crucial regulator of F-actin dynamics and network architecture in the lamellipodium and neuronal growth cones [64, 65, 133, 160–164]. EM and SMLM studies have shown that myosin II is found in both dendritic spine heads and necks [39, 66] and that knockdown or pharmacological inhibition of myosin II induces filopodium-like morphologies [165–168]. Myosin II function in dendritic spines might be more complex than just enhancing the retrograde F-actin flow [60, 169, 170]. A recent study suggests that myosin IIb can stabilize the stable F-actin pool through actin crosslinking, while its contractile activity increases the turnover rate of the dynamic F-actin pool [170]. This dual function might arise from differences in the structural organization of actin filaments in the stable versus dynamic pool. A very elegant in vitro study has shown that myosin II, as well as myosin VI, can disassemble antiparallel but not parallel F-actin bundles leading to a selective actomyosin contractility and disassembly [163]. Taken together with the localization of the parallel bundler drebrin, this suggests that the stable F-actin pool is mainly composed of parallel F-actin bundles stabilized by drebrin and myosin II.

Other ABPs, such as actin binding protein 1 (ABP1), synaptopodin and MAP1B, can be enriched in dendritic spine and regulate spine morphology [171–176]. However, the mechanism of F-actin regulation by those ABPs remains largely unclear, and precludes any speculation about their role in F-actin network organization and dynamics.

Revealing the nanoscale organization and dynamics of a subset of ABPs led to a deeper understanding of the mechanisms linking F-actin generated forces to dendritic spine motility during basal synaptic transmission. In the following sections, we will focus on how changes in synaptic activity can lead to transient and long-lasting reorganization of the F-actin network.

The interplay between the actin cytoskeleton and synaptic plasticity

The strength of synaptic transmission can be either increased or decreased on a timescale of seconds to months. These processes, called long-term potentiation (LTP) and long-term depression (LTD), correlate with the enlargement and shrinkage of dendritic spines, respectively [27–29, 31, 43]. Therefore, the organization and dynamics of the F-actin network requires to be adjusted accordingly to the strength of synaptic transmission [177]. In this section, we will describe the signaling pathways that can regulate F-actin networks during synaptic plasticity, the sequence of events leading to the morphological remodeling of dendritic spines and how the actin cytoskeleton sustains both structural and functional synaptic plasticity.

Signaling pathways that control activity-induced reorganization of the actin cytoskeleton

Neuron depolarization leads to the pre-synaptic release of glutamate and to the activation of post-synaptic receptors. During synaptic plasticity, the opening of post-synaptic GluN receptors (NMDA receptors) and voltage-gated calcium channels induces Ca^{2+} influx into dendritic spines and activation of calmodulin (CaM) sensitive proteins [153, 178]. CaM binding to CaMKII releases its auto-inhibition allowing autophosphorylation and leading to transient kinase activity [153, 155] (Fig. 2a). As mentioned previously, CaMKII autophosphorylation induces its dissociation from F-actin. Once activated, CaMKII can in turn phosphorylate several synaptic proteins, including regulators of small Rho-familly GTPases. RhoGTPases can switch from an active GTP-bound state to an inactive GDP-bound state by intrinsic GTPase activity. Guanine nucleotide exchange factors (GEFs) activate RhoGTPases by stimulating the release of bound GDP and allowing the binding of GTP. Conversely, GTP-activating proteins (GAPs) can inactivate RhoGTPases by increasing GTPase activity. Rac1, Cdc42 and RhoA are among the best characterized small RhoGTPases and have been extensively described as F-actin regulators in non-neuronal cells [179–182]. Rac1 is responsible for lamellipodium formation, while Cdc42 is more involved in the formation of conventional filopodia, and RhoA plays a role in the assembly of stress fibers and adhesion sites. CaMKII can directly phosphorylate and activate Rac1 GEFs such as Tiam1 and Kalirin7 but can also directly phosphorylate and inactivate Rac1 GAP, p250GAP, both signaling cascades leading to enhanced Rac1 activity [183–186]. CaMKII

