REVIEW ARTICLE
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The Ca^{2+} and Rho GTPase Signaling Pathways Underlying Activity-Dependent Actin Remodeling at Dendritic Spines

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Most excitatory synapses reside on small protrusions located on the dendritic shaft of neurons called dendritic spines. Neuronal activity regulates the number and structure of spines in both developing and mature brains. Such morphological changes are mediated by the modification of the actin cytoskeleton, the major structural component of spines. Because the number and size of spines is tightly correlated with the strength of synaptic transmission, the activity-dependent structural remodeling of a spine plays an important role in the modulation of synaptic transmission. The regulation of spine morphogenesis utilizes multiple intracellular signaling pathways that alter the dynamics of actin remodeling. Here, we will review recent studies examining the signaling pathways underlying activity-dependent actin remodeling at excitatory postsynaptic neurons. © 2012 Wiley Periodicals, Inc

Key Words: actin, dendritic spines, long term potentiation, structural plasticity, Rho GTPases, CaM-kinases

Introduction

The human brain consists of billions of neurons that are interconnected to form functional neuronal circuits. The accurate formation of neuronal circuits arises from the interplay between the initial genetically encoded gross wiring process during development and the subsequent activity-dependent refinement process that occurs in more mature systems [Katz and Shatz, 1996; Feller, 1999]. Such activity is required to shape, refine and integrate nascent neuronal connections into mature circuits.

The majority of excitatory synapses in the central nervous system reside on actin-rich protrusions on the dendrite known as spines [Harris and Kater, 1994; Matus, 2000; Hayashi and Majewska, 2005; Yuste, 2010; Bosch and Hayashi, in press]. The number and morphology of dendritic spines are tightly regulated during the development of neuronal circuits. In developing neurons, neuronal activity can bidirectionally regulate spine number. Pharmacological blockade of neuronal activity reduces the number of spines [Collin et al., 1997; Kossel et al., 1997; Segal et al., 2003; but also see McKinney et al., 1999], whilst enhancing neuronal activity increases [Neuhoff et al., 1999; Segal et al., 2003]. Additionally, local stimulation of the dendritic shaft of cortical neurons generates spines de novo specifically during the critical period of sensory plasticity of the area of cortex, but not before or after [Kwon and Sabatini, 2011].

Processes that regulate the number and structure of dendritic spines are also involved in the modulation of synaptic circuits in the mature brain, in both physiological and pathophysiological conditions. In mature neurons, administration of a stimulus that induces long-term potentiation (LTP) leads to the enlargement of existing spines, an event which is temporally associated with an increase in the number of AMPA type glutamate receptors (AMPAR) at the synapse [Shi et al., 1999; Matsuzaki et al., 2004; Okamoto et al., 2004; Kopec et al., 2006; Bosch et al., 2009]. In contrast, a stimulation that induces long-term depression (LTD) causes shrinkage of spines or even elimination alongside a concomitant loss of synaptic responsiveness [Nägerl et al., 2004; Okamoto et al., 2004; Zhou et al., 2004]. Thus, it is likely that the change in the number and size of dendritic spines contributes to the change in synaptic efficacy seen during synaptic plasticity.

In this article we will review the signaling pathways associated with actin remodeling. We provide particular focus to the role of Ca^{2+} and Rho-GTPase signaling during structural modification of dendritic spines, mainly within the context of LTP in mature neurons. The function of other actin binding proteins and small GTPases in neurons were recently reviewed elsewhere [Kennedy et al., 2005; Ng and Tang, 2008; Lin and Webb, 2009].

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Actin Dynamics in Spines

Filamentous actin (F-actin) is highly concentrated in dendritic spines and plays an important role in regulating spine morphology, size and number [Matus, 2000; Okamoto et al., 2009; Hotulainen and Hoogenraad, 2010]. As the size of a dendritic spine is close to the resolution limit of conventional optical microscopic techniques, a detailed examination of the structure of actin within spines can only be readily investigated by electron microscopy (EM) [Fifková and Delay, 1982; Landis and Reese, 1983; Hirokawa, 1989]. Platinum replica EM analysis combined with detergent extraction revealed that short cross-linked and branched actin filaments dominate the spine head, whereas long actin filaments are found in the spine neck. At the spine base, a delta-shaped structure is formed by linear actin filaments coming out from the dendrite to the neck of spine [Korobova and Svitkina, 2010]. Furthermore, recent advances in super resolution microscopy allowed the observation of actin in living neurons, which revealed that curvilinear actin bundles may play a role in linking the spine head to the dendritic shaft [Urban et al., 2011].

