

Update Article

Clustering and anchoring mechanisms of molecular constituents of postsynaptic scaffolds in dendritic spines

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Abstract

Recent technological progress has yielded great amounts of information about the molecular constituents of postsynaptic scaffolds in the dendritic spine. Actin filaments are major cytoskeletal elements in the dendritic spine, and they functionally interact with neurotransmitter receptors via regulatory actin-binding proteins. Drebrin A and α -actinin-2 are two major actin-binding proteins in dendritic spines. In adult brains, they are characteristically concentrated in spines, but not in dendritic shafts or cell bodies. Thus, they are part of a unique postsynaptic scaffold consisting of actin filaments, PSD protein family, and neurotransmitter receptors. Localization of NMDA receptors, actin filaments, and actin-binding proteins in spines changes in parallel with development, and in response to synaptic activity. This raises the possibility that clustering and anchoring of these characteristic molecular constituents at postsynaptic scaffolds play important roles in spine function. This article focuses on the clustering and anchoring mechanisms of NMDA receptors and actin filaments, and the involvement of actin-binding proteins, in dendritic spines, and the way in which characteristic postsynaptic scaffolds are built up. © 2001 Elsevier Science Ireland Ltd and the Japan Neuroscience Society. All rights reserved.

Keywords: Dendritic spine; Drebrin; NMDA receptors; α -Actinin-2; Clustering and anchoring; Postsynaptic scaffolds; Actin cytoskeleton

1. Introduction

Dendritic spines are the specialized structures on which the majority of excitatory glutamatergic synapses in the brain are found. Since Ramon y Cajal's first description of dendritic spines, numerous studies have shown that spine shape and density are altered by pathological and experimental influences. However, there have been no insights into the molecular constituents regulating spine function and structure.

A major cytoskeletal element in dendritic spines is actin filaments, as shown in immunoelectron microscopical studies (Matus et al., 1982; Cohen et al., 1985). Functional interaction between receptors and actin cytoskeleton was first described in 1993. Rosenmund and Westbrook (1993) reported that function of the *N*-

methyl-D-aspartate (NMDA) subtype of glutamate receptors is regulated by the actin cytoskeleton in a calcium-dependent manner. Within the past 5 years, it has been demonstrated that spine shapes are rapidly modified in response to transmembrane signals (Hosokawa et al., 1995; Yuste and Denk, 1995; Fischer et al., 1998; Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999). These rapid morphological changes most likely result from rearrangements of the actin cytoskeletons. In 1999, we showed that accumulation of an actin-binding protein, drebrin, within dendritic spines resulted in increased spine length, possibly via rearrangements of the actin cytoskeleton (Hayashi and Shirao, 1999). These observations indicate that many molecular components in dendritic spines interact with each other directly and indirectly, resulting in alteration of synaptic function.

Specialization of molecular components in dendritic spines results in unique postsynaptic scaffolds different from those in the dendritic shaft and cell body. Al-

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though, early in development, the molecular components that are observed in the mature spine are already expressed in the neurons, they are not accumulated in protrusions from the dendritic shaft such as dendritic filopodia or immature spines (Fig. 1A and C). The unique postsynaptic scaffolds are built up only in the mature spines (Fig. 1B and D). Thus, the characteristics of postsynaptic scaffolds in dendritic spines are now a focus of interest in studies of synaptic function.

Although diverse in size and morphology, spines contain the following general components: postsynaptic density (PSD), actin cytoskeleton and soluble regulatory proteins. PSD is a specialized structure on the

intracellular side of the postsynaptic membranes of synapses in the CNS. It has been proposed that PSD is a crucial element in the organization of neurotransmitter receptors (Kennedy, 1997; Ziff, 1997; Kennedy, 1998). Actin seems to provide the only structural basis for cytoskeletal organization in dendritic spines (Matus et al., 1982; Cohen et al., 1985), as spines lack microtubules and intermediate filaments (except the large branched spines on CA3 pyramidal cells, in which microtubules are clearly visible) (Chicurel and Harris, 1992). Longitudinal actin filaments have been observed in the necks of dendritic spines, and a lattice of actin filaments has been observed in the head (Landis and