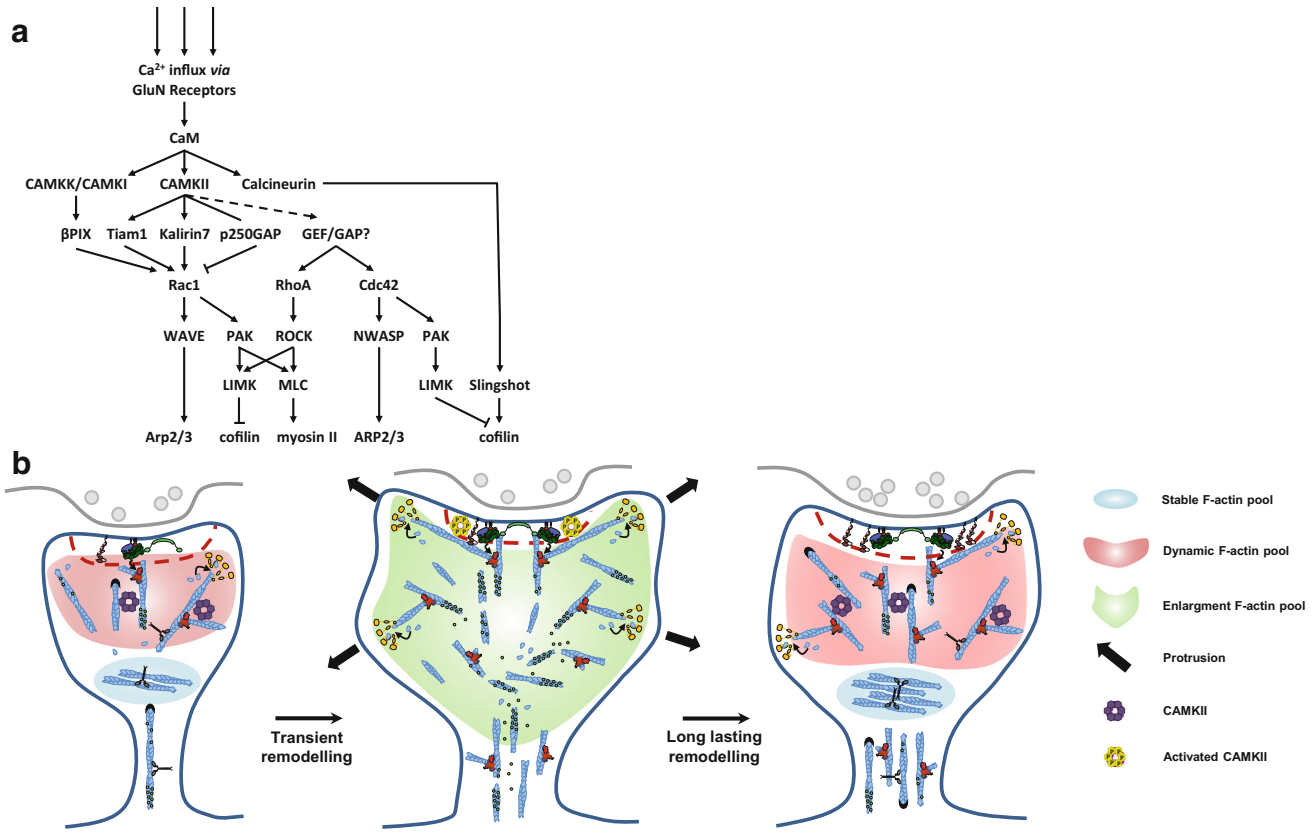

Fig. 2 Activation of ABPs and reorganization of the F-actin cytoskeleton during LTP. **a** Selected signaling cascades driving F-actin remodeling upon Ca^{2+} influx via GluN Receptors. CAMKII is a key triggering protein both involved in functional and structural LTP. CAMKII can activate small RhoGTPases (Rac1, Cdc42, RhoA) by phosphorylating GEFs (Tiam1, Karilin7) and GAPs (p250GAAP). This will control the spatiotemporal activation of several ABPs (Arp2/3, myosin II, cofilin). *Arrows* indicate positive regulation. *T-shaped bars* indicate negative regulation. *GluN* NMDA receptor, *CaM* calmodulin, *CaMK* calcium-calmodulin dependent protein kinase, *GEF* guanine nucleotide exchange factor, *GAP* GTPase activating protein, *βPIX* β PAK interacting exchange factor, *Tiam1* T cell lymphoma invasion and metastasis-inducing protein 1, *Rac1* Ras-related C3 botulinum toxin substrate 1, *Cdc42* cell division cycle 42, *RhoA* Ras homologous member A, *ROCK* Rho-associated, coiled-coil containing protein kinase, *PAK* p21-activated kinase, *LIMK* LIM-kinase, *N-WASP* neuronal Wiskott–Aldrich syndrome protein, *WAVE* WASP-family verprolin homologous protein, *MLC* myosin light chain, *Arp2/3* actin-related-protein 2/3. **b** A model for F-actin reorganization during LTP. LTP induces a transient and long-lasting increase of the spine head size, a shortening and widening of spine neck and a concomitant ABPs and CaMKII spine recruitment. Activated CAMKII will dissociate from F-actin and phosphorylate multiple proteins leading to a fast F-actin reorganization and a transient spine head enlargement. This transient reorganization is characterized by the formation of an enlargement F-actin pool, an increase in the F-actin/G-actin ratio and increased concentration of cofilin and Arp2/3. Note that inactivation of cofilin is detected only 2 min after LTP induction, suggesting that cofilin might be only transiently active before it gets inactivated to prevent further depolymerization. This might be important to only transiently sever F-actin filaments in order to generate new F-actin barbed ends, and to increase F-actin polymerization and branching. During the long-lasting spine head enlargement, most ABPs return to their basal concentration, suggesting the formation of a larger dynamic and stable F-actin pool. Those larger dendritic spines most likely provides a "tag" for the capture of newly synthetized synaptic proteins in order to sustain late LTP

