# Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy

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Abstract | Synapse regulation exploits the capacity of actin to function as a stable structural component or as a dynamic filament. Beyond its well-appreciated role in eliciting visible morphological changes at the synapse, the emerging picture points to an active contribution of actin to the modulation of the efficacy of pre- and postsynaptic terminals. Moreover, by engaging distinct pools of actin and divergent signalling pathways, actin-dependent morphological plasticity could be uncoupled from modulation of synaptic strength. The aim of this Review is to highlight some of the recent progress in elucidating the role of the actin cytoskeleton in synaptic function.

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**Long-term potentiation**
(LTP). Long-lasting (hours to days) enhancement of synaptic communication between two neurons as a consequence of their simultaneous activity. It is one of the most highly studied cellular models for learning and memory.

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Actin is one of the major components of the cellular scaffold that is essential for sculpting and maintaining cell shape, and actin dynamics support a myriad of processes ranging from cell motility, cell division and cell morphogenesis to intracellular protein trafficking. In developing neurons the actin cytoskeleton has a key role in neurite formation, extension and branching, as well as in synaptogenesis. In mature neurons, actin is the most prominent cytoskeletal protein at synapses, being present at both the pre- and the postsynaptic terminals[1–5]. Notably, actin is highly enriched at dendritic spines, specialized postsynaptic compartments that mediate most of the excitatory synaptic transmission in the brain[6–8] (BOX 1).

The efficacy of synaptic transmission is dynamically modulated in a use-dependent manner over a wide range of timescales, a property that is thought to endow the brain with the capacity for performing computations, learning and storing information[9–12]. The changes in synaptic efficacy, which involve both pre- and post-synaptic mechanisms, are thought to be accompanied by structural plasticity, at least in some instances. This is particularly evident for the durable forms of synaptic plasticity such as long-term potentiation (LTP), which involves an increase in the size of dendritic spines and can result in the formation of new synapses. Because actin is the major cytoskeletal protein found in the pre- and postsynaptic terminals, modulation of actin dynamics is likely to drive the cytoarchitectural changes that are associated with synaptic plasticity [6,13,14]. Yet, it is not clear to what extent such structural changes are related

to the expression of synaptic plasticity. In addition to its readily discernable function in processes that incur a visible shape change at the synapse, actin also contributes to morphologically undetectable but equally important activities that range from organizing the components of the junctional scaffold to facilitating the trafficking of synaptic machinery. This diversity of actin function is attributable to the dynamic turnover and remodelling of actin filaments, which are regulated by a cohort of accessory proteins and signalling machinery[13].

Actin exists in two states in the cell: as monomeric G-actin and as an asymmetric two-stranded helical filament (F-actin) composed of G-actin (BOX 2). The assembly and disassembly of F-actin can be rapid owing to the weak non-covalent interactions of G-actin. At steady-state, F-actin preferentially polymerizes at one end of the filament (the barbed end) while G-actin monomers are lost at the opposite end (the pointed end). The difference in polymerization rates between the two ends result in a net turnover of the filaments. Some actin-binding proteins (ABPs) can alter actin-filament dynamics whereas others can interlink F-actin into a variety of cytoskeletal networks[15–17] (BOX 2; FIG. 1; TABLE 1). Cellular signalling machineries exploit these properties to adapt and sculpt the synaptic cytoarchitecture in response to changes in synaptic activity.

Research efforts over the past decade have established a central role for actin regulators and cell signalling events that target actin in conferring the distinctive morphology of dendritic spines and driving their dynamic shape changes in response to activity. This Review focuses

## Box 1 | Spines and long-term synaptic plasticity

Since their discovery by Ramón y Cajal over a century ago, dendritic spines have continued to fascinate neuroscientists. These tiny protoplasmic protrusions cover the dendritic surface of many neurons and receive over 90% of all excitatory synaptic inputs in the neocortex. Most principal output neurons bear spines at high density (for example, there are up to two spines per μm in layer-5 pyramidal neurons), whereas local interneurons are generally devoid of spines. Intriguingly, synapses made by Schaffer collaterals onto spines of hippocampal CA1 pyramidal neurons are highly heterogeneous with respect to their relative content of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and NMDA (N-methyl-D-aspartate) receptors. NMDA receptors are found in every synapse and their number is proportional to the diameter of the postsynaptic density (PSD). By contrast, AMPA receptors are not detected in ~25% of synapses and their number is linearly correlated with the area rather than the diameter of the PSD in the remaining synapses. As a consequence, small spines contain only or mainly NMDA receptors whereas large spines have a higher ratio of AMPA to NMDA receptors[129–132]. Excitatory synaptic transmission under basal conditions is mostly mediated by AMPA receptors. These findings have provided a morphological correlate for the postsynaptically 'silent' synapses (as defined by electro-physiological studies). Synapses on small spines would be inactive (silent) under normal conditions because they do not contain AMPA receptors, and they might represent preferential sites for the induction and expression of long-term potentiation (LTP)[133].

Two-photon photolysis of caged glutamate allows rapid and local release of glutamate with a spatial resolution of a single spine. Thus, this technique has made it possible to probe the glutamate sensitivity of individual morphologically identified spines[134]. In agreement with previous studies, glutamate sensitivity was found to be highly correlated with spine-head volume[135]. Furthermore, LTP induction at single identified spines by repetitive uncaging of glutamate produced a long-lasting and selective enlargement of the spines that mirrored the increase in synaptic efficacy[101]. By contrast, long-term depression (LTD) induction by low-frequency stimuli resulted in a retraction or collapse of spines[96,106,136]. Interestingly, small and weak spines preferentially underwent structural and functional long-term synaptic plasticity[101]. Therefore, in agreement with previous observations, small spines are more dynamic and plastic than large spines[137–141]. In a model for learning and memory in cortical networks, small spines that either disappear or grow into large spines according to the pattern of synaptic activity could represent 'learning spines', whereas large spines that are relatively stable over time could be structural substrates for mnemonic traces.

**Vesicle docking**
The attachment of synaptic vesicles to the active zone of the presynaptic terminal.

**Vesicle priming**
ATP-dependent pre-fusion reactions that prepare docked synaptic vesicles for $Ca^{2+}$-dependent exocytosis.

**Active zone**
The portion of the presynaptic membrane where synaptic vesicle exocytosis occurs.

**Readily releasable pool**
The pool of synaptic vesicles that are immediately available for exocytosis. These vesicles are thought to be the docked and primed vesicles at the active zone and constitute ~1–2% of all vesicles.

**Recycling pool**
The pool of synaptic vesicles that engage in and support neurotransmitter release at moderate stimulation intensities. They represent ~5–50% of all synaptic vesicles.

**Reserve pool**
The pool of synaptic vesicles that can engage in neurotransmitter release during intense stimulation. They constitute the majority of synaptic vesicles (~50–90%). At some synapses, not all reserve-pool vesicles are used for neurotransmitter release, and the function of these vesicles remains to be established.

**Bouton**
The button-like swelling or small protuberance on an axon that harbours the presynaptic assembly for neurotransmitter release (that is, the presynaptic terminal).

primarily on the less well-understood aspects of actin's role in basic synaptic mechanisms and the regulation of synaptic strength, highlighting the recent progress in this area. For reviews of ABPs, intracellular signalling and dendritic-spine morphogenesis and plasticity, see REFS 14,18–21.