Experimental evidence has shown that changes in actin dynamics can influence spine morphology and the strength of a synapse [Cingolani and Goda, 2008; Saneyoshi et al., 2010]. The activity-dependent structural change of dendritic spines is attenuated by drugs that block actin polymerization such as latrunculin or those that stabilize actin filaments such as jasplakinolide [Halpain et al., 1998; Allison et al., 1998; Matsuzaki et al., 2004]. Okamoto et al. [2004] demonstrated using a Förster resonance energy transfer (FRET)-based method that actin polymerizes during LTP and depolymerizes during LTD, 30 min after induction of plasticity. Honkura et al. [2008] used photoactivatable-GFP tagged actin to monitor the turnover of actin molecules in the dendritic spine head. In unstimulated spines, existing actin molecules diffuse from the spine head into the shaft, in the order of minutes. However, induction of LTP causes a decrease in the rate of actin turnover, possibly reflecting the formation of more F-actin in the stimulated spine. From these results, the authors proposed that three distinct pools of F-actin exist in spines. A *dynamic pool* is the dominant population in unstimulated spines and has a turn-over rate (τ) of $\sim$40 s. This pool is constantly moving from the periphery to the center of the spine, possibly representing an actin treadmilling process [Honkura et al., 2008; Frost et al., 2010b]. Induction of LTP leads to spine enlargement, which is dependent on an *enlargement pool* with a slow turnover rate (τ = 2–15 min). For this enlargement to persist, the formation of a *stable pool* at the base of the spine head is required (τ = 17 min) [Honkura et al., 2008]. Occasionally, the enlargement pool escapes from the spine head without forming the stable pool. In such cases, spine enlargement cannot persist [Honkura et al., 2008].

The actin cytoskeleton not only regulates the morphology of the spine head, but also controls the organization of the postsynaptic density (PSD), the stability and trafficking of neurotransmitter receptors and local translation within spines [Kuriu et al., 2006; Korkotian and Segal, 2007; Pontrello and Ethell, 2009]. Pharmacological manipulations of actin not only affect spine morphology but also inhibit the plasticity of synaptic currents [Kim and Lisman, 1999; Krucker et al., 2000], indicating that actin may have dual functions in regulating both the structure of spines and synaptic transmission.

Actin regulates the structure and function of spines partially through myosin, an actin binding motor protein superfamily. Myosin molecules are able to interact, traffic and produce tension to actin filaments [Sweeney and Houdusse, 2010]. Nonmuscle myosin II is involved in regulating spine morphology and actin dynamics during LTP. Treatment of neurons with a myosin II specific inhibitor, blebbistatin, reduced spine formation as well as excitatory synaptic transmission in developing hippocampal neurons [Zhang et al., 2005; Ryu et al., 2006; Hodges et al., 2011]. In adult hippocampal slices, knockdown of myosin heavy chain IIb (MHCIIb) by shRNA or acute blebbistatin treatment reduced the stability of LTP [Rex et al., 2010]. Furthermore, examination using fluorescence recovery after photobleaching (FRAP) showed that blebbistatin treatment reduced the recovery rate of actin in spines, indicating that myosin II directly contributes to the dynamics of actin [Rex et al., 2010]. In LTP-induced spines, synaptic incorporation and retention of AMPAR contributes to the increase in synaptic currents. Myosin V transports AMPAR-containing recycling endosomes along with F-actin into spines during LTP [Correia et al., 2008; Wang et al., 2008]. Therefore, myosin contributes to the dynamics of actin and synaptic transmission mediated by AMPARs. The next major question to understand is how the actin cytoskeleton is dynamically regulated during the induction and maintenance of synaptic plasticity.

