Transmitting on actin: synaptic control of dendritic architecture

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Summary

Excitatory synaptic transmission in the central nervous system mainly takes place at dendritic spines, highly motile protrusions on the dendritic surface. Depending on the stimuli received, dendritic spines undergo rapid actin-based changes in their morphology. This plasticity appears to involve signaling through numerous proteins that control the organization of the actin cytoskeleton (actin regulators). At least in part, recruitment and activation of these depends on neurotransmitter receptors at the post-synapse, which directly link neurotransmission to changes in dendritic spine architecture. However, other, non-neurotransmitter-receptors present at dendritic spines also participate. It is likely that several receptor types can control the activity of a single actin-regulatory pathway and it is the complex integration of numerous signals that determines the overall architecture of a dendritic spine.

Key words: Dendritic spine, Actin cytoskeleton, Rho GTPase, Plasticity, Excitatory synapse, Neurotransmitter receptor

Introduction

Accurate functioning of the brain can be seen as the consequence of both local and distant effects of neurotransmitter (NT) release at synapses. Distant effects include changes in gene expression, as well as mRNA and protein synthesis and transport. How such effects are produced and their relation to nerve circuit maturation, learning and memory are the subject of numerous reviews (Gulledge et al., 2005; Kandel, 2001; Kelleher, 3rd et al., 2004). Local effects of synaptic transmission, by contrast, comprise changes that mainly but not exclusively take place at the post-synapse and, from a rather simplistic point of view, can be divided into immediate and mediate changes. Immediate changes are those occurring within milliseconds of NT release, modulating the membrane potential that in the end is responsible for the propagation of neurotransmission. Excellent reviews on this matter can be found elsewhere (Lai and Jan, 2006; Vogels et al., 2005). Mediate changes occur within seconds of NT release, one consequence being a change in the morphology of dendritic spines, the membranous protrusions on the dendritic surface where excitatory synaptic activity most commonly occurs (Gray, 1959).

Filamentous actin represents the major cytoskeletal component of dendritic spines (Cohen et al., 1985; Fifkova and Delay, 1982). It therefore appears obvious that changes in spine shape, size and number are determined by local actin dynamics. In fact, observations made in living neurons showed that spines are capable of rapid (within seconds) actin-based morphological plasticity (Fischer et al., 2000; Matus et al., 1981). Our current view is that this morphological plasticity reflects changes in synaptic strength and facilitates a neuron's response to alterations in neural activity (Lippman and Dunaevsky, 2005). Moreover, the actin-dependent reorganization of spine morphology is believed to play a key role in the processes underlying memory and learning in the mammalian brain (Kasai et al., 2003). Support for this comes from studies showing that the activity levels of glutamate receptors at the excitatory postsynaptic density (PSD) influence local actin dynamics and, therefore, spine morphology (Fischer et al., 2000; Halpain et al., 1998; Matsuzaki et al., 2004). Additionally, depolymerization of F-actin reduces the number of glutamate receptors at the excitatory post-synaptic density (Allison et al., 1998). Finally, repetitive quantal release of glutamate at dendritic spines on hippocampal neurons induces selective enlargement of the stimulated spine, which is accompanied by increased AMPA-receptor-mediated currents and can be prevented by latrunculin A, an inhibitor of actin polymerization (Matsuzaki et al., 2004). This demonstrates that the interplay between synaptic activity and local actin dynamics is bidirectional because neurotransmission-dependent modulation of spine actin dynamics is required for alterations in synaptic strength.

NTs are thought to regulate actin dynamics at spines by controlling the recruitment and activation of actin regulators at the excitatory PSD through NT receptors (NTRs). Rho-family GTPases such as RhoA, Rac1 and Cdc42 are the best studied among these regulators (Hall, 1998). These are activated by guanine-nucleotide-exchange factors (GEFs) that stimulate exchange of GDP for GTP, thus rendering the protein active. GTPase-activating proteins (GAPs), by contrast, inhibit them by accelerating GTP hydrolysis. The Rho family proteins are responsible for a wide variety of cytoskeleton-mediated events, including dendrite development and spine morphogenesis (Luo, 2002). In mature neurons, expression of constitutively

active RhoA decreases dendritic spine density and length (Nakayama and Luo, 2000; Pilpel and Segal, 2004; Tashiro et al., 2000). Constitutively active Rac1 leads to a reduction of spine size together with an increase in spine density (Luo et al., 1996; Nakayama and Luo, 2000; Pilpel and Segal, 2004; Tashiro et al., 2000). The effect of activated Cdc42 is less clear (Tashiro et al., 2000); however, different Cdc42 GEFs and GAPs, as well as downstream effectors of Cdc42, have been implicated in the regulation of dendritic spine morphology.