phosphorylation of neurabin-II leads to the recruitment of a RhoA GEF, Lfc ("Lbc's first cousin") [187, 188]. CaMKII activity is also partially involved in Cdc42 activation, although the signaling pathway has not been described in dendritic spines [189]. An increase in intracellular Ca^{2+} can activate other CaM sensitive proteins involved in the regulation of F-actin networks. CaMKK and CaMKI form a multiprotein complex with another Rac1 GEF, βPIX ("Pak-interacting exchange factor"), resulting in Rac1 activation [190, 191].

Many targets of RhoGTPases are present in dendritic spines and are critical regulators of the actin cytoskeleton (Fig. 2a). The WAVE complex and N-WASP, NPFs of the Arp2/3 complex, are two main downstream targets of Rac1 and Cdc42, respectively [192–194]. p21-activated kinase (PAK) is a downstream target of Rac1 and Cdc42, while Rho-associated, coiled-coil-containing protein kinase (ROCK), is a downstream target of RhoA [180, 195–197]. Both PAK and ROCK can activate LIM-kinase (LIMK) that can in turn phosphorylate ADF/cofilin preventing its

binding and severing activities. This inhibition of ADF/cofilin can be counterbalanced by other signaling pathways. CaM activates protein phosphatase 2B (PP2B or calcineurin) triggering slingshot activation, which dephosphorylates and activates ADF/cofilin leading to enhanced F-actin severing [198, 199]. PAK and ROCK can also phosphorylate the myosin light chain (MLC) leading to increased myosin II activity [177, 195]. Finally, the Arp2/3 complex inhibitor PICK1 can also be regulated by synaptic activity since it is a low-affinity Ca^{2+} sensor, which binds to Rac1 and Cdc42 and is regulated by the GTPase ADP-ribosylation factor 1 (Arf1) [200–202]. Although many studies revealed the complex signaling pathways leading to F-actin network reorganization, their spatiotemporal integration during synaptic plasticity is far from being understood [181, 189, 203–205].