### Actin in presynaptic mechanisms

*Actin and vesicle-pool organization.* The presynaptic terminal is a subcellular axonal compartment dedicated to releasing neurotransmitters through synaptic vesicle exocytosis (FIG. 2). The exocytic fusion of synaptic vesicles with the active zone membrane is supported by the synaptic vesicle cycle, the major steps of which are vesicle docking, priming, fusion and recycling (endocytosis)[22,23]. Numerous synaptic vesicles are found in the centre of the terminal, and some have to be brought to the active zone in a process that is referred to as 'docking'. Docking is followed by a priming step, which represents a series of reactions that confer 'fusion competence' to the docked vesicles, perhaps involving the assembly and maturation of the membrane fusion machinery. Primed vesicles undergo exocytosis upon $Ca^{2+}$-influx (triggered by the arrival of an action potential), and the fusion event is followed by endocytosis to reclaim vesicles for reuse or to repopulate the central cluster of vesicles.

Synaptic vesicles are organized into three functionally distinct pools: the readily releasable pool, the recycling pool and the reserve pool[24,25]. The extent to which these pools are spatially segregated in the presynaptic terminal is not clear. For instance, vesicles that are associated with a particular pool might be identifiable by their unique molecular composition or by the state of modification of their proteins, irrespective of their location. Nevertheless, by definition, the readily releasable pool, which represents primed vesicles, must consist of at least some of the morphologically docked vesicles at the active zone. By contrast, the reserve pool is believed to comprise vesicles that are found in the core region of the presynaptic bouton. The recycling pool, which consists of vesicles that are not necessarily primed but that actively participate in the synaptic vesicle cycle, could be located closer to the active zone than the reserve pool[26], although this might not be a strict requirement[27,28]. The spatial segregation of vesicle pools would require a topographical divide or a constraint in the bouton, and the actin cytoskeleton seems to be highly suited for this role. During the synaptic vesicle cycle, vesicles would be mobilized between pools across such a constraint by either active movement or passive diffusion.

Actin is abundantly present at the presynaptic terminal[1,4,29,30]. From its subcellular organization (FIG. 1b,c), actin has been implicated in maintaining and regulating vesicle pools — on the one hand by serving as a scaffold to restrict vesicle mobility and on the other hand by providing a conduit to direct the transfer of vesicles between the pools[13]. At the core of the bouton, actin is associated with short filaments of synapsin which, in turn, are linked to the vesicles[1,2,31–33]. It has been suggested that such a meshwork of actin–synapsin–vesicles sequesters the reserve vesicle pool[32–34]. Upon increased neuronal activity, phosphorylation of synapsin releases the reserve vesicles from the actin meshwork and synapsin itself is dispersed[35,36]. The resulting mobilization of reserve-pool vesicles sustains increased exocytosis by replenishing the readily releasable pool.

# REVIEWS

## Box 2 | Actin dynamics

Monomeric G-actin is the building block of F-actin. G-actin bound to ATP (panel **a** of the figure, blue) spontaneously self-associates through weak non-covalent interactions to form asymmetric filaments that have distinct ends because of the polarity of their constituent actin monomers (ADP-bound G-actin is shown in red). Polymerization occurs preferentially at the barbed (or 'plus') end over the pointed (or 'minus') end. At steady-state and at a given cellular G-actin concentration, the difference in polymerization rates at the two ends gives rise to a net loss of actin monomers at the pointed end and a simultaneous gain of monomers at the barbed end. This creates a net flow of newly acquired G-actin through the filament in a phenomenon that is known as actin treadmilling, resulting in a dynamic turnover of actin filaments while filament length is maintained.

A number of naturally occurring toxins that have been isolated from marine sponges and fungi directly bind to actin[126–128]. Many of these are cell-permeable and have had major utility in the study of actin's cellular roles. Commonly used toxins affect actin dynamics by binding to F-actin and stabilizing (phalloidin, jasplakinolide) and/or promoting (jasplakinolide) polymerization; by sequestering G-actin to promote F-actin depolymerization (latrunculins A and B (see figure, part **a**)); or by binding to the barbed end of F-actin to prevent the addition of monomers (cytochalasins B and D (see figure, part **a**)).

A variety of actin-binding proteins (ABPs) influence the structure and organization of the actin cytoskeleton. Capping proteins (for example, tropomodulin and CapZ) bind to filament ends and can modify filament turnover to affect their length, whereas crosslinking proteins (for example, α-actinin (see figure, part **b**), filamin (see figure, part **c**) and spectrin) can arrange F-actin into distinct networks, such as actin bundles and gels (see figure, parts **b**,**c**). Other ABPs affect F-actin by promoting its depolymerization (for example, ADF/cofilin), its severing (for example, gelsolin and ADF/cofilin) or its polymerization (for example, profilin) (see figure, part **a**). The ABPs are targeted by a wide range of signal transduction cascades that regulate actin remodelling to meet the changing demands of cellular functions[15–17].
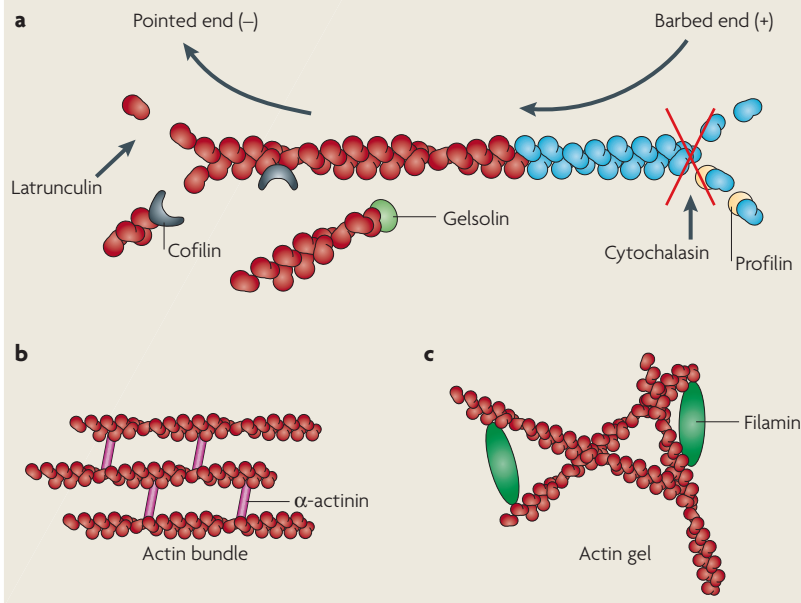
Biochemical and morphological evidence suggests that actin is also a major constituent of the active zone[2,3,37], where it might serve a dual function. Actin might guide the arriving vesicles to facilitate their docking and thus positively regulate the size of the readily releasable pool. In addition, actin could form a physical or molecular barrier for the priming reaction, and thereby serve as a brake to prevent facile fusion of vesicles. Functional studies have provided evidence for both such positive and negative roles for actin at the active zone, and the

role that predominates might be dependent on the particular synapse type[13] or the state of the synaptic activity. For instance, boutons that are large and/or release neurotransmitters at a fast rate might be highly dependent on actin for recruiting vesicles into the readily releasable pool to support exocytosis, whereas boutons that are small or release neurotransmitters with low probability would not be heavily dependent on active mechanisms for vesicle mobilization but might use actin filaments as a physical barrier to limit vesicle fusion. In such a model of dual actin function, the intracellular signalling machinery that is predominant in a particular synapse type or that is engaged by synaptic activity would modify the activity of ABPs or regulatory proteins to control how actin modulates neurotransmitter release.