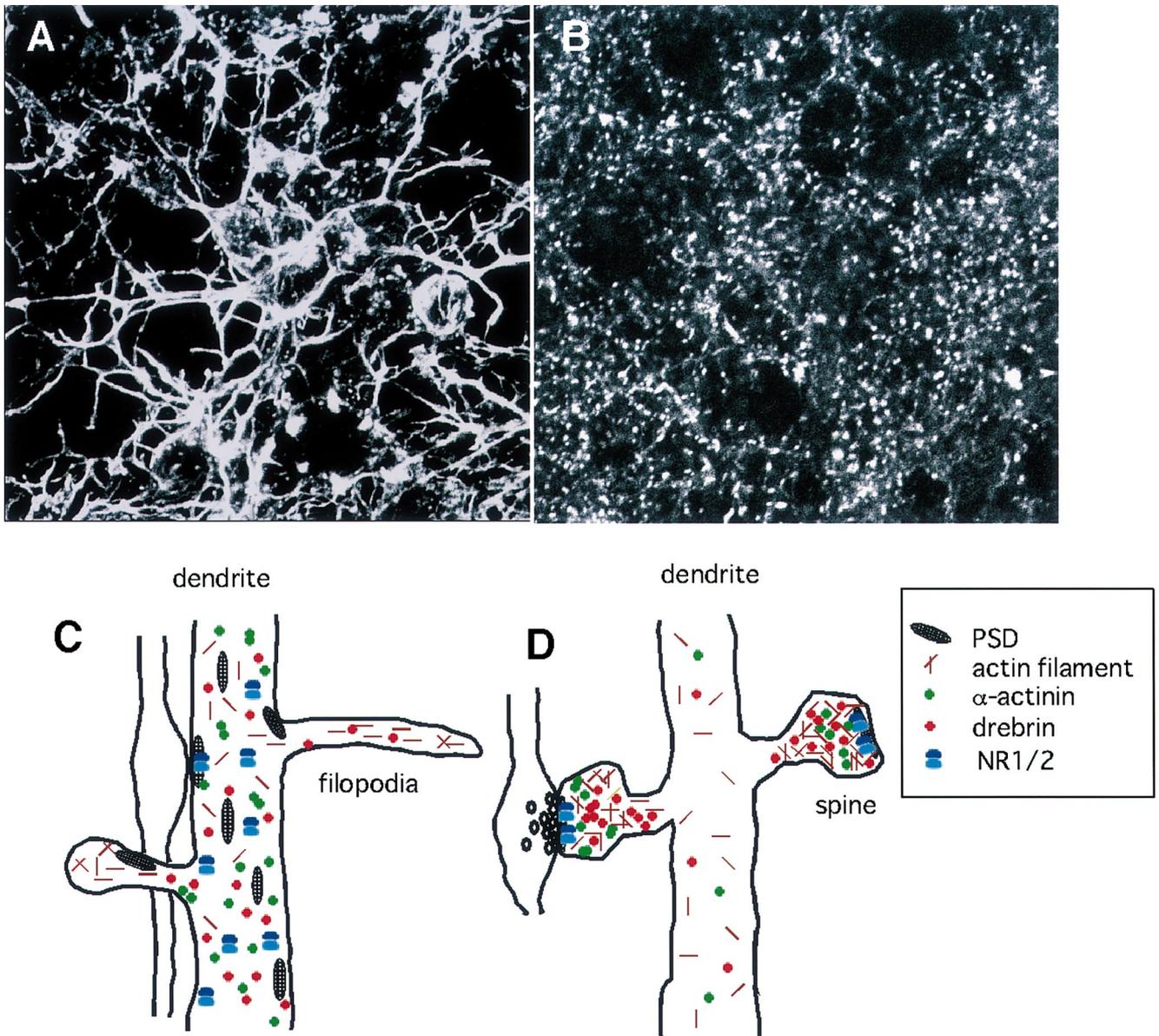


Fig. 1. Developmental changes in clustering and anchoring of molecular constituents within dendritic spines. A–B, Immunofluorescence of drebrin within the dendritic spine of cortical neurons at 1 day (A) and 3 weeks (B) in culture. At day 1, drebrin was present diffusely throughout cell bodies and dendritic shafts. In contrast, 3 weeks later, drebrin was concentrated within dendritic spines. C–D, Diagrammatic summary of clustering and anchoring of molecular constituents in dendritic spines, in early (C) and late (D) development.