Morphological remodeling of dendritic spines and F-actin reorganization during LTP and LTD

Early EM studies already reported that LTP induction would increase dendritic spine head and PSD size [23, 206–208], increase the number of dendritic spine protrusions [25] and induce wider and shorter spine necks [207]. However, EM studies cannot provide longitudinal measurements for single spines during LTP induction. Kasai and co-workers performed two-photon glutamate uncaging to release glutamate at single dendritic spines [28, 209]. This stimulation induced spine head enlargement, GluA1 recruitment and an increase in GluA-mediated current [28, 210]. More recently, time-lapse STED microscopy revealed that shortening and widening of the spine neck occurred in a concerted fashion with spine head enlargement [31]. These changes in spine head volume display two distinct phases, a transient large volume increase (<5 min; Δvolume 200–400 % increase) followed by long-lasting smaller volume increase for small dendritic spines (60 min; Δvolume 50–150 % increase), whereas spine neck changes seem to display only a long-lasting change (Δ with 30 % increase; Δlength 30 % decrease; [31]). Consistent with previous studies demonstrating that F-actin dynamics supports spine motility, pharmacological treatments that increased F-actin depolymerization (latrunculin-A) prevented transient and long-lasting changes of dendritic spine head [28].

In vivo, LTP induction was shown to correlate with increased F-actin content in dendritic spines visualized by EM [211]. Fluorescence resonance energy transfer (FRET) also revealed an increased F-actin/G-actin ratio after presynaptic fiber tetanic stimulation, suggesting that LTP promotes F-actin polymerization [75]. Subsequently, a very elegant study explored F-actin reorganization by probing photoactivable actin dynamics after two-photon glutamate uncaging [77]. Single spine activation, which triggered spine enlargement, induced the formation of a transient enlargement F-actin pool distributed throughout the spine head, displaying a distinct turnover compared to the stable and dynamic F-actin pools (Fig. 2b). The localization and dynamics of this enlargement pool often synchronized with spine membrane protrusions, as if F-actin polymerization drove the enlargement. However, increased volumes triggered by LTP last longer than the life-time of the enlargement pool, suggesting that the long-lasting spine enlargement might be sustained by transfer of F-actin from the enlargement into the stable pool.

GluN receptor-dependent Ca^{2+} influx during LTP, which leads to activation of the intracellular signaling pathways, could increase F-actin polymerization and reorganization [153, 212]. CaMKII is necessary and sufficient for LTP induction; it is therefore not surprising that pharmacological inhibition of CaMKII or expression of a CaMKIIα kinase dead mutant in knock-in mouse disrupt the long-lasting volume increase of dendritic spines [28, 205, 213]. Combining FRET with two-photon glutamate uncaging, Yasuda and co-workers demonstrated that CaMKII activity was only transiently increased (∼2 min) during spine head enlargement [205]. As mentioned in "Actin organization in dendritic spines" and "The interplay between the actin cytoskeleton and synaptic plasticity", active CaMKIIβ will dissociate from F-actin and phosphorylate multiple proteins. Recently, Okamoto and colleagues showed that CaMKIIβ dissociation from F-actin is required for structural and functional plasticity of spines, and that this dissociation is not sufficient to induce structural plasticity but permits ADF/cofilin binding to F-actin [158]. The small RhoGTPases Cdc42 and RhoA showed increases in transient (∼2 min) but also long-lasting (∼40 min) activity [189]. These increased activities are dependent on CaMKII and GluN receptor. Although this latter study did not study Rac1 activity pattern during LTP, a previous study reported that kalirin-7 phosphorylation by CaMKII and subsequent Rac1 activation would lead to dendritic spine enlargement [186]. Overall, those studies suggest that the sustained activities of Rac1, Cdc42 and RhoA might be the main mechanism to relay the transient CaMKII activity for efficient F-actin remodeling.

The main downstream targets of those proteins will regulate F-actin branched nucleation by Arp2/3, ADF/cofilin severing activity and myosin II function (Fig. 2a). Studying the spatiotemporal coordination of various proteins, Hayashi and co-workers have provided new insights into F-actin cytoskeleton reorganization during two-photon glutamate uncaging-induced LTP [49] (Fig. 2b).