A recent report examined the three-dimensional organization of the presynaptic terminal of excitatory hippocampal synapses[38]. The authors of the study used a high-pressure freezing method[39–41] that allows the cytomatrix to be preserved without the need for fixatives. The resulting electron tomograms revealed the intricate organization of the presynaptic architecture with an exceptional level of detail. Strikingly, vesicles that are interlinked by short filaments are also connected to longer filaments that mostly extend from the active zone (FIG. 1c). At the active zone, docked vesicles are preferentially associated with presynaptic dense elements from which the long filaments extend. Thus, there seems to be a vertical subgrouping of vesicle clusters (running perpendicular to the plane of the active zone) that are associated with different subdomains of the active zone. Although the molecular composition of the filaments has not been directly examined, a prior demonstration of actin and synapsin immunolabelling of similar filaments suggests the potential involvement of both of these proteins in such columnar grouping of vesicles[3]. Surprisingly, the overall vesicle organization and the short filament links between vesicles remain largely unaltered in the absence of three synapsin isoforms, I, II and III, raising questions about the function of synapsins in sequestering the reserve-pool vesicles[38]. However, a lack of effect could be due to compensation by other proteins with scaffolding activity. Even though the exact nature of the short filaments and the functional implications of the organization of vesicle clusters linked to the active zone remain to be explored, the findings described above encourage detailed ultrastructural characterization of the involvement of the actin cytoskeleton in regulating the presynaptic cytoarchitecture under basal conditions and in response to synaptic activity. Furthermore, if it can be combined with the photoconversion of synaptic vesicles that have been labelled by activity-dependent fluorescent probes[26,28,42,43], this approach will provide further insights into the relationship between cytoskeletal organization and synaptic vesicle dynamics.

*Actin and vesicle mobilization.* The proposed role for actin in restraining the reserve-pool synaptic vesicles would suggest that interfering with actin dynamics might alter vesicle mobility. Consistently, imaging of fluorescently labelled synaptic vesicles in cultured
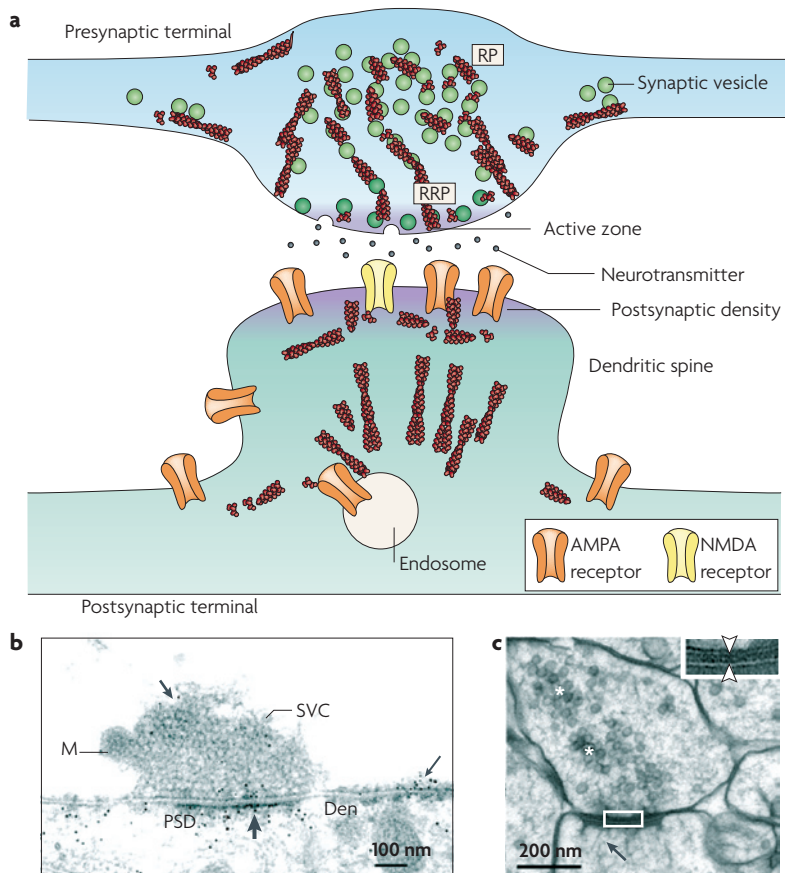
Figure 1 | **Overview of actin at the excitatory synapse. a** | At the presynaptic terminal (top), some synaptic vesicles (those belonging to the readily releasable pool (RRP); dark green) are found docked at the active zone, where they undergo exocytosis to release neurotransmitters. Numerous vesicles that presumably belong to the reserve pool (RP; light green) are located centrally, where they are interlinked to each other by short actin filaments (shown in red) and by synapsin (not shown) in a manner that suggests subgrouping within the cluster (see part **c**). The subgroups are linked to longer filaments that extend from the plasma membrane, some of them from the active zone. At the postsynaptic terminal (bottom), the dendritic spine harbours AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid ) and NMDA (N-methyl-D-aspartate) receptors at the postsynaptic density (PSD), which lies opposite the presynaptic active zone. A sub-membraneous actin network that interlinks scaffolding proteins (not shown) organizes the PSD. Actin filaments are also found longitudinally along the spine axis. Actin is a key component of dendritic spines that defines their shape. Furthermore, actin filaments regulate surface-receptor diffusion and the exo–endocytic trafficking of receptors to the surface, thus determining their abundance on the postsynaptic membrane (a key parameter of postsynaptic efficacy). By altering actin dynamics, activity that elicits synaptic plasticity could remodel both the pre- and the postsynaptic actin scaffold, the organization of synaptic vesicle pools or the organization of the postsynaptic receptors that are supported by the scaffold. In addition, altered actin dynamics could modulate steps of the synaptic vesicle cycle and postsynaptic receptor activity or traffic, which are directly regulated by actin turnover. Overall, these changes would affect the efficacy of synaptic transmission and, thus, of synaptic plasticity. **b** | An electron micrograph of a lamprey reticulospinal synapse, showing actin localization by immunogold labelling. The arrows indicate gold particles and thus indirectly label actin. Actin is present scattered within the synaptic vesicle cluster (SVC) (indicated by intermediate-thickness arrow) and below the PSD (indicated by the thickest arrow) in the dendrite (Den). It is also present at the endocytic zone that surrounds the active zone (indicated by the thinnest arrow). A mitochondrion (M) is found adjacent to the vesicle cluster. **c** | An electron micrograph of a hippocampal synapse from a sample that was subjected to high-pressure freezing. Synaptic vesicles are interlinked by small filaments and are grouped into smaller clusters (indicated by asterisks). Long filaments extend from the PSD (indicated by the arrow). Part **b** reproduced, with permission, from REF. 3 © (2003) Rockefeller University Press. Part **c** reproduced, with permission, from REF. 41 © (2006) Blackwell Science.

hippocampal neurons using methods of fluorescence recovery after photobleaching (FRAP) or fluorescence correlation spectroscopy shows that vesicle mobility is generally low but is increased upon treatment with actin depolymerizing agents[44,45]. However, recent FRAP studies at the presynaptic boutons of frog and mouse neuromuscular junctions (NMJs) have revealed no effect of actin agents on the inherently low vesicle mobility[46,47]. This bolsters the idea that different types of boutons might rely on actin to different extents for sequestering their reserve vesicle pool.