Reese, 1983). There are some soluble proteins, such as calcineurin (Halpain et al., 1998), drebrin A (Shirao, 1995) and α -actinin-2 (Wyszynski et al., 1998), which are specifically concentrated within spines. It has been suggested that some soluble proteins are kept in place by protein–protein interaction with the actin cytoskeleton, and that these proteins play regulatory roles in the function of neurotransmitter receptors (Rosenmund and Westbrook, 1993) and in spine morphology (Hayashi et al., 1996; Hayashi and Shirao, 1999).

What kind of mechanism is responsible for the specialization of molecular constituents at postsynaptic scaffolds within dendritic spines? This article focuses on recent advances in the understanding of the clustering and anchoring mechanisms of NMDA receptors and actin filaments, and involvement of actin-binding proteins, in dendritic spines.

2. Clustering and anchoring of NMDA receptors within dendritic spines

In cultured neurons, NMDA receptors are clustered in the dendritic shafts and in cell bodies, early in development, but the number of NMDA receptor clusters increases later in development, and most of them are anchored within dendritic spines (Rao and Craig, 1997). The NMDA receptors are complexes of NR1 and NR2A-D subunits. NR2A-D subunits have distinct expression profiles that are regulated developmentally (Monyer et al., 1994; Okabe et al., 1998). Chronic treatment with an NMDA receptor antagonist (MK801 or APV) in cultured neurons has been shown to result in up-regulation of the number of NMDA-binding sites (Williams et al., 1992) and the expression levels of NR2A and NR2B, accompanied by a dramatic shift in concentration of NMDA receptor oligomers from dendritic shafts and cell bodies to dendritic spines (Rao and Craig, 1997). These observations raise the possibility that expression of NR2 subunits plays a role in the anchoring of NMDA receptors.

However, in the above experiments, the ratios of NR2A and NR2B subunits to NR1 subunits at the primarily non-synaptic clusters of dendritic shafts in control cells were not different from the ratios at primarily synaptic clusters of dendritic spines in APV-treated cells (Rao and Craig 1997). Moreover, it has recently been reported that NR2A and NR2B colocalize with NR1 at all stages of development from 2 days to 5 weeks in culture (Rao et al., 1998). These findings demonstrate that increased expression of NR2 cannot be solely responsible for the anchoring of NMDA receptors in dendritic spines, although it may play some roles in the clustering of NMDA receptors, as indicated in the following discussion.

It has been suggested that molecular constituents of PSD play roles in the clustering and anchoring of NR1/NR2 heteromeric NMDA receptors (Kornau et al., 1995; Kim et al., 1996). Core components of PSD, such as PSD-95 and Chapsyn-110, bind to NR2 subunits and cocluster with them upon coexpression in non-neuronal cells, but neither PSD-95 nor NR2 clusters when expressed alone. Although the NR1 subunit does not cocluster with members of the PSD family, triple transfection with NR1, NR2 and members of the PSD family results in coclustering. It has been demonstrated, in a developmental study using cultured hippocampal neurons, that PSD-95 clusters at presumptive postsynaptic sites several days before NMDA receptors cluster at such sites (Rao et al., 1998). These observations lead us to speculate that PSD family proteins cluster at postsynaptic sites, resulting in the anchoring of NMDA receptors in spines.

Such speculation is supported by the results of an experiment using mutant mice carrying carboxy terminal-truncated NR2B (Mori et al., 1998). Reduced immunostaining of NR2B in the synaptic sites of the mutant mice suggests that the interaction between NR2 and PSD-95 is important for the anchoring of NMDA receptors. However, results of an experiment using mutant mice expressing a truncated 40 K PSD-95 protein demonstrate that NMDA receptors are synaptically localized in the absence of intact PSD-95 (Migaud et al., 1998). This is consistent with experimental results which show that suppression of PSD-95 expression in cultured neurons does not affect NMDA currents or Ca^{2+} loading (Sattler et al., 1999). These observations raise the possibility that molecules other than PSD-95 may be more important in the synaptic localizing of NMDA receptors. Furthermore, we suggest that the molecular mechanism responsible for clustering of NMDA receptors may be different from their anchoring mechanism.