Concomitantly with spine enlargement, ABPs and CaMKII were translocated into spines. However, while the concentration of cofilin, AIP1 and Arp2/3 in spines increased during the transient spine enlargement phase, the concentration of the crosslinking proteins CaMKIIβ, α-actinin and drebrin A decreased (relative to the spine volume) [49]. This qualitative switch in ABPs concentration in spines suggests that the F-actin network undergoes a fast reorganization that might be important for the formation of the F-actin enlargement pool. Phosphorylation and inactivation of cofilin is detected only 2 min after LTP induction [214], suggesting that cofilin might be only transiently active before it gets inactivated by LIMK to prevent further F-actin severing [49]. Activation of Rac1 downstream pathways might be the key for this short time window activation of ADF/cofilin. Indeed, activation of Rac1 will lead to increased F-actin branching via activation of WAVE and Arp2/3 complexes but also decreased F-actin severing via phosphorylation of ADF/cofilin by PAK/LIMK pathways. A delayed activation of the PAK/LIMK pathway as compared to the fast increase of AIP1 and ADF/cofilin concentrations might provide this 2-min time window for efficient F-actin severing [120]. Interestingly, this transient activation fits with the CaMKII time window of activity and its dissociation from the F-actin network [158, 189]. This model is in agreement with the role that ADF/cofilin might play to trigger membrane protrusions [83, 215]. In that model, transient F-actin severing generates new F-actin barbeds ends, increasing F-actin branching and polymerization without totally depolymerizing the F-actin network. Thus, during these 2-min time windows, the actin cytoskeleton in spines could experience drastic remodeling. Our recent findings provided some insights into the links between spine enlargement and the nanoscale reorganization of ABPs [67]. SMLM experiments demonstrated that Rac1 activation correlates with its immobilization in spines, spine enlargement and delocalization of the WAVE complex from the PSD. In addition, we found that Shank3 overexpression, which causes spine enlargement and neuropsychiatric disorders [129, 216], also induces delocalization of the WAVE complex from the PSD [67]. Thus, alteration of spine morphology could rely on the long-lasting or transient nanoscale relocalization of ABPs, e.g., the WAVE complex, leading to remodeling of the entire dendritic spine structure.

During the long-lasting spine volume increase, most ABPs returned to their basal concentration, whereas cofilin showed a persistent accumulation at the base of the spine and increased F-actin binding [49]. Actin filaments fully decorated by ADF/cofilin are stable, promoting F-actin bundle stabilization in the absence of AIP1 [120, 121, 217]. The long-lasting ADF/cofilin pool might therefore be associated with the stable F-actin pool. Accordingly, drebrin-A is also slightly enriched at potentiated spines during the long-lasting spine head enlargement [49, 218]. Maintaining high-concentration levels of ADF/cofilin during a precise time window could be an interesting mechanism to efficiently depotentiate newly potentiated synapses [49, 219].

Although GluN receptor antagonist and F-actin depolymerization (latrunculin-A) totally disrupt transient and long-lasting spine remodeling, inhibition of CaMKII, Cdc42, ADF/cofilin or Arp2/3 blocks only the long-lasting volume changes [28, 49, 93, 189, 205]. Thus, Ca^{2+} influx might remodel the F-actin network via distinct signaling pathways such as activation of CaMKI or release of internal calcium stores [174, 220]. Activation of GluN receptor can also recruit and stabilize β-catenin in dendritic spines [221]. According to the molecular clutch hypothesis, connection of N-cadherin/β-catenin to the acto-myosin network could transmit forces that will lead to spine enlargement [60, 70, 222, 223]. Supporting the requirement of a clutch mechanism for dendritic spine enlargement during LTP, inhibition of RhoA and ROCK, needed for myosin II activity, also disrupted transient and long-lasting volume changes [60, 189]. All these studies highlight the need for a spatiotemporal coordination of F-actin polymerization, branching, severing and bundling for transient and long-lasting remodeling of the different F-actin pools in order to sustain structural LTP.