Additionally, other mechanisms affect spine morphology by directly modulating the activity of several actin regulators in response to NT release. One major example of this is Ca^{2+}, which, aside from its role as a charge carrier, modulates the activity of various postsynaptic proteins, including Arp2/3, LiMK, spinophilin, myosin light chain kinase (MLCK) and myosin, some of which are also subject to Rho GTPase signaling (Ethell and Pasquale, 2005; Sabatini et al., 2001). Below, we highlight recent findings that shed light on how Rho GTPase-meditated signaling and other actin-regulatory mechanisms link NTRs in the post-synaptic membrane with the underlying actin.

Organization of the excitatory post-synapse

Excitatory synapses contain clusters of a minimum of two ionotropic glutamate receptor types in the postsynaptic membrane, of which α-amino-3-hydroxy-5-methyl-4-isoazolepropionate (AMPA)-type receptors (AMPARs) and N-methyl-D-aspartate (NMDA)-type glutamate receptors (NMDARs) are the most prominent (Nusser, 2000). Both receptors are ligand-gated ion channels but have distinct structural and, therefore, functional properties. A characteristic of NMDARs is their direct anchorage to the PSD and thus to the actin cytoskeleton. Every receptor comprises heteromultimers containing at least one NR1 subunit and an unknown number of NR2 subunits. Each subunit extends its long C-terminus into the cytoplasm, acting as an attachment site for proteins of the PSD.

Membrane-associated guanylate kinases (MAGUKs), e.g. PSD95, are a prominent part of the PSD scaffold (Garner et al., 2000; Kennedy, 2000). PSD-95 localizes to membranes via palmitoylation and binds to NMDARs through two of its N-terminal PDZ domains. MAGUKs contain repetitive PDZ-domain motifs, which allow the formation of homo-multimers and therefore contribute to the clustering of NMDARs (Topinka and Bredt, 1998). Additionally, MAGUKs interact with a number of multidomain scaffold proteins, e.g. A-kinase anchoring protein (AKAP) 79/150 and membrane associated protein/guanylate kinase associated proteins (SAPAP/GKAPs). AKAP is targeted to dendritic spines by its N-terminal basic region, which binds phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2], F-actin and the actin-linked cadherin adhesion molecules. AKAP recruits cAMP-dependent protein kinase (PKA) and calcineurin/protein phosphatase 2B (CN/PP2B) to the PSD (Bauman et al., 2004; Smith et al., 2006). Activity and recruitment of PKA and CN/PP2B is modulated in an NMDA-dependent manner and it is the balance between the two proteins that regulates many phosphorylation-dependent events that influence synaptic plasticity (e.g. activation/inhibition of actin-regulators as well as excitability and anchorage/endocytosis of receptors at the synapse).

SAPAP/GKAPs connect the PSD complex to the cytoskeleton by recruiting ProSAP/Shank (Ehlers, 1999; Naisbitt et al., 1999; Tu et al., 1999). Shank proteins possess numerous protein-protein interaction domains, including N-terminal ankyrin (ANK) repeats, a Src homology 3 (SH3) domain, a PDZ domain, as well as proline-rich clusters (PRC) and a specific cortactin-binding domain, thus interlinking the postsynaptic scaffold with a variety of signaling molecules.

How ionotropic glutamate receptors 'talk' to actin regulators

A conspicuous actin-binding protein in the central nervous system is cofilin. Cofilin regulates actin dynamics by severing filamentous actin and by increasing the dissociation rate of actin subunits from the pointed end of filaments (Bamburg et al., 1999; DesMarais et al., 2005). Its activity depends on its phosphorylation state, which is regulated by various kinases (e.g. Ca^{2+}/calmodulin-dependent kinase II, CaMKII) and phosphatases (e.g. calcineurin and PP1), and the activity of Rho GTPases. The latter act via p21-activated kinase (PAK). Interestingly, PAK is recruited to excitatory synapses through formation of a complex with PIX (a Rac GEF) and Shank (Park et al., 2003). PAK activation requires autophosphorylation, which can be triggered by either active Rac1 or Cdc42 (Bokoch et al., 1998). Active PAK phosphorylates and activates LIM kinase, which then phosphorylates cofilin at serine 3, which results in its inactivation (Edwards et al., 1999; Manser et al., 1994).