Rao and Craig (1997) reported that APV treatment induces a redistribution of NMDA receptors from non-synaptic sites to synaptic sites which already contained PSD-95, although the distribution of PSD-95 itself was not affected. Moreover, Allison et al. (1998) observed that NMDA receptor clusters partially migrate from synapses to non-synaptic sites through disruption of actin filaments in dendritic spines by latrunculin A, which sequesters G-actin and brings about net actin filament depolymerization. Therefore, the presence of postsynaptic core proteins, such as PSD family proteins, at postsynaptic scaffolds may not always be sufficient to induce synaptic anchoring of NMDA receptors. Regulatory steps other than clustering, such as association with actin filaments, seem necessary for the anchoring of NMDA receptors at postsynaptic scaffolds.

3. Anchoring of actin filaments

Although actin is a major cytoskeletal element of dendritic spines, the mechanism by which actin filaments are anchored in the spines remains unclear. A mechanism for enrichment of actin within a subcellular compartment was suggested in 1993. Hill and Gunning (1993) showed that the 3'-untranslated sequences of actin guided the sorting of actin isoform mRNAs in non-neuronal cells. The transport of actin mRNA may provide a mechanism for enrichment of actin within a subcellular compartment via local synthesis. Data which supports this idea has been obtained in studies of developing neurons; actin mRNA was enriched in growing neurites and growth cones. (Bassell et al., 1998) However, Kaech et al. (1997) have reported that protein, rather than mRNA, is involved in the targeting of actin to spines. They used epitope-tagged actin isoform cDNA without the 3'-untranslated cytoplasmic actin sequence. In transfection studies using these constructs and 4~5-week mature neurons in culture, β - and γ -cytoplasmic actins, which are expressed in the brain, were correctly targeted to spines, whereas α -cardiac muscle actin, which is normally absent from neurons, aggregated in dendrites. Moreover, C-terminal actin sequences were shown to determine spine targeting. Results of these studies indicate that certain specific sequences of actin may play important roles in targeting actin to spines. The sequences might form sites involved in association with other unique components of postsynaptic scaffolds. Such a mechanism may be responsible for the anchoring of actin filaments in dendritic spines.

Postsynaptic scaffolds consist of two structural and correspondingly functional levels of molecular constituents: stable core components of PSD, and dynamic actin cytoskeletons with their binding proteins. (Adam and Matus, 1996) Two of these actin-binding proteins, drebrin A and α -actinin-2, have been reported to be specifically accumulated within dendritic spines (Shirao et al., 1987; Hayashi et al., 1996; Wyszynski et al., 1998). Since actin filaments in spines are frequently associated with such actin-binding proteins, they may play important roles in anchoring actin filaments in them. These actin-binding proteins may turn out to be the hypothetical regulatory proteins that mediate receptor-cytoskeleton interaction, as mentioned by Rosenmund and Westbrook (1993).

It has recently been reported that the actin-binding protein SH3P7 functions in lymphocytes as an adaptor protein, and that it has motifs mediating specific protein-protein interaction and linked antigen-receptor signaling to components of the cytoskeleton (Larbolette et al., 1999). Because the N-terminal domain of the drebrin polypeptide is very similar to that of SH3P7, drebrin may act as an adaptor protein in neurons,

linking synaptic activation to the actin cytoskeleton and modifications of its organization.

4. Characteristics of actin filaments in dendritic spines

Drebrin is an F-actin-binding protein (for review, see Shirao, 1995) which has ADF-H domain in its N-terminal region (Lappalainen et al., 1998). Drebrin A is a neuron-specific isoform. Drebrin E is a ubiquitous isoform (Shirao and Obata 1986; Keon et al., 2000). Drebrin competitively inhibits the actin-binding activity of tropomyosin, fascin and α -actinin (Ishikawa et al., 1994; Sasaki et al., 1996), and imparts a unique character to the actin cytoskeleton bound to it (Shirao, 1995). Biochemical studies have indicated that drebrin is one of the regulators of actin cytoskeleton organization (Shirao et al., 1992; Ishikawa et al., 1994; Shirao et al., 1994 Hayashi et al., 1996). In adult cerebral cortex, drebrin A is the major isoform expressed, and is highly concentrated in the dendritic spines (Shirao et al., 1987 Hayashi et al., 1996).