Induction of synaptic LTD by electrical or chemical stimulation can lead to dendritic spine shrinkage [29, 30, 75, 224, 225]. Adapting the two-photon glutamate uncaging to induce LTP, Zito and co-workers established a low frequency uncaging (LFU) protocol to induce LTD at individual spines [30]. Although LFU does not allow the probing of structural remodeling at early time scales, the authors were able to induce a long-lasting and saturable volume shrinkage of small and large dendritic spines (Δvolume 25 % decrease). Combining two-photon uncaging of glutamate and GABA to induce a distinct form of LTD, Kasai and coworkers could induce larger volume changes (ΔVolume 40 % decrease) and even spine elimination [226]. Spine shrinkage was first shown to correlate with a decreased F-actin/G-actin ratio after pre-synaptic fiber tetanic stimulation, suggesting F-actin depolymerization [75], which, during GluN receptor-dependent LTD, would be powered by activation of ADF/cofilin by calcineurin via slingshot [29, 123, 198, 224]. Inhibition of GluN receptors and calcineurin and also a phospho-cofilin peptide can therefore abolish spine shrinkage [224, 226]. However, shrinkage of large spines requires additional signaling from mGluR and internal calcium stores [30]. Dendritic spine shrinkage is also blocked after knockdown of the Arp2/3 inhibitor PICK1 or expression of a PICK1

mutant unable to bind Arp2/3 [106]. Therefore, as for LTP, regulation of F-actin organization and dynamics might be critical mechanisms for structural LTD.

How does the actin cytoskeleton sustain functional LTP and LTD?

Dendritic spine structural plasticity and functional plasticity share similar triggering molecules, such as CaMKII for LTP and calcineurin for LTD. However, structural plasticity and functional plasticity can be dissociated as they do not strictly rely on the same intracellular signaling pathways [43, 199, 224, 227–230]. Although regulation of F-actin network assembly and disassembly is not sufficient to induce synaptic plasticity, it is required for its formation in vitro and in vivo [211, 231–233]. Likewise, knocking out proteins involved in F-actin network organization and dynamics, such as Rac1, IRSp53, subunits of WAVE or Arp2/3 complexes, PAK1, PAK3, LIMK, ADF/cofilin and Eps8 displayed impaired LTP and/or LTD and also spatial and working memory deficits [93, 98, 100, 117, 122, 234–239]. Neurological disorders associated with abnormal spine morphologies such as autism spectrum disorders (ASD) and schizophrenia can also be triggered by genetic deregulation of proteins such as IRSp53, Shank3 and FMRP (fragile X mental retardation protein) that directly interact with subunits of the WAVE and Arp2/3 complexes [129, 216, 239–242]. What could therefore be the mechanisms behind F-actin cytoskeleton sustainment of synaptic plasticity?

GluN and GluA receptors play a major role in the induction and maintenance of synaptic plasticity, respectively [199, 243, 244]. Trafficking of GluN and GluA in and out of dendritic spines, but also their anchorage and stabilization via interaction with PSD scaffold proteins, are crucial mechanisms for LTP and LTD [245–248]). PSD dynamic architecture and receptor trafficking are supported and regulated by the F-actin cytoskeleton [43, 86, 249]. F-actin can regulate in many ways the dynamics of key PSD scaffold proteins such as PSD-95, GKAP, Shank and Homer [35]. First, actin filaments are in close proximity with the PSD [36]. Second, the Arp2/3 and WAVE complexes and cortactin can directly bind Shank3 [101, 129]. Third, proteins involved in the activation of the WAVE complex, such as IRSp53 and the Rac1 GEF βPIX, can interact with Shank family proteins and PSD95 [126, 127, 250–252]. Acute pharmacological treatment inducing F-actin depolymerization (latrunculin-A) disrupts PSD-95 nanoscale organization and dynamics but only slightly affects its content in dendritic spines, probably because of its direct association with the plasma membrane [253–255]. A similar treatment decreases GKAP, Homer1C and Shank2 content in immature but not mature spines [253, 254]. Thus, PSD scaffold matrix remodeling relies on F-actin dynamics, but the intermolecular assembly between PSD proteins might be strengthened during dendritic spine maturation to become independent from the F-actin network [256]. Interestingly, as for PSD95 proteins, the WAVE complex and IRSp53 are still present in dendritic spines after F-actin depolymerization, highlighting the tight functional coupling between the PSD and F-actin regulators [67]. Pharmacological treatments which induce F-actin depolymerization also disrupt GluA dynamic architecture, increasing their surface diffusion, decreasing their spine content and preventing their spine insertion during LTP [230, 238, 254, 257]. Furthermore, inhibiting F-actin dynamics without disrupting the F-actin network (low concentrations of latrunculin-A) does not decrease GluA content but still prevents insertion of new GluA receptors upon synaptic stimulation [228, 230, 232]. Altogether, these findings suggest, first, that PSD reorganization by F-actin controls the anchorage of a synaptic pool of GluA receptors; second that PSD remodeling provides constant accessibility of new binding sites for GluA; and finally that maintenance and insertion of GluA receptors relies on the F-actin cytoskeleton [230, 238, 254, 258–260].