At larval *Drosophila* NMJ boutons, F-actin has been demonstrated to be required for recruiting synaptic vesicles into the readily releasable pool[48,49]. Interestingly, analysis of a *Drosophila* strain with mutant N-ethylmaleimide-sensitive factor (NSF), a protein that is essential for the SNARE-complex disassembly and recycling that drives synaptic vesicle fusion[22,50], has uncovered an unexpected relationship between NSF activity and F-actin[51]. Dominant-negative and loss-of-function *Nsf2* mutants show substantially decreased vesicle mobility and reduced F-actin levels at their NMJ boutons. This is in contrast to the findings in hippocampal boutons, where vesicle mobility is increased upon depolymerization of F-actin. Thus, at the *Drosophila* NMJ, synaptic vesicle mobility probably reflects the NSF-activity-dependent recruitment of vesicles along F-actin to the readily releasable pool, whereas in hippocampal boutons increased vesicle mobility corresponds to the release of restrained vesicles. The positive coupling between the synaptic vesicle fusion–recycling machinery and vesicle mobilization into the readily releasable pool at the *Drosophila* NMJ might serve to moderate vesicle traffic according to the efficacy of the SNARE machinery. That such coordination between the fusion machinery and vesicle mobilization along F-actin could be extended to other systems has been suggested by a study in chromaffin cells[52]. Here, a t-SNARE (syntaxin) and a plus-end-directed actin-dependent motor protein (myosin V) have been shown to interact in a Ca²⁺-dependent manner. Based on the observed interaction, upon neuronal stimulation and elevated intracellular Ca²⁺, vesicles that are trafficked along F-actin by myosin V could be captured at the active zone by binding of myosin V to syntaxin localized on the target membrane. Subsequently, syntaxin could help prime the vesicle upon its assembly into the SNARE complex (FIG. 2).

If actin is generally involved in vesicle recruitment then, similar to the situation at *Drosophila* NMJ boutons, interfering with actin-filament turnover at central synapses should compromise neurotransmitter release, especially under conditions of enhanced release, when vesicle mobilization to the readily releasable pool might become limiting. Indeed, at the large mammalian calyx of Held synapses, actin depolymerization inhibits neurotransmitter release by reducing the recovery from vesicle pool depletion[53]. This has also been observed in the NMJs of snakes[54]. By contrast, studies at small central synapses, such as hippocampal boutons, have not found a consistent contribution of actin to vesicle mobilization[55–58]. This could result from the opposing contributions of actin to

Table 1 | **Synaptic actin-binding proteins**

| Protein | Cellular function | Synaptic function | Refs |
|---|---|---|---|
| α-actinin | Crosslinking and bundling of actin filaments | Regulates the length and density of dendritic spines | 138 |
| ADF/cofilin | Depolymerization and severing of actin filaments | Involved in regulating spine volume and required for the late phase of LTP | 95,103 |
| Cortactin | Stabilization and branching of actin filaments | Knockdown results in depletion and, when overexpressed, in elongation of dendritic spines | 139 |
| EPS8 | Capping barbed ends | Knockout results in increased NMDAR currents, altered NMDAR-dependent actin remodelling and increased resistance to ethanol | 120 |
| Drebrin | Bundling of actin filaments | Increases spine length and induces PSD95 clustering; severely depleted in Alzheimer's disease patients | 140,141 |
| Gelsolin | Severing actin filaments and capping barbed ends | Stabilization of actin filaments during synaptic plasticity. Null mutation results in increased NMDAR currents | 84,121 |
| Myosin II | Contractile activity and cell motility | Control of spine shape and motility | 142 |
| Myosin V | Plus-end-directed actin-based motor | Binds to syntaxin. Null mutation results in aberrant neurotransmitter release in photoreceptors and in climbing-fibre inputs onto Purkinje cells | 52,143,144 |
| Myosin VI | Minus-end-directed actin-based motor | Loss of expression accompanies a decreased number of synapses, shorter spines and deficits in synaptic vesicle recycling and AMPA receptor internalization | 63,87 |
| Profilin II | Promotes actin polymerization | Stabilization and enlargement of dendritic spines during synaptic plasticity | 115,116 |

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; EPS8, epidermal growth factor receptor pathway substrate 8; LTP, long-term potentiation; NMDAR, N-methyl-D-aspartate receptor; PSD95, postsynaptic density protein 95.

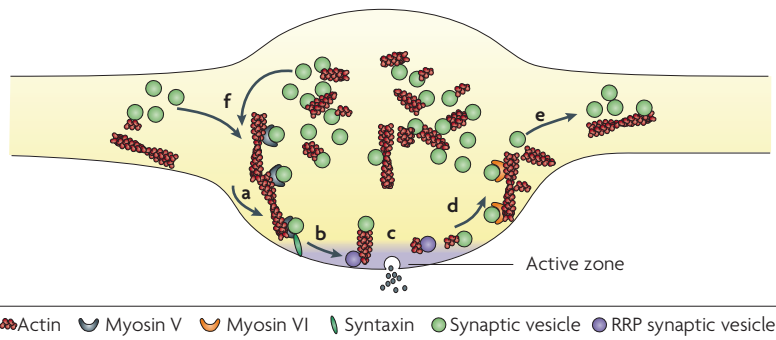**Fluorescence recovery after photobleaching**
(FRAP). A technique that provides measurements of the lateral mobility and dynamics of fluorescently labelled molecules in living cells.

**Neuromuscular junction**
(NMJ). A synapse between a motor neuron axon and the muscle that it innervates.

**SNARE complex**
(Soluble NSF attachment protein (SNAP)-receptor complex). A trimeric complex formed between synaptobrevin on synaptic vesicles and syntaxin and SNAP-25 on the plasma membrane that is essential for membrane fusion.

the sequestration/restraint of the reserve pool vesicles and the mobilization of vesicles from the reserve pool. Whereas the actin dynamics that serve the two distinct functions could be differentially regulated by the endogenous machinery, pharmacological perturbation of F-actin might cancel out both effects. Alternatively, as described above, the relative importance of the two opposing functions of actin could be dependent on the properties of a particular synapse type.

A new study in adult mouse hippocampal slices that has carefully examined synaptic responses to stimulus trains gives fresh insight into the issue[59]. Upon prolonged repetitive stimulation, a delayed transient component of enhanced synaptic release is observed. This delayed component requires F-actin and synapsins I and II. Notably, the delayed component is evident at physiological temperatures but not at room temperature, a condition that was used in many previous experiments that investigated a role for actin[55–58]. Thus, at mature hippocampal synapses, physiological temperature seems to be a crucial factor for uncovering actin-dependent properties of the presynaptic machinery. At low temperatures boutons might switch to a distinct operational mode in which the cytomatrix contributes little.

*Axonal vesicle trafficking and synaptic plasticity.* In addition to regulating vesicle availability by sequestering the reserve pool and facilitating its vesicles' mobilization, actin is proposed to replenish the reserve pool. In the classical view of the synaptic vesicle cycle, the reserve pool is refilled by the endocytic recycling of synaptic vesicles at individual boutons[60,61]. Actin and myosin VI, a minus-end-directed motor protein, seem to facilitate this retrieval[3,62,63]. Accordingly, fluorescence microscopy shows actin localization surrounding the vesicle cluster, and electron micrographs reveal actin filaments extending from periactive zone regions where vesicle endocytosis is thought to occur[3,57,62]. A recent study in cultured hippocampal neurons demonstrated a novel actin-dependent component of synaptic vesicle recycling. Synaptic vesicles that are endocytosed at one bouton are recruited into the functional pool of nearby boutons, where they undergo exocytosis, and the sharing of vesicles between neighbouring boutons requires actin turnover[28]. Whether actin dynamics are crucial for the transport of vesicles along the axon, the release and entry of vesicles to the bouton, or both remains to be established. Interestingly, recycled synaptic vesicles seem to travel along the axon together with vesicles from the reserve pool[28]. Thus, small units of vesicle clusters might form a single mobile entity and, as described above, the columnar subgrouping of vesicle clusters could help in forming the mobile packets[38]. If the spatial segregation of vesicle pools is associated with the vesicles' distance from the active zone, then the vertical columns of vesicle clusters will include not only the recycling-pool but also the reserve-pool vesicles. Columns that extend from the outermost edges of the active zone could preferentially partake in the shedding and acquiring of travelling clusters of vesicles in an actin-dependent manner.