Within spines, cofilin is thought to be critically involved in the architectural, and thus functional, changes triggered by stimuli leading to stable modifications in synaptic responses, e.g. long-term potentiation (LTP) and long-term depression (LTD) (Fukazawa et al., 2003; Racz and Weinberg, 2006; Zhou et al., 2004). By examining the F-actin content in synaptic layers of the dentate gyrus of un-anesthetized rats, Fukazawa et al. found that the amount of F-actin in dendritic spines increases during late-LTP (L-LTP), possibly because of NMDAR signaling. This is accompanied by increased phosphorylation, and thus inactivation, of cofilin (Fukazawa et al., 2003). Zhou et al. observed, that in hippocampal slices, low-frequency-stimulation-induced LTD triggers shrinkage of dendritic spines through activation of NMDARs and calcineurin. Loading of the cells with a synthetic phosphorylated cofilin peptide, which competes with endogenous phosphorylated cofilin for protein phosphatases, reduced endogenous cofilin activity and prevented LTD-induced spine shrinkage (Zhou et al., 2004). These studies, together with other evidence (e.g. Meng et al., 2004; Morishita et al., 2005), indicate that the activity of cofilin is required for the stimulus-dependent reorganization of the postsynaptic actin cytoskeleton triggered by stimuli such as NMDA. This could lead to phosphorylation of cofilin via the PIX-Rac1-PAK-LIMK pathway and also to its dephosphorylation via calcineurin as a consequence of increased Ca^{2+} levels (Fig. 1).

It thus appears that cofilin-controlled actin dynamics in spines tightly depend on receptor signaling intensity, which in the end balances the phosphorylating and dephosphorylating activities at the PSD. An additional level of complexity comes from the fact that the binding of cofilin to actin induces changes in the conformation of actin filaments, which can affect the actin-binding properties of other proteins. The actin affinity of the actin stabilizer drebrin (see below), for instance,