The Role of Rho Family Small G-Proteins in the Regulation of Actin Remodeling

Neuronal depolarization leads to an increase in intracellular calcium by influx through NMDA-type glutamate receptors (NMDAR) and/or voltage-gated calcium channels. A crucial question is: how is the increase in intracellular calcium decoded into a signal that can regulate the dynamics of the actin cytoskeleton. Comprehensive proteomic approaches have identified multiple actin-regulating proteins at the synapse [Husi et al., 2000; Peng et al., 2004]. The specific role of some of these proteins is now becoming clear.

Several lines of evidence indicate that the Rho family small G-proteins, including RhoA, Rac1 and Cdc42, play critical roles in the activity-dependent formation of the

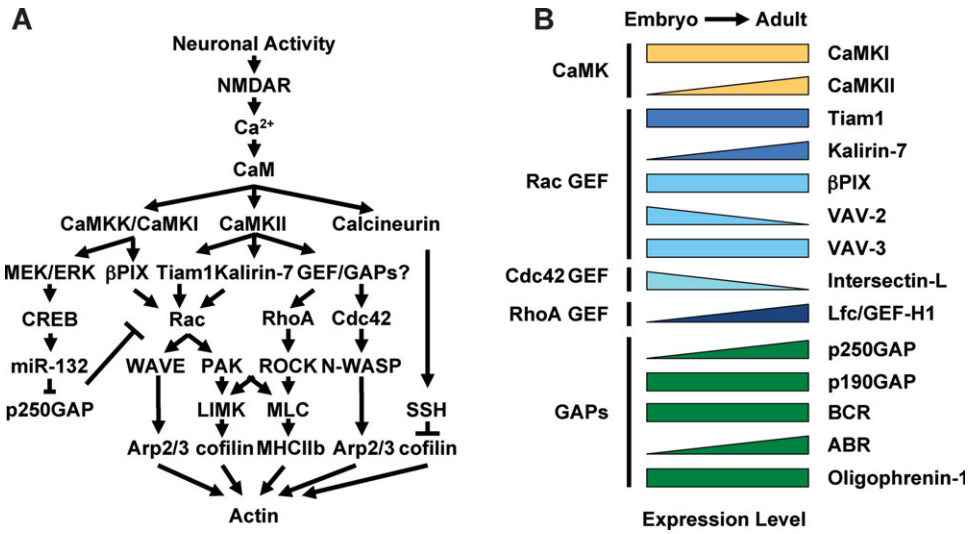

Fig. 1. Signaling cascades involved in regulating actin cytoskeletal dynamics. (**A**) Selected signaling cascades involved in the regulation of the actin cytoskeleton. Arrows indicate positive regulation. T-shape bars indicate negative regulation. NMDA-R, NMDA-type glutamate receptor; CaM, calmodulin; CaMK, calcium-calmodulin dependent protein kinase; ERK, extracellular signal-regulated kinase; MEK, MAPK/ERK kinase; CREB, cAMP-response element binding protein; GEF, guanine-exchange factor; GAP, GTPase-activating protein; ROCK, Rho-associated coiled-coil containing protein kinase; PAK, p21-activated protein kinase; βPIX, β PAK interacting exchange factor; Tiam1, T-cell lymphoma invasion and metastasis-inducing protein 1; LIMK, LIM-domain-containing protein kinase; N-WASP, Neuronal Wiskott-Aldrich syndrome protein; WAVE, WASP-family verprolin homologous protein; MLC, myosin light chain; MHCIIb, myosin heavy chain IIb; mi-R, microRNA; SSH, slingshot; Arp, Actin related protein. See text for details. (**B**) Relative expression of CaMK and the Rho-GTPase signaling molecules in developing and adult brain tissue. Box shapes represent constant expression throughout developmental stages and triangle shapes indicate a graded expression during development. CaMKI [Kamata et al., 2007; Saneyoshi et al., 2008], CaMKII [Bayer et al., 1999; Fink et al., 2003], Tiam1 [Yoshizawa et al., 2002; Ehler et al., 1997], Kalirin-7 [Ma et al., 2003; Xie et al., 2007], βPIX [Kim et al., 2000; Saneyoshi et al., 2008], VAV2/VAV3 [Cowan et al., 2005; Hale et al., 2011], intersectin-L [Nishimura et al., 2006], Lfc/GEF-H1 [Ryan et al., 2005; Kang et al., 2009], oligophrenin-1 [Govek et al., 2004], BCR/ABR [Oh et al., 2010], p250GAP [Hayashi et al., 2007], p190GAP [Brouns et al., 2000].