What might be the functional significance of vesicle sharing between boutons? Such a sharing mechanism

**Figure 2 | Actin and the synaptic vesicle cycle. a |** Synaptic vesicle docking. Vesicles are transported along F-actin by myosin V and, upon elevation of intracellular Ca²⁺, binding of myosin V to syntaxin at the active zone facilitates the docking and replenishment of the readily releasable pool (RRP) of vesicles. **b |** Vesicle priming defines entry into the RRP. **c |** Vesicle fusion with the active-zone membrane. Actin in the active zone could regulate vesicle priming and/or fusion to directly affect neurotransmitter release. **d |** Vesicle recycling after neurotransmitter release. Myosin VI might use F-actin in the perimeter of the active zone to guide recycling vesicles back to the centre of the bouton. Some vesicles exit (**e**) or (re-)enter (**f**) the presynaptic terminal in an actin-dependent process that shares the recycling vesicle pool between neighbouring presynaptic boutons.

might allow individual synapses to quickly shrink or expand their vesicle pool sizes to accommodate changes in synaptic strength during synaptic plasticity. Accumulating evidence also points to a requirement for vesicle clusters in the building of new boutons during activity-induced synapse remodelling, a process that also requires actin dynamics. Notably, in cultured hippocampal neurons, brain-derived neurotrophic factor (BDNF), a key protein that is involved in various forms of activity-dependent synaptic plasticity[64,65], promotes the shedding of vesicles from existing synapses; these vesicles are then used to form vesicle clusters at new boutons[66]. BDNF releases vesicles by disrupting the cadherin/β-catenin adhesion complex, which is integral for maintaining the presynaptic vesicle cluster[67]. Whether other synaptic components, such as the active-zone elements, travel together with the released vesicles needs to be determined. More importantly, the precise roles for actin in the sequence of events that leads to the formation of new synapses await further elucidation.

*Actin and presynaptic awakening.* Although the activity-driven induction of functional presynaptic boutons requires actin, it does not always involve new presynaptic assembly. Two groups recently reported an activity- and actin-dependent conversion of presynaptic boutons from silent (mute) non-functional release sites to active boutons that are capable of neurotransmitter release in young hippocampal neurons in culture[68,69]. In immature neurons, many presynaptic boutons (identified by their labelling with synaptic vesicle markers) are not yet functional. Repetitive stimulation converts such non-functional boutons into active boutons and, surprisingly, promoting actin polymerization with jasplakinolide treatment is sufficient to trigger this unsilencing[68]. In a related study that was carried out in young cultured hippocampal neurons, BDNF and

activation of Cdc42 were shown to act upstream of actin polymerization to induce the stimulus-dependent rapid activation of immature presynaptic boutons[69]. Exactly how actin polymerization converts silent boutons into mature boutons that can support robust, stimulus-coupled vesicle recycling is not clear. One possible scenario involves restructuring the presynaptic cytomatrix to organize the vesicle clusters for efficient release and recycling. The requirement for actin in presynaptic functional maturation is in agreement with the strong dependence of the structural integrity of nascent boutons on actin[70]. Nonetheless, the fast speed with which synapses are unsilenced (within minutes[69], unlike the developmental maturation of the presynaptic assembly, which occurs slowly) suggests that all of the necessary components are already in place and that actin polymerization acts as the switch that activates synaptic transmission. It would be of interest to examine the extent to which such a mechanism of enhancement of presynaptic efficacy is preserved in more mature cultures under physiological conditions (for example, at physiological temperatures) and how such a process relates to actin-dependent assembly of new boutons during durable forms of synaptic plasticity[71–73].

In summary, despite recent advances, our knowledge of the presynaptic roles of actin remains fragmentary, and controversies abound. Before we can gain further insights, we need to develop a basic understanding of how actin and its dynamic regulation contribute to the structural organization of the presynaptic terminal, paying particular attention to the unique properties of individual synapse types and the experimental conditions that best preserve the physiological milieu. We can then begin to address how the interrelationship between actin regulation and presynaptic organization is shaped by synaptic activity.

### Actin in postsynaptic mechanisms
*Actin and receptor anchoring.* In postsynaptic terminals, neurotransmitter receptors are clustered at the postsynaptic density (PSD), which lies opposite the active zone, and receptor abundance is a key determinant of synaptic strength[74]. The surface-receptor number at the PSD can be dynamically modified by lateral diffusion of receptors along the membrane, and such a mechanism is targeted during synaptic plasticity to alter the postsynaptic efficacy[75–77]. Actin is highly enriched at the PSD, where it anchors receptors by interacting with a cohort of scaffolding proteins[78]. Accordingly, F-actin depolymerization disperses AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and NMDA (*N*-methyl-D-aspartate) receptors at excitatory synapses[79] and reduces the clusters of gephyrin, a glycine receptor scaffolding protein, at inhibitory synapses[80,81]. The role for actin at the PSD, however, extends beyond the anchoring of receptors. Recent studies illustrate an active contribution of the actin cytoskeleton to the regulation of postsynaptic receptor mobility both in and out of synapses.

Using an elegant single-particle tracking method, researchers examined how the cytoskeleton affects

**Postsynaptic density**
(PSD). The electron-dense region of the postsynaptic membrane that is directly apposed to the active zone. It contains neurotransmitter receptors, scaffold proteins, cell-adhesion molecules and signalling proteins.

the behaviour of surface glycine receptors in spinal cord neurons[82]. In addition to reducing the clusters of receptors and the levels of gephyrin, disruption of F-actin enhanced the rate of exchange of synaptic and extrasynaptic receptors while decreasing receptor 'dwell' time at synapses. In particular, actin depolymerization significantly increased the diffusion of a subset of glycine receptors within synapses (some remained resistant to cytoskeletal disruption). These findings indicate that, rather than being a 'molecular glue', actin contributes to the differential organization of distinct pools of postsynaptic receptors to promote the potential sub-cluster associations within the post-synaptic specialization. A similar function for actin might extend to excitatory synapses that harbour AMPA and NMDA receptors. These two types of glutamate receptors are differentially sensitive to actin depolymerization[79], and their synaptic surface abundance is modulated distinctly by synaptic activity[77,83,84], yet they generally share the same spine.

*Actin and receptor trafficking.* Whereas their differential association with actin might give rise to heterogeneous anchoring of postsynaptic receptors, actin itself could be heterogeneously present in postsynaptic terminals. Previous studies have supported the existence of such heterogeneous populations of actin filaments, which have different arrangements and stabilities in the dendritic spine[41,85,86]. Moreover, a recent detailed study of spine actin dynamics that used actin that had been tagged with a photoactivatable green fluorescent protein (GFP) provided further confirmation of the existence of at least two distinct actin pools[87]. The differences in the turnover rates and structures of these filaments could be tuned to serve different postsynaptic mechanisms. In addition to the lateral traffic of receptors along the membrane surface discussed above, the exo–endocytic cycling of receptors, which mirrors the synaptic vesicle cycle in the presynaptic terminal, is also crucial for regulating synaptic receptor abundance and synaptic strength. For instance, the dynamic sub-plasmalemmal actin network at the postsynaptic scaffold could anchor and organize receptors and have a key role in the plastic remodelling of spine head size. By contrast, stable longitudinal filaments that are present along the core of spines could facilitate exo–endocytic trafficking of surface and internal-pool receptors while providing overall stability to the spine.