Recently, a study of expression of drebrin A cDNA in cultured cortical neurons has yielded results which demonstrate that drebrin A autonomously accumulates in dendritic spines and causes their elongation (Hayashi and Shirao, 1999). In this study, green fluorescent protein (GFP)-tagged drebrin A cDNA was transfected into 1-week culture of cortical cells dissected from 20-day-old fetal rats. Transfected neurons showed very prominent fluorescence at the dendritic spines, with low levels of diffuse fluorescence in the dendritic shafts and cell bodies. Furthermore, spines labeled with GFP-drebrin A were markedly longer than those of the control DiI-labeled or GFP-labeled spines, although there was no difference in the number of spines between transfected neurons and control neurons. These data suggest that drebrin A is involved in regulation of spine morphology.

Does drebrin A play some roles in anchoring actin filaments in dendritic spines? It has been reported that actin filaments in the spines of cultured neurons show particular stability against the actin-depolymerizing reagent cytochalasin D (Allison et al., 1998). Since cytochalasin D caps the fast growing plus end of an actin filament and induces depolymerization of actin filaments, stability against cytochalasin D indicates a low rate of turnover of actin filaments in dendritic spines. We reported that cytochalasin D cannot disrupt drebrin-binding actin filaments in neuronal cell line SY5Y, although it can disrupt tropomyosin-binding actin filaments (Asada et al., 1994). Furthermore, results of a study of expression of drebrin A cDNA in fibroblasts demonstrated that the retraction-process formation induced by cytochalasin D is inhibited in transfected cells, and suggest that drebrin-expressing

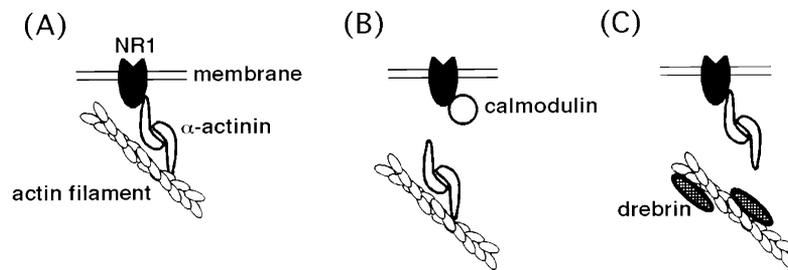


Fig. 2. Protein–protein interactions between the NR1 subunit, α -actinin and actin filaments can be regulated in dendritic spines by calmodulin and drebrin. (A) NR1 is anchored to acting filaments via α -actinin at resting intracellular Ca^{2+} . (B) Activated calmodulin dissociates α -actinin from NR1 by competitive displacement. (C) Even at resting intracellular Ca^{2+} levels, the binding of α -actinin to actin filaments can be competitively inhibited by drebrin.

cells form cytochalasin-resistant adhesion plaques (Ikeda et al., 1996). These observations indicate that the cytochalasin D-resistant stability characteristics of actin filaments within spines might result from a high concentration of drebrin in them. Thus, drebrin may play a role in anchoring actin filaments by maintaining a low rate of turnover of actin filaments within dendritic spines.

In the past 4 years, there have been many reports that drebrin is associated with a structurally and functionally distinct pool of actin cytoskeletons that exhibit polarity in their subcellular localization within a cell. (1) Cells of the neuronal cell line SY5Y contain two types of actin filaments (Asada et al., 1994); one is bound to drebrin E, and the other is a stress fiber bound to tropomyosin. (2) Drebrin-E-binding actin filaments and fascin-binding actin filaments are differentially localized in the neuronal growth cone of PC12 cells (Sasaki et al., 1996). (3) In the acid-secreting parietal cells of the gastric gland, drebrin E is distributed in association with the actin filaments in the microvilli and submembranous regions of the canaliculi (Keon et al., 2000). (4) In the acid-secreting type A intercalated cells of the kidney, drebrin E is specifically concentrated among actin filaments at the apical membrane, but not at the basal membrane (Keon et al., 2000). (5) Fucini et al. (2000) reported that activation of the ADP-ribosylation factor (ARF) stimulates the assembly of two distinct pools of actin on Golgi membranes; one contains drebrin E, and the other contains spectrin. (6) Peitsch et al. (1999) have recently reported that drebrin forms actin-anchoring junctional plaques distinct from the vinculin-based microfilament system. These data clearly show that drebrin can be associated with a distinct actin pool which is found in different locations from other actin pools within a cell. Therefore, we favor the hypothesis that drebrin A forms a distinct pool of actin filaments, and that this actin pool is targeted to the dendritic spines, resulting in a high concentration of actin filaments anchored within the dendritic spine.