synapse and the structural modification of dendritic spines [Govek et al., 2005; Saneyoshi et al., 2010] (Fig. 1A). The Rho family small G-proteins switch from an active GTP-bound form to an inactive GDP-bound form by its intrinsic GTPase activity. Guanine nucleotide exchange factors (GEFs) activate the GTPase activity by stimulating the release of bound GDP and allowing binding of new GTP molecules. Conversely, GTPase-activating proteins (GAPs) can inactivate G-proteins by accelerating GTPase activity that hydrolyzes the GTP into GDP, to render the protein inactive [Hall, 1998]. The dynamic regulation of their activity suggests that Rho family small G-proteins can act as molecular switches in signal transduction processes.

Although belonging to the same Rho family small G-proteins, RhoA, Rac1 and Cdc42 appear to have very different mechanistic roles in regulating different aspects of dendritic spine function and structure. In developing neurons, expression of a dominant negative form of Rac1 or RNAi-mediated suppression of Rac1 leads to the decrease or elimination of spines [Tashiro et al., 2000; Zhang et al., 2005; Impey et al., 2010]. Conversely, a constitutively active form of Rac1 leads to an increase in spine density [Nakayama et al., 2000; Tashiro et al., 2000; Pilpel and Segal, 2004; Zhang and Macara, 2006]. RNAi-mediated suppression of Cdc42 decreases spine density in developing cultured hippocampal neurons [Kang et al., 2008]. However, studies have shown that overexpression of a dominant negative form of Cdc42 leads to inconsistent changes in spine density, with some reports showing a reduction [Irie and Yamaguchi, 2002; Wegner et al., 2008] and others showing no clear effect [Tashiro et al., 2000]. Neurons expressing an active form of Cdc42 show an increase in spine density [Kang et al., 2008]. In contrast, a constitutively active form of RhoA can decrease dendritic spine density and increase spine length in immature neurons [Nakayama et al., 2000; Tashiro et al., 2000; Zhang and Macara, 2006; Impey et al., 2010]. A dominant negative form of RhoA can increase spine density [Zhang and Macara, 2006; Impey et al., 2010]. Thus, these reports suggest that Rac and Cdc42 play a role in the induction and maintenance of spines, while RhoA regulates spine shrinkage and elimination, at least during development.

Using more mature hippocampal slice cultures, Yasuda and colleagues measured the activity of RhoA and Cdc42 within a single spine during LTP [Murakoshi et al., 2011]. In this preparation, as the dendritic spine expands, the activity of both RhoA and Cdc42 were elevated. This increase was dependent on NMDAR and CaMKII

activation and lasted at least 30 min. The activation of Cdc42 is localized specifically to stimulated spines whereas the activity of RhoA diffuses out from stimulated spines. Knocking-down either Cdc42 or RhoA (and also RhoB) partially reduces the enlargement of dendritic spine. These results indicate that in mature neurons, RhoA plays an important role in spine enlargement. Interestingly, the role of RhoA in regulating spine morphogenesis seems to undergo a developmental switch, with RhoA causing shrinkage during early development to controlling enlargement in more mature states. Understanding the mechanisms underlying this functional switch in RhoA signaling will be of future interest.

The involvement of Rac in modulating synaptic function in mature neurons has also been investigated. Mutant mice lacking the *rac1* gene displayed impaired synaptic plasticity as well as deficits in learning and memory [Haditsch et al., 2009]. Inhibitors of Rac1 can block the formation and maintenance of LTP [Martinez and Tejada-Simon, 2011; and see Rex et al., 2009], suggesting that Rac is a key regulator of synaptic strength and plasticity. However, these studies do not provide information about the duration of Rac1 activation during LTP.