Several observations support the existence of different pools of actin that are specialized for receptor anchoring and receptor trafficking. A study in cultured hippocampal neurons showed that actin depolymerization by latrunculin elicits AMPA receptor internalization, similar to the NMDA-receptor-dependent internalization of AMPA receptors that is induced by glutamate application[88]. By contrast, stabilization of F-actin by jasplakinolide prevents AMPA receptor endocytosis. These observations are consistent with a likely requirement for actin filaments in the tethering of synaptic receptors at the PSD. Upon disruption of these filaments, receptors diffuse laterally along the plasma membrane (see above)

and are free to be accessed by the endocytic machinery. The endocytic machinery in turn requires the presence of stable F-actin, because myosin VI, an actin-dependent motor protein, is required for this AMPA receptor endocytosis[89]. These results suggest that the rapid latrunculin-induced depolymerization affects actin filaments that sequester postsynaptic receptors at the surface, while leaving F-actin that is used by myosin VI intact. Thus, it seems that there are at least two distinct populations of F-actin that serve different functions in dendritic spines.

In addition to its role in AMPA receptor endocytosis, analysis of myosin-VI-knockout mice has revealed another feature of myosin VI function at the postsynaptic terminal[89]. Loss of myosin VI or exogenous over-expression of a dominant-negative myosin VI decreased synapse number in the hippocampus. Furthermore, in the knockout mice, the dendritic spines on hippocampal neurons were abnormally short. Therefore, myosin VI has a role in conferring dendritic spine morphology while directing AMPA receptor traffic along F-actin. The mechanism by which loss of myosin VI results in spine loss and altered morphology of the remaining spines remains to be addressed. Nevertheless, its simultaneous involvement in regulating spine morphology and synaptic strength suggests that myosin VI could serve as a target for coordinating synapse structure and function.

*Actin and synaptic plasticity versus morphological spine plasticity.* Dendritic spines can take on a great variety of shapes (for example, stubby, thin, mushroom-like), and their size can range over two orders of magnitude (from $0.01 \, \mu m^3$ to $0.8 \, \mu m^3$) (for a review, see REF. 90). Anatomical studies indicate that there is a positive correlation between the dimensions of the spine head and the PSD area, and between the PSD area and the number of synaptic glutamate receptors. Consequently, spine head size and synaptic efficacy are highly correlated. However, such a relationship cannot be taken for granted as the PSD occupies only a fraction of the spine head — generally the distal tip — and as only a subpopulation of AMPA receptors in the PSD are believed to be close enough to the site of vesicle fusion to respond to neurotransmitter[91–95]. Therefore, simply enlarging dendritic spines or even inserting more AMPA receptors into an enlarged PSD without changing their density does not automatically translate into a stronger synaptic connection. How, then, are morphological and functional synaptic plasticity coordinated?

Actin is the main cytoskeletal component of dendritic spines and has been extensively studied as a determinant of spine morphology and plasticity. It is highly enriched in the spine head, where microfilaments of actin are seen to protrude from the PSD (FIG. 1c). However, in the PSD itself actin is found at relatively low levels[41]. Studies using fluorescently labelled actin have been immensely valuable in elucidating actin dynamics in living neurons. In particular, the use of FRAP and photoactivatable GFP has shown that actin in spines is surprisingly dynamic, with ~85% of it being exchanged within 2 minutes[85,87]. Moreover, the
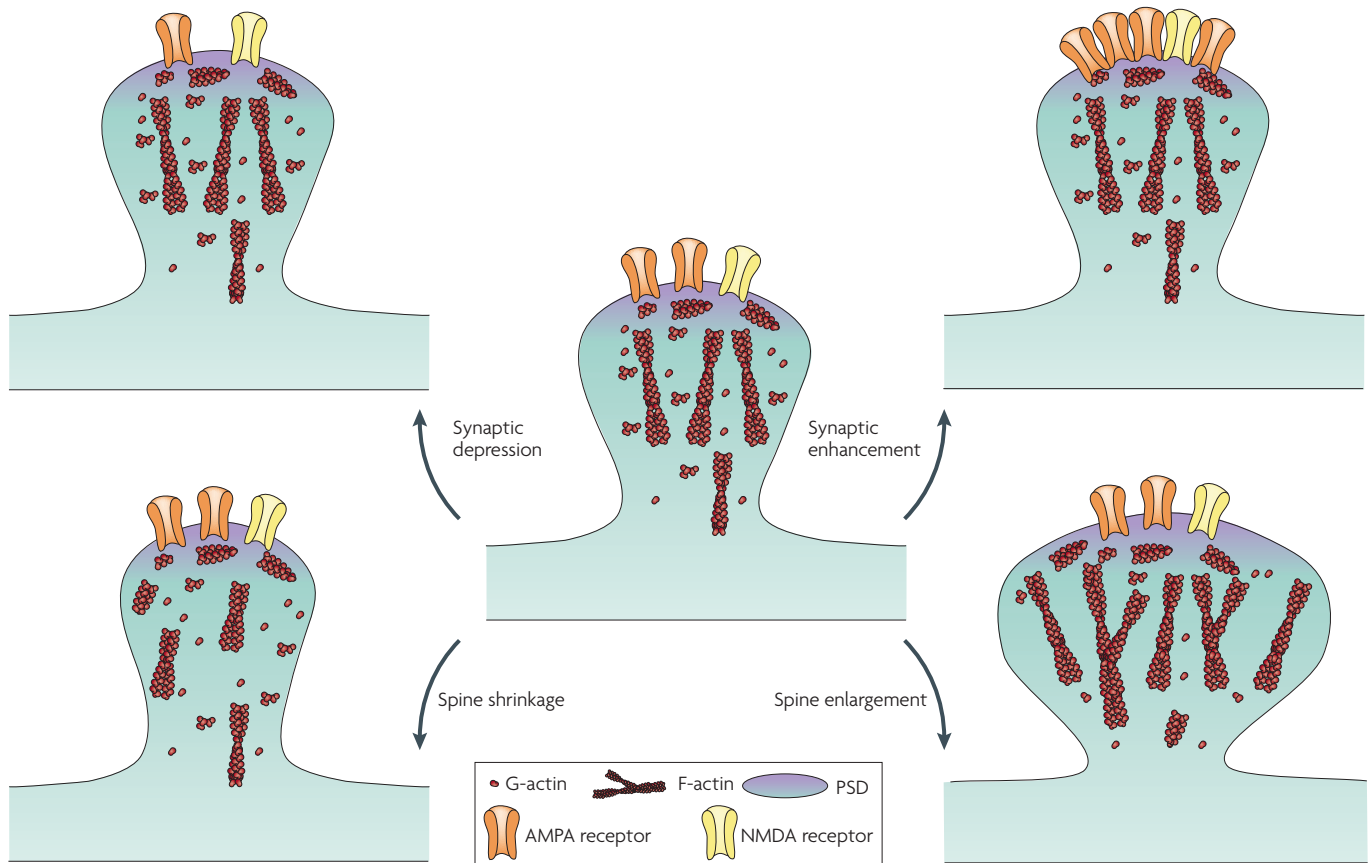
Figure 3 | **A model for structural and functional spine plasticity.** Recent findings demonstrate decoupling between changes in spine size and synaptic strength under certain conditions. Relative to the central illustration, the top left illustration shows synaptic depression (a reduction in the number of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors) occurring independently of spine shrinkage[105,107]; the bottom left illustration shows spine shrinkage that is not associated with altered synaptic currents[107]; the top right illustration shows synaptic potentiation (characterized by the insertion of AMPA receptors) occurring without a change in spine size[110]; and the bottom right illustration shows spine enlargement without potentiation of synaptic transmission[96]. NMDA, *N*-methyl-D-aspartate; PSD, postsynaptic density.