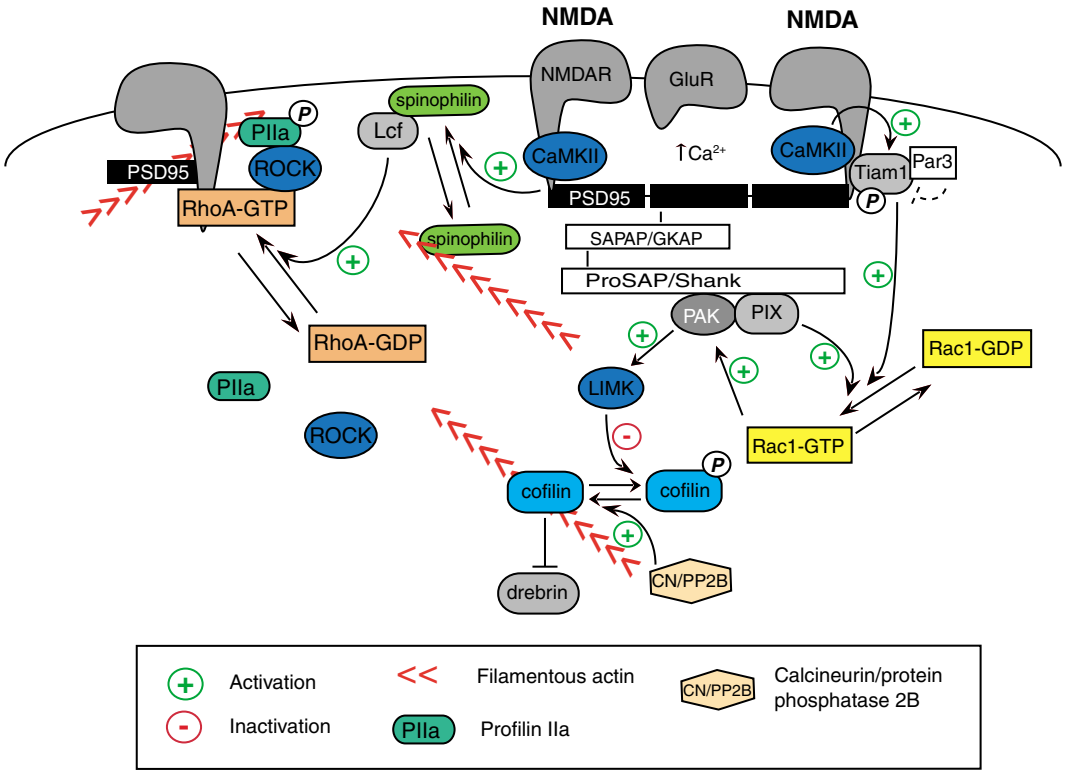

Fig. 1. Putative model of actin-regulatory pathways controlled by ionotropic glutamate receptors. Active RhoA interacts with NMDARs at the excitatory PSD and recruits and activates the ROCK/PII complex, thus stabilizing actin. High levels of Ca^{2+} induce CaMKII-dependent phosphorylation of spinophilin, detaching it from actin and recruiting it to the membrane, where it interacts with Lcf, which in turn activates RhoA. The actin-severing activity of cofilin is controlled by different kinases and phosphatases. LIMK, for example, is a negative-regulator of cofilin activity. Activation of LIMK can occur in a Rac1-dependent manner through its downstream effector PAK. NMDA stimuli could trigger an increase in local Rac1-activity levels through the Rac1-GEFs PIX and Tiam1, consequently increasing the activity of cofilin, which eventually results in higher actin-turnover rates.

is reduced by active, actin-bound cofilin and triggers its detachment from these sites (McGough et al., 1997; Zhao et al., 2006). This broadens the mechanistic possibilities by which cofilin phosphorylation within spines can regulate spine size in response to NT action: directly through actin filament severing and indirectly through regulation of the activity of other actin-binding proteins.

The second major actin-regulatory protein with a clearly identified role in the control of spine architecture by NMDARs is profilin. Profilin is an actin-monomer-sequestering protein that binds to G-actin, increases ADP-for-ATP exchange and thus promotes actin polymerization. Experiments performed in hippocampal neurons expressing profilin-II–GFP gave the first indications of an NTR-mediated profilin regulatory pathway. Electrical or chemical stimulation of these cells induces the recruitment of profilin-II–GFP to dendritic spines in an NMDAR-dependent manner (Ackermann and Matus, 2003). A series of biochemical and immunofluorescence analyses revealed that profilin is recruited to excitatory synapses by its upstream regulators RhoA and Rho-kinase (ROCK). A pool of endogenous active RhoA directly interacts with NMDARs, thus recruiting the ROCK-profilin complex to the PSD (Schubert et al., 2006). Excessive activation of the receptor detaches the Rho GTPase from the receptors, concomitantly inactivating the protein and its downstream players ROCK and profilin, which eventually results in depolymerization of the spine actin cytoskeleton (Schubert et al., 2006). Although these data await confirmation by in vivo experiments, they strongly suggest that neurotransmission events modulate RhoA activity levels in dendritic spines, which consequently alters local actin stability, possibly in a profilin-mediated manner.

Recent work has shown that other actin regulators might modulate the activity of RhoA and thus its effect on spine actin. Ryan et al. showed that the Rho GEF Lcf interacts with the actin-binding protein spinophilin (Ryan et al., 2005). Spinophilin is localized to actin filaments by its actin-binding domain and has crosslinking activities (Grossman et al., 2002; Satoh et al., 1998). The affinity of spinophilin for F-actin is regulated by phosphorylation of the actin-binding domain, which can be mediated by PKA and CaMKII (Grossman et al., 2004; Hsieh-Wilson et al., 2003). In neurons, Ca^{2+}-dependent phosphorylation by CaMKII reduces the affinity of spinophilin for actin and targets the protein to synaptic membrane fractions (Grossman et al., 2004). Expression studies in hippocampal neurons showed that Lcf localizes to the cell body and the dendritic shaft, where it associates with microtubules. Stimulation of these neurons with high concentrations of KCl, NMDA or glutamate, or field stimulation rapidly induces translocation of Lfc into dendritic spines. This movement of Lfc depends on NMDAR activation and NMDAR-mediated

Ca^{2+} influx, and results in colocalization of spinophilin and Lcf (Ryan et al., 2005). Spinophilin-bound Lcf in spines could then activate the RhoA-ROCK–profilin pathway, thus facilitating spine morphogenesis (Fig. 1).

Another prominent signaling mechanism is via CaMKII (Erondu and Kennedy, 1985; Kennedy et al., 1983). CaMKII is most abundant in the PSD, where it is reversibly recruited to the C-terminal domain of the NR2b subunit of NMDARs, depending on its phosphorylation state, which is determined by the levels of Ca^{2+} (Leonard et al., 1999; Shen and Meyer, 1999; Shen et al., 2000; Strack et al., 1997a; Strack et al., 1997b; Yoshimura and Yamauchi, 1997). CaMKII interacts with and/or phosphorylates a number of synaptic proteins, e.g. the NR2 subunit of NMDARs (Omkumar et al., 1996) and many other PSD components, including cytoskeletal elements (Yoshimura et al., 2000; Yoshimura et al., 2002). Tiam1, for instance, a Rac-GEF important for neuronal polarization, localizes to dendritic spines, where it directly interacts with NMDARs at the excitatory PSD (Tolias et al., 2005). Stimulation of NMDARs leads to Ca^{2+}-dependent phosphorylation and activation of Tiam1, which in turn activates Rac1 (Fig. 1) (Fleming and Busse, 1999; Tolias et al., 2005).