Biochemical evidence also suggests that there are further reciprocal interactions between members of the Rho-family small G-proteins, with RhoA and Rac having opposing roles to each other, whilst Cdc42 can activate Rac [Tsuji et al., 2002; Burridge and Wennerberg, 2004; Nishimura et al., 2005; Ohta et al., 2006]. Given the complex regulation of multiple Rho-family small G-proteins, it will be of interest to compare the spatiotemporal activity of different Rho-family small G-proteins in a spine during LTP using simultaneous live imaging [Machacek et al., 2009].

Down-Stream Effectors of Small G-Protein Signaling

Different members of the Rho-family small G-proteins can activate different kinase signaling cascades. RhoA can activate Rho-associated, coiled-coil containing protein kinase (ROCK), while Cdc42 and Rac can activate p21-activated kinase (PAK). Pharmacological inhibitors of PAK and ROCK can block the structural enlargement of dendritic spines associated with LTP-induction [Murakoshi et al., 2011]. Consistent with this, mice lacking *rock2* and *pak1* genes or those expressing a dominant negative form of PAK display a deficiency in LTP [Hayashi et al., 2004; Asrar et al., 2009; Zhou et al., 2009].

The detailed mechanisms by which effectors of the Rho family G-proteins (including ROCK or PAK) can regulate the dynamics of the actin cytoskeleton during LTP remain to be clarified. One of the effectors, cofilin, is of particular interest. Cofilin is a member of the actin depolymerization factors and can sever F-actin. The activity of cofilin is regulated by phosphorylation at amino acid residue serine 3 by LIM-kinase (LIM-K) and by dephosphorylation by slingshot [Bernstein and Bamburg, 2010]. Serine 3 phosphorylation renders cofilin inactive and therefore incapable of binding and severing F-actin, whereas dephosphorylation triggers its activity. Cofilin is localized within dendritic spines and a LTP-inducing stimulus can elevate the levels of phosphorylated cofilin (p-cofilin) in spines for 15–30 min [Racz and Weinberg, 2006; Chen et al., 2007]. High resolution, single spine imaging studies have shown that cofilin rapidly accumulates in the spine after LTP induction with focal uncaging of glutamate [Bosch et al., 2009].

Cofilin has also been implicated in regulating spine morphology during LTD [Zhou et al., 2004; Wang et al., 2007]. LTD-induced spine shrinkage requires activation of NMDA-R and calcineurin [Zhou et al., 2004]. Wang et al. [2007] demonstrated that postsynaptic introduction of a p-cofilin peptide (which is expected to prevent actin depolymerization) can inhibit LTD-induced spine shrinkage but does not alter the reduction in synaptic currents. This result indicates that LIM-K-cofilin-mediated regulation of actin may modulate changes in spine shape but not changes in synaptic efficacy during LTD.

The regulation of cofilin activity is a complex matter. Since LIM-K is regulated via upstream kinases including ROCK, PAK, PKA, as well as CaMKIV, the final activity of cofilin can be viewed as a result of the integration of multiple signaling pathways [Edwards et al., 1999; Maekawa et al., 1999; Ohashi et al., 2000; Nadella et al., 2009; Takemura et al., 2009]. Additionally, an activity-regulated brain specific microRNA (miR)-134, a member of the noncoding RNA species that controls protein synthesis, has been shown to regulate LIM-K translation [Schratt et al., 2006]. Moreover in mice lacking *rock2,* the amount of p-cofilin at basal states is significantly decreased compared to wild type mice, but the levels of p-cofilin attained by stimulation with NMDA plus glycine is comparable to wild type animals [Zhou et al., 2009]. In contrast, *pak1* mutant mice have normal basal p-cofilin levels, but do not respond to NMDA plus glycine stimulation, suggesting that PAK1 can only respond to strong stimulation [Asrar et al., 2009]. Thus, the activity of cofilin in spines is tightly regulated by different kinases and phosphatases and this regulation depends on the strength of the synaptic input, as basal neuronal activity and strong stimulation show different effects. Crucially, mutant mice lacking cofilin1 show impaired LTP and LTD and have abnormal spine morphology [Rust et al., 2010]. It will be intriguing to further determine the dynamics of cofilin phosphorylation within spines during LTP and LTD.