α -actinin is a member of the spectrin/dystrophin family of proteins, which cross-link and bundle actin filaments (Bubb, 1999). Rao et al. (1998) reported that, in cultured hippocampal neurons, α -actinin-2 colocalizes with the NMDA receptor only at synaptic clusters in dendritic spines, and not at shaft non-synaptic or synaptic clusters. Wyszynski et al. (1997) demonstrated that α -actinin-2 binds by its central rod domain to the cytoplasmic tail of both the NR1 and NR2B subunits of the NMDA receptor, and that this interaction is directly antagonized by Ca^{2+} /calmodulin. Since α -actinin-2 binds to actin filaments *in vitro*, α -actinin-2 may bind NMDA receptors to actin filaments *in vivo*. Krupp et al. (1999) proposed a model for the interaction between NR1 and actin filaments. At rest, NR1 is anchored to actin filaments via α -actinin (Fig. 2A). Calcium influx during receptor activation activates calmodulin, resulting in the dissociation of NR1 from the α -actinin, possibly by competitive displacement of α -actinin (Fig. 2B). As a result, NR1 is dissociated from actin filaments. However, NR1 could be dissociated from actin filaments by another molecular mechanism without activating calmodulin. The balance between the levels of drebrin and α -actinin likely regulates the interaction of NR1 with actin filaments, because binding between α -actinin and actin filaments is competitively inhibited by drebrin (Ishikawa et al., 1994) (Fig. 2C). Taken together, this indicates that α -actinin-2 and drebrin A have a modulatory role in the anchoring of molecular constituents in dendritic spines.

5. Activity-dependent anchoring of actin filaments in dendritic spines

Recent developments in the study of dendritic spine cytoskeleton support the idea that rapid reorganization of actin cytoskeleton is the basis for rapid morphological change of dendritic spines in response to synaptic activity (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999). Although NMDA receptor activation results in an increase of intracellular calcium, it is not yet

known whether this increased calcium actually induces reorganization of actin cytoskeleton in dendritic spines.

It has recently been reported that NMDA receptor activity disrupts the anchoring of actin filaments and actin-binding proteins in dendritic spines (Halpain et al., 1998). Cultured hippocampal neurons exposed to 50 μ M NMDA for as little as 5 min exhibit a rapid and selective loss of actin filaments from dendritic spines, with no observed changes in NMDA receptors or PSD-95 at the postsynaptic sites. There is no apparent change in actin filaments in cell bodies or in dendritic shafts or axons. This rapid loss of actin filaments from spines seems related to destabilization of the actin cytoskeleton, because pretreatment with the actin-stabilizing compound jasplakinolide attenuates this effect of NMDA receptor activation (Halpain et al., 1998). It has also been shown that the calcium influx through NMDA receptors is responsible for this reduction in actin filaments in spines. An agonist of metabotropic glutamate receptor (ACPD) and an L-type calcium channel agonist (BAYK-50861) had little effect on spine actin, although they induced a decrease in actin-filament staining in the cell bodies.

Calcineurin is a calcium- and calmodulin-dependent protein phosphatase. Although calcineurin immunoreactivity is present throughout the neuron, intense punctate staining of calcineurin is observed in dendritic spines. Such calcineurin punctate staining is disrupted in parallel with actin punctate staining in response to NMDA, although direct binding activity of calcineurin to actin filaments has not been observed. NMDA-induced loss of actin filaments from dendritic spines is attenuated by preincubation with the calcineurin antagonist cyclosporin. These observations lead us to imagine that dephosphorylation of regulatory proteins, such as drebrin A and α -actinin-2, by calcineurin (induced by a rise in intracellular Ca^{2+} through the NMDA receptor) leads to the disruption of mechanisms for the anchoring of actin filaments.

6. Conclusion

Recent technological progress (e.g. cDNA cloning, expression studies of tagged proteins, and immunofluorescence analysis using confocal microscopy) has yielded great amounts of information about unique molecular constituents of dendritic spines. Over 100 years after Ramon y Cajal's first description of these spines, we can now monitor dynamic changes in spine morphology in relation to synaptic activity and synaptic plasticity. We can even trace translocations of specific proteins in living cells. The major molecular players in the workings of postsynaptic scaffolds are already being characterized, as described in this article. Elucidation of clustering and anchoring mechanism of

molecular constituents at postsynaptic scaffolds will result in new insights into spine function.

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