Zhang et al. have recently discovered that the product of partitioning-defective gene 3 (PAR-3) (Zhang et al., 2005), which is important for diverse cytoskeleton-mediated cell polarization processes (Munro, 2006), localizes to excitatory post-synapses and directly interacts with Tiam1 (Zhang and Macara, 2006). Knocking down Par-3 by RNA interference (RNAi) in hippocampal neurons causes the formation of filopodia-like structures, similarly to expression of constitutively active Rac1. Knocking down Tiam1 as well rescues these effects on spine morphology. Co-expressing dominant negative Rac1 yields a similar result (Zhang and Macara, 2006). Par-3 thus appears to be a negative regulator of Tiam-1 activity, sequestering the protein and restricting its availability for Rac1 activation. However, experiments performed in other cell types support contradictory mechanisms for Par3-Tiam1-mediated modulation of Rac1 activity, suggesting there are cell-type-specific effects of Par-3 on Tiam1 activity (Munro, 2006).

Metabotropic glutamate receptors

Metabotropic glutamate receptors (mGluRs) are G-protein-coupled receptors and can be divided into three subgroups (Knopfel and Grandes, 2002). Group I mGluRs consist of mGluR1 and mGluR5. Activation of these typically triggers the release of intracellular Ca^{2+} and activation of PKC via phospholipase C (PLC), inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3], and 1,2-diacylglycerol (DAG) (Berridge et al., 1984; Rebecchi and Pentyala, 2000). Group II (mGluR2 and mGluR3) and group III (mGluR4 to mGluR8) mGluRs inhibit adenylate cyclase, which leads to a reduction of cAMP levels (Knopfel and Grandes, 2002).

Group I mGluRs reside at the outer rim of the excitatory synapse and are connected to NMDAR-linked PSD complexes through Homer-proteins that interact with PSD95 via the linker proteins Shank and GKAP (Mateos et al., 2000; Naisbitt et al., 2000; Tu et al., 1999). Homer1c, for example, interacts with type I mGluRs via an enabled/VASP homology 1 (EVH1) domain and a leucine zipper motif and is involved in recruiting Shank and F-actin to the PSD (Kato et al., 1998; Tadokoro et al., 1999; Usui et al., 2003; Xiao et al., 1998). Interestingly, type I mGluRs are connected to the synaptic actin cytoskeleton in an activity-dependent manner. In cerebellar granule cells mGluR1 recruits cupidin, a Homer2 isoform, which in turn directly interacts with the actin stabilizer drebrin A, thus associating with filamentous actin (Shiraishi et al., 2003a; Shiraishi et al., 2003b). Activation of the receptors triggers de-clustering of cupidin in spines and dispersal of the protein within the cell (Shiraishi et al., 2003b). Other isoforms of the Homer/vesl family (Homer1b/c, cupidin/Homer 2a/b, Homer 3a/b) behave similarly in hippocampal neurons (Shiraishi et al., 2003b).

Immunoprecipitation experiments using synaptic fractions revealed that active RhoA also directly interacts with mGluR1. Stimulation of the receptor increases the amount of associated RhoA, illustrating that metabotropic glutamate receptors autonomously recruit actin-regulators to the PSD (Schubert et al., 2006). Moreover these data provide evidence that mGluRs can integrate Ins(1,4,5)P_3-dependent Ca^{2+}-signaling and actin remodeling at the PSD. Increased Ca^{2+} levels trigger activation or inhibition of the various regulatory proteins discussed above, thereby generating the actin dynamics required for morphological changes and functional plasticity.

Other receptor types

NTRs are not the only receptors to regulate actin dynamics in dendritic spines. Receptor tyrosine kinases (RTK), such as members of the Trk and ephrin families of receptors, as well as cell-cell adhesion/recognition molecules (e.g. cadherins), also play a part in the morphological plasticity at the synapse.