development of a fluorescence resonance energy transfer (FRET)-based technique for actin has allowed optical monitoring of the steady-state balance of F-actin and G-actin at rest and upon neuronal stimulation[96]. In an emerging view, long-term plasticity is associated with a rapid and persistent reorganization of the spine actin cytoskeleton: LTP induction shifts the G-actin/F-actin ratio towards F-actin and increases spine volume, whereas long-term depression (LTD) induction shifts the ratio towards G-actin and results in spine shrinkage[96–98]. Importantly, reorganization of the actin cytoskeleton is integral to the expression of synaptic plasticity, because actin-depolymerizing agents block both structural and functional LTP[97,99–101]. Upon inducing LTP, actin polymerization could endow enlarged dendritic spines with a stable scaffold that has an increased capacity for anchoring structural and signalling molecules, such as neurotransmitter receptors[79,88,102], Ca$^{2+}$/calmodulin-dependent protein kinase II (CaMKII)[96,103] and the immediate-early-gene product activity-regulated cytoskeleton-associated protein (ARC; also known as Arg3.1)[104], which are required for stably enhancing the

synaptic connection. Nevertheless, actin-polymerizing agents, despite being able to enlarge spine heads, do not potentiate synaptic transmission on their own[88,96,105]. Thus, actin polymerization in spines is required but not sufficient for functional LTP. To what extent, then, are actin polymerization and changes in synaptic strength coordinated (FIG. 3)? Do the signalling pathways that trigger structural and functional plasticity overlap or do they diverge at some point?

As for the induction of LTP or LTD, structural rearrangements of actin filaments in dendritic spines are dependent on NMDA receptor activation[97,106]. However, recent studies indicate that the subsequent signalling pathways diverge considerably and, in some instances, structural and functional changes are dissociable. For example, whereas LTD is dependent on both the phosphatase calcineurin and protein phosphatase 1 (PP1), only calcineurin is necessary for the spine shrinkage that is associated with LTD. By contrast, the actin depolymerizing protein ADF/cofilin is specifically required for spine shrinkage but not involved in LTD[84,105,106] (BOX 3). Thus, synaptic depression can be uncoupled

**Fluorescence resonance energy transfer**
(FRET). A spectroscopic technique that uses the direct transfer of energy from one fluorophore to another to measure the distance between the fluorophores.

**Long-term depression**
(LTD). Long-lasting (hours to days) weakening of synaptic communication between two neurons, generally as a consequence of persistent weak neuronal activity. Along with LTP, LTD is intensively studied as a cellular model for learning and memory.

## Box 3 | Actin signalling in dendritic spines

Synaptic activity regulates actin dynamics and spine morphology through multiple signalling pathways. Key regulators of actin polymerization are the GTPases of the Rho family and serine/threonine kinases, which ultimately target actin-binding proteins (TABLE 1). In dendritic spines, opening of the $Ca^{2+}$-permeable NMDA (N-methyl-D-aspartate) receptors by repetitive synaptic stimulation leads to the elevation of intracellular $Ca^{2+}$ and the subsequent activation and translocation of $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII) to the postsynaptic density[142]. Once activated, CaMKII can phosphorylate and activate multiple downstream signalling targets. In addition, CaMKII, which is present in dendritic spines at high levels, has been proposed to also function as a structural molecule that can bind to and bundle F-actin through its β subunit[103]. Among the intracellular signalling events that are relevant for actin dynamics, of particular interest is the CaMKII-mediated activation of the calmodulin-dependent kinase kinase (CaMKK)–CaMKI signalling pathway (the components of this pathway are shown in the figure). These kinases form a multimolecular complex with the guanine-nucleotide exchange factor (GEF) βPIX. CaMKI-mediated phosphorylation of βPIX increases its GEF activity, resulting in the activation of the small GTPase Rac1 (REF. 143). βPIX also interacts with p21-activated kinase 1



(Pak1)[144], which can be activated by Rac1-triggered autophosphorylation. In turn, Pak1 phosphorylates several downstream signalling molecules that directly modulate F-actin. For instance, Pak1-mediated phosphorylation activates LIM kinase (LIMK). The only known targets of LIMK are the members of the ADF/cofilin family (actin-depolymerizing proteins), which are inactivated upon LIMK-mediated phosphorylation[145]. Alternatively, Rac1 can be activated by a second GEF, kalirin 7, which interacts with and is phosphorylated by CaMKII[146]. The activation of LIMK can also be promoted by the small GTPase RhoA, through RhoA-specific kinase (ROCK)[147]. Interestingly, RhoA interacts with NMDA receptors, ROCK and the actin-polymerizing protein profilin II in an activity-dependent manner[148]. Also, α-actinin, which is involved in crosslinking and bundling actin filaments, associates with NMDA receptors in an activity-dependent manner[149]. When NMDA receptors open in response to stimuli delivered at low frequency to induce long-term depression, the effects on actin dynamics are opposite to those of CaMKII-initiated pathways, as ADF/cofilin family proteins are turned on following the activation of the phosphatase calcineurin[106]. In the figure, actin-binding proteins are shown in red, Rho GTPases are shown in blue, kinases are shown in pale orange, phosphatases are shown in green and GEFs are shown in dark orange.
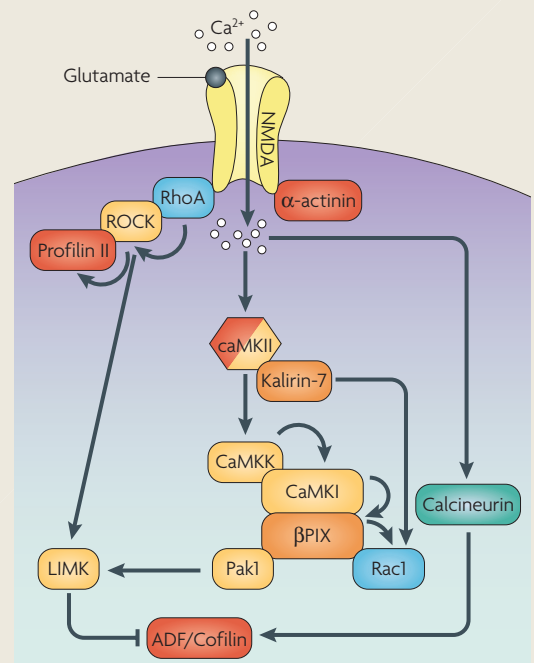
from spine shrinkage. Furthermore, in a surprising recent finding, durable reductions of synaptic AMPA receptors induced by NMDA-receptor-independent mechanisms occur without an appreciable change in spine size. For example, LTD of the parallel fibre/Purkinje cell synapse in the cerebellum is not associated with shrinkage of dendritic spines. Conversely, stimuli that produce a global retraction of Purkinje cell spines are not accompanied by changes in synaptic currents[107]. Similarly, in hippocampal pyramidal neurons, constitutive trafficking of AMPA receptors, insulin-induced internalization of AMPA receptors and lateral movement of AMPA receptors to extrasynaptic sites all occur independently of spine shape changes[105]. Spine shrinkage is therefore not always associated with the reduction of synaptic AMPA receptors, but it seems to be specific to NMDA-receptor-dependent LTD.