Disrupting F-actin dynamics could also lead to impaired exocytosis and endocytosis therefore preventing insertion and removal of GluA receptors during LTP and LTD, respectively [249, 261–265]. The plasma membrane t-SNARE syntaxin 4 binds to actin and is involved in exocytosis in dendritic spines and LTP [261, 266]. However, F-actin depolymerization does not change the frequency and proportion of exocytotic events in the somatodendritic compartment [267]. Spine-localized endocytosis of GluA receptors is, on the other hand, well established and relies on the actin cytoskeleton [249]. The Arp2/3 inhibitor PICK1 binds to GluA2/3 subunits and plays a critical role in their surface expression. Preventing PICK1 binding to Arp2/3 disrupts GluA internalization during LTD induction therefore occluding LTD [96, 106]. Interestingly, PICK1 is a Ca^{2+} sensor, and the PICK1-mediated inhibition of Arp2/3 is enhanced by GluA2-PICK1 interaction thus providing spatiotemporal control of GluA endocytosis during LTD [96, 200]. Since PICK1 knockdown also prevents LTP, branched F-actin nucleation and its regulation by PICK1 could be more generally involved in the recycling endosomal pathway and in the constant supply of GluA receptors [249, 268, 269].

Intracellular transport of recycling endosomes (RE) into and out of dendritic spines relies on myosin motors moving on actin filaments: myosin V and myosin VI walking towards the barbed- and pointed-end, respectively. Spine necks are composed of both branched and non-branched long F-actin filaments of mixed orientation, thus providing

tracks for myosin V and VI to enter and exit dendritic spines [39, 66]. As mentioned previously, LTP induction induces shortening and widening of the spine neck and could therefore facilitate in and out trafficking mediated by myosin motors [31]. Myosin VI was first shown to be involved in stimulation-induced GluA1 endocytosis [270]. Myosin Vb was also shown to be recruited to RE upon Ca^{2+} influx and LTP induction, triggering translocation of RE into spines and GluA1 surface insertion [271]. Furthermore, acute blockade of Myosin Vb ATP activity, needed for its movements along F-actin, attenuates LTP maintenance [271].

While the mechanisms described above were shown to be essential to sustain the early phase of LTP and LTD (1–4 h), protein synthesis is required to sustain the later phases (≥4 h) [49, 199, 272–277]. Late LTP (L-LTP) requires, for instance, the supply of newly synthetized PSD proteins to increase the number of available binding sites for GluA receptors in order to prevent synaptic saturation [278]. Interestingly, inhibiting CaMKII activity only during LTP induction or inhibiting F-actin dynamics during LTP induction up to 30 min after induction are sufficient to occlude L-LTP [233, 279]. Therefore, activation of the CaMKII signaling cascade, reorganization of the F-actin cytoskeleton and dendritic spine morphological remodeling could all represent a synaptic "tag" for efficient delivery and capture of newly synthetized proteins in order to sustain long-term synaptic plasticity and memory storage [49, 280].