How RTKs 'talk' to actin in spines

Stimulation of neurons with brain-derived neurotrophic factor (BDNF) induces Trk-triggered tyrosine phosphorylation and recruitment of cortactin to spines (Iki et al., 2005). Cortactin is an actin-binding protein that, upon binding to F-actin, forms a stable complex with the Arp2/3 complex and neuronal Wiskott-Aldrich syndrome protein (N-WASP) (Lua and Low, 2005). Cortactin and the G-actin-monomer-binding protein N-WASP simultaneously modulate the actin-polymerizing properties of the Arp2/3 complex and facilitate its binding to the sides of actin filaments (Lua and Low, 2005; Mullins et al., 1998; Svitkina and Borisy, 1999). Subsequently, the Arp2/3 complex initiates de novo actin polymerization by mimicking the pointed end of the filament, thus creating branched actin filaments. Phosphorylation of cortactin by serine/threonine kinases (e.g. MAPK) increases binding and activation of N-WASP, whereas phosphorylation of tyrosine residues by Src-family kinases has the opposite effect, inactivating the protein (Martinez-Quiles et al., 2004). N-WASP in turn recruits G-actin monomers, rendering them available for the Arp2/3 complex and therefore favoring actin polymerization (Takenawa and Miki, 2001). In fact, reduction of cortactin protein levels in cultured hippocampal neurons by RNAi reduces dendritic spine density, whereas overexpression of the protein leads to spine elongation (Hering and Sheng, 2003).

Cortactin activity is also affected by NT-mediated signaling, however. Thus, activation of NMDARs induces Src-kinase-mediated phosphorylation of cortactin and subsequent loss of the protein from postsynaptic sites (Hering and Sheng, 2003;

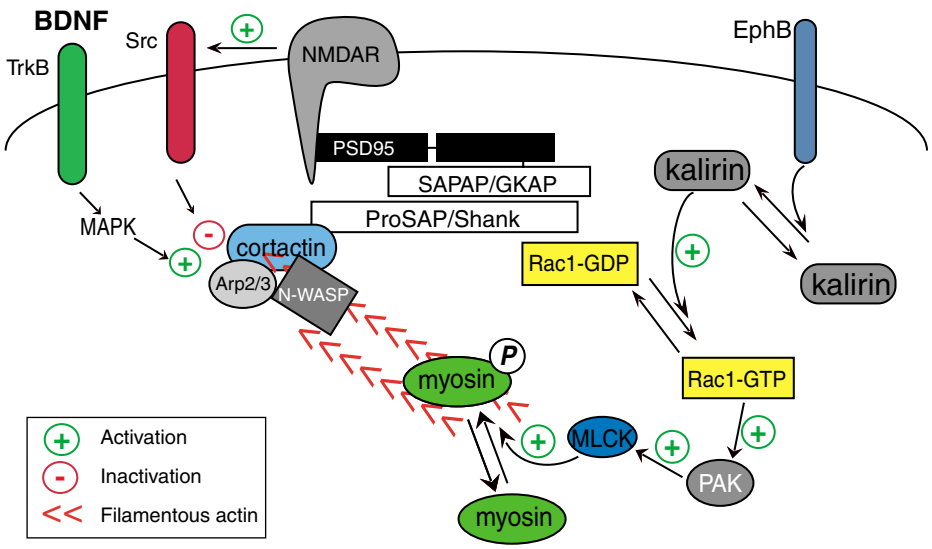

Fig. 2. Putative model of actin regulatory pathways mediated by non-glutamate receptors. The actin-polymerizing activity of Arp2/3 and N-WASP depends on cortactin phosphorylation levels, which are controlled in a TrkB- and Src-dependent manner. Activation of EphB recruits and activates kalirin to spines, which through Rac and PAK results in activation of myosin.

Iki et al., 2005). Since BDNF is a crucial factor for synaptic transmission, and numerous plasticity events occurring at mature synapses (Bramham and Messaoudi, 2005; Nagappan and Lu, 2005), the integration and propagation of both NMDA- and BDNF-triggered signals at a common actin regulator, cortactin, appears to be a very effective tool for tight control over actin-mediated morphogenetic events (Fig. 2).

Ephrin receptors (Ephs) are another family of RTKs important for many cellular processes, including neuronal differentiation and synaptic plasticity (Klein, 2004). The cytosolic signal-transducing C-terminal domain of the receptor bears a SAM protein-interaction domain and a binding site for PDZ-domain proteins, allowing interaction with a range of proteins. According to their binding-specificities they can be divided into two sub-families, EphA and EphB. EphA receptors interact with ephrin-A ligands, which lack a cytosolic domain and are attached to the plasma membrane through a glycosylphosphatidylinositol (GPI) anchor. EphB receptors bind to transmembrane ephrin B ligands, which (like the receptor) bear a PDZ-binding motif in their cytosolic C-terminus and thus are capable of retrograde signaling. Both anterograde and retrograde Eph-ephrin signaling play an important part in morphogenetic events leading to dendritic spine formation and synapse maturation.