Myosin is also located downstream of Rho-family small G-protein signaling. The activity of myosin is increased by phosphorylation of the light chain (MLC) of the regulatory subunit at residues Thr18 and/or Ser19 by PAK and ROCK, as well as other kinases [Amano et al., 1996;

Chew et al., 1998; Vicente-Manzanares et al., 2009]. ROCK also indirectly modulates the phosphorylation of these sites by inhibiting myosin light chain phosphatase (MLCP) [Kimura et al., 1996]. Therefore, myosin II function can be either directly or indirectly regulated by Rho-family small GTPase signaling cascades. Treatment of hippocampal neurons with ROCK inhibitor, Y27632, increased spine length, which is rescued by expression of a phospho-mimetic mutant of myosin light chain (MLC18D19D) [Hodges et al., 2011]. In adult hippocampal slices, treatment with NMDA or theta-burst stimulation induced phosphorylation of MLC, which is blocked by ROCK inhibitor H1152 [Rex et al., 2010]. Furthermore, overexpression of active Pak3 in hippocampal neurons increased spine density, which was prevented by treating the neurons with a myosin II inhibitor, blebbistatin. In contrast, overexpression of a kinase-dead mutant of Pak1 lead to a reduction in spine density, which was rescued by coexpression of MLC18D19D [Zhang et al., 2005]. These results suggest that Pak also plays a role in spine formation by regulating myosin activity in spines.

In addition to kinases that are directly activated by Rho-family small G-proteins, WAVE and N-WASP are other well-studied downstream effectors of Rac and Cdc42, respectively [Takenawa and Suetsugu, 2007]. WAVE1 and N-WASP belong to the Wiskott-Aldrich syndrome protein family, which stimulate actin polymerization through the Arp2/3 complex. WAVE1 knockout mice display reduced spine density and impaired LTP and LTD, as well as learning [Soderling et al., 2003, 2007; Kim et al., 2006]. Application of an inhibitor of N-WASP or knockdown of N-WASP by shRNA can reduce spine and synapse formation. Overexpression of a N-WASP fragment lacking actin and Arp2/3 binding domains also caused a reduction in spine density, suggesting that the Arp2/3 complex mediates actin polymerization during spine formation through N-WASP [Wegner et al., 2008; Hotulainen et al., 2009].

Mechanisms Involved in Translating Ca^{2+}-Signals Into Small G-Protein Signaling

An elevation in intracellular Ca^{2+}concentration can activate calmodulin (CaM) sensitive molecules including CaM-dependent protein kinases (CaMKs). CaMKs have been implicated in modifying both spine structure and synaptic plasticity [Wayman et al., 2008]. In rats, the expression of αCaMKII is low during development and reaches a plateau 2 weeks after birth [Bayer et al., 1999; Fink et al., 2003]. In contrast, CaMKI isoforms are expressed at similar levels in embryonic and adult brains [Kamata et al., 2007; Saneyoshi et al., 2008]. Members of the Rho GEFs and GAPs also appear to be controlled by development and neuronal activity. The ontogeny of these molecules are listed in Fig. 1B. Therefore, the activity-dependent actin remodeling in dendritic spines could be regulated by different subsets of CaM-kinases in the developing and mature neuron.

In developing neurons, CaMKI phosphorylates and activates βPIX, a protein with RacGEF activity, resulting in Rac1 activation [Saneyoshi et al., 2008]. CaMKI also activates a MEK-ERK signaling cascade [Schmitt et al., 2005]. The MEK-ERK signaling cascade increases the transcription of CREB-regulated genes through the phosphorylation of CREB [Vo et al., 2005]. One such gene is a micro RNA, miR-132. miR-132 can suppress the translation of p250GAP, a protein with RacGAP activity, leading to facilitation of the Rac-PAK pathway [Impey et al., 2010].