Conclusion/outlook

In this review, we have highlighted that, as for many other motile sub-cellular compartments, dendritic spine motility and remodeling are sustained by F-actin cytoskeleton dynamics. The unique F-actin dynamics of dendritic spines results from the nanoscale organization of various ABPs within different sub-spine compartments [67]. However, to completely understand how the nanoscale organization of ABPs can lead to the coexistence of several F-actin pools with distinct dynamic properties will require further studies. Correlative live cell and super-resolution microscopy could, for instance, enable correlation between F-actin dynamics and ABP nanoscale organization [281]. Because of the heterogeneity of dendritic spine morphologies, multiplex SMLM could represent a powerful tool to visualize membrane, ABPs, PSD proteins and post-synaptic receptors within an individual spine. Multiplex SMLM can be achieved by linking DNA-PAINT (DNA-Point Accumulation for Imaging in Nanoscale Topography) docking strands to antibodies [282, 283]; by using virtual grids and sequential immunostaining and imaging [284]; or more recently by spectrally resolving single molecules from different dyes [285]. This last approach might, however, be challenging regarding the density of proteins in dendritic spines.

Regulation of the spatiotemporal activity of ABPs is a fundamental mechanism to induce spine structural plasticity during LTP and LTD. Recent work has provided a detailed picture of this spatiotemporal coordination [49, 189, 286]. However, those studies could not provide the nanoscale organization of ABPs at sub-spine resolution. So far, SMLM has reached the highest resolution for fluorescence microscopy on both live and fixed biological samples in 2D and 3D [53, 80, 287, 288]. Deep tissue and in vivo imaging of dendritic spines is, however, still a challenge [289, 290]. Super-resolution microscopy techniques, such as simulated emission depletion microscopy (STED), can perform live recordings of dendritic spines in brain slices and in vivo [22, 31, 291]. However, high-power lasers used for STED induce high photobleaching and requires high labeling density or replenishment of a cytosolic fluorophore. Reversible saturable optical fluorescence transitions microscopy can overcome those drawbacks by exploiting reversibly photoswitchable fluorophores [292, 293]. Structured illumination microscopy is also well suited for three-dimensional multi-color live imaging, and recent improvements of its spatial resolution in combination with light sheet microscopy could represent a valuable tool to study the spatiotemporal reorganization of synaptic proteins in brain slices and in vivo during synaptic plasticity [294–299].

Spine structural plasticity and the underlying reorganization of F-actin networks could represent a synaptic "tag" required for the maintenance of functional plasticity [75, 153, 233, 280]. Recently, Kasai and co-workers have demonstrated that motor task learning could be disrupted by optical shrinkage of the potentiated spines, therefore establishing a direct link between dendritic spine morphology and the formation of a memory trace in vivo [13]. The exact mechanism by which the F-actin cytoskeleton sustains functional plasticity is, however, still unclear. Most likely, it involves efficient delivery and capture of newly synthetized proteins as well as the regulation of PSD proteins and post-synaptic receptors dynamics and stability. The coordinated structural remodeling of pre- and post-synaptic structures could also be a key mechanism for maintenance of functional plasticity and might require tight coordination between the F-actin cytoskeleton and trans-synaptic adhesion proteins [286, 300]. Further development in optogenetics will allow the manipulation of synaptic protein interactions in vitro and in vivo and could tackle those hypotheses to refine the model behind the sustainment of functional synaptic plasticity by F-actin cytoskeleton reorganization [301, 302]. However, how this knowledge, acquired at a single spine and neuron level, can

be implemented in neural networks models that can generate behavior, cognition and mental disease remains a main challenge in neuroscience [303, 304].

Acknowledgments We would like to thank Dr. Harold D. McGillavry and Dr Laura F. Gumy for helpful comments on the manuscript. We acknowledge financial support from the French Ministry of Research and CNRS, ANR grant Nanomotility, LabEx BRAIN, Conseil Régional Aquitaine, Fondation pour la Recherche Médicale, and from Marie Skłodowska-Curie fellowship to Anaël Chazeau.

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