Studies of mutant mice indicated that ephrinB-EphB2 signaling participates in dendritic spine plasticity and behavior (Grunwald et al., 2001; Henderson et al., 2001). Penzes et al. subsequently showed that dendritic spine morphogenesis in hippocampal neurons requires the activation of an actin-regulatory pathway stimulated by EphB in response to ephrinB (Penzes et al., 2003). Stimulation of 10DIV neurons with ephrin B rapidly triggers the formation of synapse-containing dendritic spines. Moreover, it leads to recruitment of the Rho-GEF kalirin to dendritic clusters and spines in an EphB-kinase-dependent manner. This activates Rac1 and consequently its downstream effector PAK, which subsequently activates MLCK. Active MLCK phosphorylates the regulatory light chain of myosin II, which results in higher actomyosin contractility, thus facilitating spine morphogenesis (Fig. 2) (Zhang et al., 2005).

Further evidence for a regulatory role of myosin II in spine morphology was provided by experiments illustrating that pharmacological or genetic inhibition of myosin IIB leads to alterations in shape and protrusive motility of dendritic

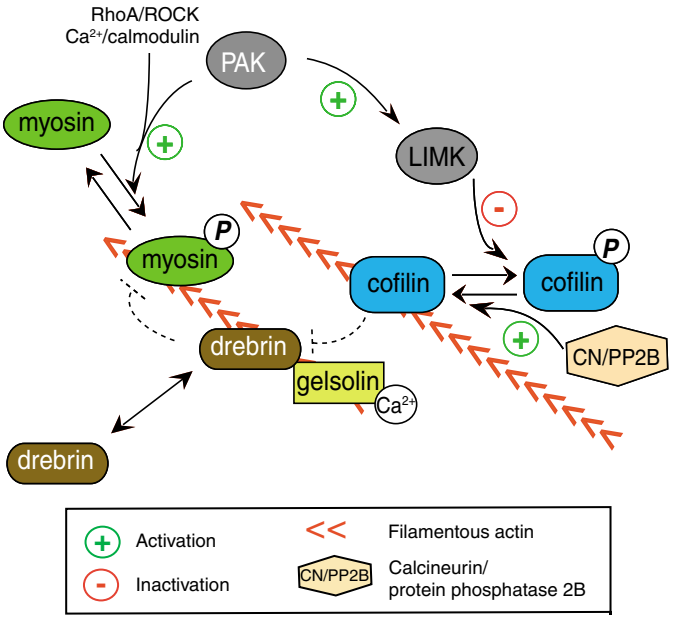

Fig. 3. Putative network of actin-regulatory proteins present in spines. The actin-severing activity of cofilin depends on a balance of various kinases and phosphatases (e.g. LIMK and CN/PP2B). The binding of cofilin to actin affects filament structure and lowers the actin affinity of drebrin at these sites. The actin stabilizer drebrin prevents actin reorganization by negatively regulating the binding of myosin to actin filaments and by interacting with gelsolin. Myosin stabilizes and contracts F-actin structures. Myosin motor activity can be triggered through PAK but also by other means. Gelsolin severs actin in a Ca^{2+}-dependent manner and caps the barbed ends of filaments, allowing control of actin polymerization at these sites.

spines and impairs synaptic activity (Ryu et al., 2006). The signals derived from ephrins and NTs thus impinge, at least in part, on identical actin regulatory pathways and this suggests that the combined signaling of RTKs and NTRs is required for synaptogenesis and dendritic spine morphogenesis.

Cadherin-dependent signaling to actin

Classical cadherins (e.g. N-cadherin) are a subfamily of the cadherin superfamily of cell-cell adhesion proteins that engage in homophilic protein interactions in a Ca^{2+}-dependent manner. They each contain a single transmembrane domain and tandem repeats of an extracellular cadherin domain, which is responsible for homophilic interactions (Tepass et al., 2000). The cytoplasmic domain is highly conserved and binds to catenins, which connect the protein to the actin cytoskeleton (via β- and α-catenin) and to the PSD (via δ-catenin) (Takeichi and Abe, 2005). The cadherin-catenin complex contributes to embryonic and neural morphogenesis and there is increasing evidence for its importance in later developmental processes, including synapse formation. N-cadherin and β-catenin localize to nascent synapses in cultured hippocampal neurons and blockage of cadherin during synaptogenesis alters spine shape and attenuates the synaptic recruitment of PSD95 (Togashi et al., 2002).