In mature neurons, spine enlargement is also partly mediated by the CaMKI-Rac-PAK pathway but through a distinct mechanism [Fortin et al., 2010]. LTP-induced activation of the Rac-PAK pathway is sensitive to IEM-1460, a specific inhibitor for calcium-permeable AMPA-R (CP-AMPAR), as well as latrunculin A, an inhibitor of actin polymerization. An earlier study showed that LTP-induced CP-AMPAR expression is CaMKI-dependent [Guire et al., 2008]. Spine enlargement induced by glycine is sensitive to STO-609, CaMKK inhibitor. Also active CaMKI increased spine head size as well as surface AMPAR expression. From these observations, it was concluded that CaMKI modulates spine shape through expression of CP-AMPAR during LTP [Guire et al., 2008; Fortin et al., 2010]. These studies support the view that the actin cytoskeleton is regulated via cross-talk between CaMKI and Rac signaling in neurons.

It is widely accepted that CaMKII is a critical molecule for synaptic plasticity, learning and memory [Wayman et al., 2008; Okamoto et al., 2009]. However, various roles have been suggested for this kinase. One mechanism by which CaMKII regulates actin remodeling in spines is through its capacity to bind and bundle F-actin and stabilize actin in the dendritic spine head [Okamoto et al., 2007]. The ability of CaMKII to bundle F-actin is further regulated by Ca^{2+}/calmodulin binding [O'Leary et al., 2006; Okamoto et al., 2007; Lin and Redmond, 2008]. CaMKII can also regulate the actin cytoskeleton through activation of Rac pathways by phosphorylating the Rac-GEFs including Tiam1 and Kalirin-7 during spine formation [Tolias et al., 2005; Xie et al., 2007; Penzes et al., 2008]. Since CaMKII directly phosphorylates and inactivates p250GAP, CaMKII can act as a positive regulator of Rho signaling [Okabe et al., 2003], in contrast to CaMKI, which regulates p250GAP translation [Impey et al., 2010].

Perspective

Actin remodeling is the major mechanism that regulates spine morphology, a factor that is critical for synapse

function. To understand the molecular mechanisms underlying neuronal activity-driven spine morphogenesis in greater detail, it is important to investigate the spatiotemporal regulation of synaptic molecules. Live-cell imaging and optical manipulations have allowed us to dissect the signaling and functions of actin in dendritic spines [Okamoto et al., 2004; Rana and Dolmetsch, 2010; Frost et al., 2010a]. However, the resolution limit attained by conventional light microscopy is already close to the size of dendritic spines. With recent advances in new microscopic technologies, so called super-resolution fluorescent microscopy such as STORM/PALM and STED, it will be possible to provide an even greater and more detailed view of the function of F-actin in dendritic spines [Toomre and Bewersdorf, 2010; Frost et al., 2010b; Izeddin et al., 2011; Urban et al., 2011]. Actin within spines is not comprised of only G-actin molecules and linear F-actin filaments, but there are many additional different suprastructures, including meshes, branches, and bundles [Watanabe, 2010]. Emerging studies using super-resolution imaging techniques have already demonstrated that F-actin flows constantly from the head to the base of spines under basal neuronal activity conditions [Tatavarty et al., 2009; Frost et al., 2010b]. Furthermore, it will be intriguing to visualize the dynamics of actin using super-resolution imaging coupled with FRET-techniques, as this will allow a more extensive characterization of distinct F-actin pools and its regulators [Okamoto et al., 2004; Honkura et al., 2008]. Since the proposal that formation of new F-actin in potentiated spines may act as a synaptic tag during LTP consolidation [Okamoto et al., 2009], a major aim of the field has been to gain a more thorough understanding of the structure and function relationship of actin dynamics in spines, which will provide further insights into the mechanisms underlying memory. In the future, it will be important to confirm that learning can induce changes in actin dynamics in spines in vivo.

In this review, we largely focused on the mechanisms underlying the structural modification of dendritic spines at mature hippocampal synapses during LTP. However, it remains to be elucidated whether these mechanisms are universally adopted to regulate spine structure during different forms of plasticity such as non-NMDA-receptor dependent LTP, LTD, agonist-induced plasticity and homeostatic plasticity or during development. For example, in cerebellar Purkinje cells, neither synaptic nor chemical LTD induction is associated with changes in spine number and size [Sdrulla and Linden, 2007]. Therefore, at least in cerebellar LTD, spine structure is not always associated with synapse function. It will be intriguing to see how the molecules and signaling pathways described above are involved in the regulation of synaptic structure and function during different activity conditions and during specific developmental stages.

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