In the case of LTP also, it is becoming clear that enhanced synaptic transmission and structural changes do not always go together. Using a chemical protocol to induce LTP, researchers showed that spine enlargement

precedes synaptic surface insertion of the AMPA receptor subunit GluR1 by ~4 minutes[108]. Thus, delivery of AMPA receptors to the synapse is apparently not necessary for the initial phase of spine enlargement. Moreover, as for LTD, synaptic strength and spine size can be uncoupled. For example, overexpression of the synaptic scaffolding protein postsynaptic density protein 95 (PSD95) can drive GluR1 into synapses and increase synaptic strength[109], yet spine size remains unchanged[110]. Therefore, synaptic insertion of GluR1 per se is not sufficient to drive spine structural plasticity. Interestingly, when LTP is induced in PSD95-expressing neurons, dendritic spines get bigger without a concomitant increase in synaptic strength[110,111], presumably because PSD95-dependent GluR1 insertion occludes LTP. These findings suggest that spine enlargement is caused by a signalling pathway that is specifically activated during LTP induction and that probably involves NMDA receptor activation, ADF/cofilin and actin reorganization[97]. If the events that trigger changes in spine morphology and LTP are distinct, how are they balanced so that in normal

conditions stronger connections always occur on bigger spines? A clue to this question is provided by the finding that LTP induction does not cause spine enlargement when the translocation of GluR1 carboxy-terminal tails into the PSD is prevented[108]. Thus, a simple and elegant mechanism that neurons might exploit to keep synaptic strength and spine size in check during LTP is to use the same AMPA receptors that are delivered to the synapse to both increase synaptic strength and stabilize spine structure.

### From synapse regulation to behaviour

Alterations in actin dynamics, particularly in dendritic spines, can have significant consequences for cognition, as exemplified by the various neurological disorders that are associated with mutations or other changes in synaptic actin-binding or signalling proteins[112–116]. In addition to these well-established links between abnormal spine morphology and mental retardation or dementia, recent studies reveal a surprising contribution of actin dynamics to complex behaviour.

Profilins are G-actin-binding proteins that facilitate actin polymerization, and they have been suggested to have a primarily postsynaptic function in stabilizing dendritic spines during synaptic plasticity and fear learning[117,118]. Whereas profilin 1 is ubiquitously expressed, profilin 2 is expressed at higher levels in the brain than in other tissues. In profilin-2-knockout mice, the gross anatomical structure of the brain is normal, as are long-term synaptic plasticity and learning behaviour, presumably because of compensation by profilin 1[119]. However, in cortical synaptosomes from profilin-2-knockout mice, depolarization-induced synaptic actin polymerization is impaired, and in striatal neurons neurotransmitter release is increased without a significant change in postsynaptic responses. The observed presynaptic defect resulting from loss of profilin 2 is consistent with the unique association of profilin 2 with the presynaptic cytomatrix[119]. Moreover, in accord with increased excitation, profilin-2-knockout mice show hyperstimulation of the striatum accompanied by hyperactivity and increased novelty-seeking behaviour. Thus, dysfunction of presynaptic actin turnover seems to have behavioural consequences that are independent of its well-appreciated contribution to dendritic spine abnormalities, and subtle changes in neurotransmitter release might have substantial effects on complex behaviour.

In another study, researchers investigated the function of EPS8, which regulates actin dynamics by capping the barbed end of F-actin or activating the small GTPase Rac through growth factor signalling[120,121]. Like profilin-2-knockout mice, EPS8-knockout mice are viable and fertile and show no obvious phenotype, presumably owing to functional compensation by other family members (EPS8L1, EPS8L2 and EPS8L3) that are expressed in most tissues. A careful behavioural analysis of EPS8-knockout mice, however, has revealed their remarkably elevated tolerance for ethanol[122]. Compared with wild-type mice, EPS8-knockout mice can withstand high levels of ethanol before their motor coordination is compromised; moreover, they show increased

voluntary consumption of alcohol. What might be the cellular basis for the observed behavioural change? Contrary to expectations, EPS8 is not appreciably expressed in the brain areas that are associated with the mesolimbic reward circuit that has been implicated in alcohol-related behaviour; rather, it is highly expressed in scattered neurons in the hippocampus, the amygdala and the prefrontal cortex. EPS8 is also abundantly expressed in the cerebellum, consistent with the altered motor coordination that is observed in the knockout mice. In particular, in cerebellar granule neurons, which express EPS8 but not any of the other EPS family members, loss of EPS8 results in increased NMDA receptor currents and F-actin stability. Furthermore, paralleling the behavioural effects, EPS8-knockout cerebellar granule neurons are more resistant to ethanol-induced actin depolymerization and do not show a component of ethanol-dependent NMDA receptor modulation. Biochemical experiments further support the link between NMDA-receptor-dependent signalling and altered ethanol tolerance. Yet, because EPS8 is also abundant presynaptically, potential changes in the regulation of neurotransmitter release could also contribute to the altered behaviour; this scenario remains to be studied. Interestingly, similarly to EPS8-knockout mice, gelsolin-knockout mice also show stabilized actin filaments and increased NMDA receptor currents, at least in cultured hippocampal neurons[123]. It would be of interest to examine the cellular and behavioural effects of ethanol in gelsolin-knockout mice to see to what extent one can generalize the role of actin dynamics in responses to ethanol.

### Concluding remarks

In the couple of decades since actin was first reported to be present at synapses, there has been tremendous progress in understanding the molecular mechanisms that underlie synapse regulation. On the presynaptic side, this includes the orchestration of the synaptic vesicle cycle, which is essential for neurotransmitter release. On the postsynaptic side, it includes the organization and trafficking of postsynaptic receptors and their scaffolds and the relationship of these proteins to synaptic strength and the morphology of the spines that house them. Yet, much remains to be understood about how all the parts fit together: how the dynamic changes in the presynaptic release machinery are coupled to the similarly dynamic behaviour of the postsynaptic assembly during synaptic plasticity, and how such functional plasticity is coordinated with structural plasticity. Moreover, the plasticity of intracellular organization might not always be reflected in the overall visible morphological changes, even if it involves the cytoskeleton. For instance, recent studies have uncovered a surprising dissociation between changes in spine size and synaptic strength, and it remains to be seen whether and how non-spiny synapses display structural reorganization in association with functional plasticity.

Given that actin is the major cytoskeletal synaptic component, it might hold the key to unravelling the coordination of synapse structure and function. It is

# REVIEWS

becoming clear that actin is engaged in multiple aspects of synapse regulation, and this might be accomplished, at least in part, through divergent signalling pathways that target different pools of synaptic actin. Actin is also involved in other aspects of neuronal cell function; therefore, we need to develop novel tools to examine actin regulation exclusively at synapses. One strategy might be to use FRET-based probes, similar to those that have been used for small GTPases[124,125], in conjunction with manipulation of functional synaptic activity to visualize the spatiotemporal activation of actin regulatory proteins. Photoactivatable GFP probes, as recently reported for examining actin pools at single spines[87], could also be useful for gaining insights into the dynamics of actin and its upstream regulators and downstream targets, as are genetic model systems that are amenable to the identification of mutations that impact actin regulation in association with synapse dysfunction. Synapses will continue to provide a rich arena for neurobiologists and cell biologists alike in their pursuit of understanding how the fundamental element of communication in the nervous system operates.

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## DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
α-actinin | βPIX | ADF | ARC | BDNF | CaMKI | CaMKK | cofilin | EPS8 | filamin | gelsolin | gephyrin | kalirin 7 | LIMK | myosin V | myosin VI | NSF | Pak1 | profilin 1 | profilin 2 | PSD95 | Rac1 | RhoA | ROCK | spectrin | synapsin | tropomodulin

## FURTHER INFORMATION
Yukiko Goda's homepage:
www.ucl.ac.uk/LMCB/research-groups/goda.htm

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