Cadherin-catenin complexes also participate in the maintenance of synaptic structure. Time-lapse video imaging of cultured hippocampal neurons showed that activity-induced remodeling of dendritic spines requires both adhesive and actin-binding activity of the cadherin-catenin complex (Okamura et al., 2004). Additionally, Abe et al. demonstrated that cultured hippocampal neurons obtained from αN-catenin-deficient mice exhibit enhanced spine mobility, whereas overexpression of αN-catenin in wild-type neurons increases spine density. Furthermore, blockage of neural activity with tetrodotoxin (TTX) reduces immunofluorescence intensity of endogenous αN-catenin in dendritic spines, but increasing neural activity with bicuculline has the opposite effect. Overexpression of αN-catenin prevents the TTX-induced turnover of dendritic spines into filopodia observed in non-transfected neurons (Abe et al., 2004). These data lead to the idea of a crucial role for cadherin-mediated cell-cell adhesion in the developmental processes of synaptogenesis and spine formation, and indicate a regulatory role in neurotransmission-dependent morphological plasticity.

Synaptic activity affects other actin regulators: the importance of drebrin A

Drebrin A is a neuron-specific isoform of the actin-filament-binding protein drebrin (Keon et al., 2000; Shirao and Obata, 1986). Drebrin stabilizes filamentous actin, uniquely modifying its structure, and affects actin dynamics by competitively inhibiting the actin-binding activity of tropomyosin and α-actinin (Asada et al., 1994; Ikeda et al., 1996; Ishikawa et al., 1994; Shirao, 1995). In neurons, drebrin A localizes to nascent and mature excitatory synapses, where it associates with filamentous actin (Aoki et al., 2005). Studies performed in hippocampal neurons in which drebrin A is overexpressed or downregulated revealed that drebrin A not only regulates F-actin dynamics during synaptogenesis but also plays a crucial role recruiting components of the excitatory post-synapse, e.g. PSD95 (Mizui et al., 2005; Takahashi et al., 2003).

Recently a role for drebrin A in neural-activity-dependent actin remodeling events in mature spines has been described. In wild-type neurons, under conditions of spontaneous activity, the NR1 subunit of the NMDAR clusters only slightly at synaptic sites. Blocking NMDAR activity, by incubation with its antagonist AP5, triggers accelerated accumulation of the receptor at synapses (Rao and Craig, 1997). Suppression of drebrin A protein levels does not affect the basal level of NR1 clustering at synapses, but it prevents the accelerated targeting of the receptor normally observed upon AP5 treatment (Hayashi et al., 1996; Takahashi et al., 2006). Moreover, activation of NMDARs by glutamate shifts drebrin into the dendritic shaft, away from spines, whereas blockage of NMDARs increases the F-actin and drebrin content in spines without affecting spine morphology (Sekino et al., 2006).

Further evidence for the involvement of drebrin in spine remodeling comes from studies showing that active cofilin induces the detachment of drebrin A from actin filaments (McGough et al., 1997; Zhao et al., 2006). Additionally, drebrin A forms a complex with other actin-binding proteins, such as myosins and gelsolin, and inhibits actomyosin interaction in vitro (Hayashi et al., 1996). These studies suggest another model for neurotransmitter-mediated reorganization of actin in spines. In this, the increase in cofilin activity leads to a reduction in the amount of drebrin A on actin, leading to a less stable actin network (a higher level of actin turnover) in the spine that, in turn, allows reorganization of the PSD. By contrast, a reduction in cofillin activity should allow stabilization of F-actin by drebrin A and negatively regulate the actions of actin-binding proteins, such as myosins and gelsolin (Fig. 3).

Conclusions

Here, we have discussed recent findings concerning only a few of the many actin regulators implicated in synaptic plasticity. Space constrains forced us to exclude many interesting results. Yet we believe that even this simplified view illustrates that control of spine architecture must be a fundamental process in central nervous system function. Only this would justify why so many molecules are employed to synchronize, in a spatially and temporally restricted manner, the numerous actin-regulatory pathways present in spines. In addition, we hope we have made clear that each single actin-signaling cascade can be activated by several receptor types at the postsynaptic membrane, and that it is the sum of signals derived from different receptors impinging on downstream players that in the end determine overall spine architecture. It would be simplistic to think that a single molecular pathway is responsible for synaptic plasticity, as many papers seem to imply. Considering the natural environment of a central nervous system neuron, it appears most plausible that developmental processes, such as synaptogenesis, and maintenance and remodeling of synaptic structures, are determined by the combined signaling of numerous factors. Only this could explain why spine development, memory formation, learning, and behavior of mammals are susceptible to developmental, environmental, hormonal, and pathological factors (Calabrese et al., 2006; Kolb and Whishaw, 1998; Schrott, 1997).

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