Review

Structure and molecular organization of dendritic spines

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Summary. Dendritic spines mediate most excitatory synapses in the CNS and are therefore likely to be of major importance for neural processing. We review the structural aspects of dendritic spines, with particular emphasis on recent advances in the characterization of their molecular components. Spine morphology is very diverse and spine size is correlated with the strength of the synaptic transmission. In addition, the spine neck biochemically isolates individual synapses. Therefore, spine morphology directly reflects its function. A large number of molecules have been described in spines, involving several biochemical families. Considering the small size of a spine, the variety of molecules found is astounding, suggesting that spines are paramount examples of biological nanotechnology. Single-molecular studies appear necessary for future progress. The purpose of this rich molecular diversity is still mysterious but endows synapses with a diverse and flexible biochemical machinery.

Key words: Synapse, Computation, LTP, Learning

Ultrastructure of dendritic spines

Dendritic spines are small protrusions which arise from dendrites of various types of neurons in the central nervous system (Fig. 1), including pyramidal neurons in the cortex and the hippocampus and Purkinje cells in the cerebellum. Dendritic spines were first described by Cajal (Ramón y Cajal, 1888), who argued against many of his contemporaries that they were not artifacts of the Golgi stain. Not only did he proposed that they were real, but he also postulated that spines were the primary site of synaptic contact between dendrites and axons (Ramón y Cajal, 1891) and were involved in learning and memory (Ramón y Cajal, 1893). Later, Gray (1959) confirmed that dendritic spines are indeed the sites of neuronal connection using electron microscopy (EM).

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At the EM level, dendritic spines appear as small protrusions (<2 μm length, from dendrite to the tip) typically having a spherical head (0.5-1.5 μm diameter) connected by a narrow neck (<0.5 μm diameter) to the dendritic shaft. Spine morphology is highly variable from spine to spine, even in the same dendrites. Spines have been morphologically categorized into three types: thin, mushroom and stubby spines, according to Peters and Kaiserman-Abramof (1970) (Fig. 1C). Thin spines are most common and have a thin, long neck and a small bulbous head. A smaller subset of spines with a large head are called mushroom spines. Stubby spines are devoid of a neck (Jones and Powell, 1969; Peters and Kaiserman-Abramof, 1970) and are particularly prominent during postnatal development (Harris et al., 1992).

A majority of spines have single heads but a few of them are branched with multiple heads. In dentate granule cells (Trommald and Hulleberg, 1997) and CA1 pyramidal neurons (Harris et al., 1992; Fiala and Harris, 1999), only ~2% of all spines are branched. The morphologies of two heads show variations similar to those found in simple spines. There have been no cases reported where their two heads of branched spines make synapses with the same axon (Harris et al., 1992; Trommald and Hulleberg, 1997; Sorra et al., 1998). In contrast to typical spines, thin long protrusions without a bulbous head are called filopodia and are developmentally transient structures disappearing after the peak period of synaptogenesis (Miller and Peters, 1981; Fiala et al., 1998).

Most spines receive an asymmetric excitatory synapse on their heads (Fig. 1A,B). On the EM level, these synapses are characterized by a thick, postsynaptic density (PSD) located in the cytoplasmic surface of the synaptic membrane, a relatively wide intermembrane distance between pre- and postsynaptic membranes, and thinner presynaptic densities (active zones) and vesicles (Gray, 1959). The PSDs seem to represent proteins located beneath postsynaptic membranes (Kennedy, 2000) and are either disk-like continuous, "macular" PSDs (Fig. 1A,D), or large and irregular-shape, "perforated" PSDs (Fig. 1B,D). In single sections, perforated PSDs are often found as multiple separate

PSDs (Fig. 1B) but, in many cases, they are found to be connected following 3-D reconstruction (Peters and Kaiserman-Abramof, 1969; Cohen and Siekevitz, 1978). Most thin spines have macular PSDs, whereas more than 80% of mushroom spines have perforated ones (Harris et al., 1992). Additionally, some spines receive inhibitory symmetric synapses on their neck or near the base of spines. In contrast to excitatory synapses on the spine heads, the pre- and postsynaptic densities in these synapses have similar thickness (Gray, 1959).

Spines are often associated with polyribosomes, which represent protein synthesis machinery. Polyribosomes are found most commonly in the bases of spines, but sometimes in spine heads or necks (Steward and Levy, 1982; Spacek, 1985). A growing body of evidence shows that protein synthesis may occur locally in dendrites (Steward and Schuman, 2001). Since many of the locally synthesized proteins, such as CaMKIIa, FMRP and Arc, are synthesized depending on synaptic activity, close association of polyribosomes with spines could in principle enable synthesized proteins to be limited to single activated spines (Steward and Schuman, 2001).

Many spines also contain smooth endoplasmic reticulum (SER), which is continuous to the SER in dendritic shafts and can extend through the spine neck to head. In the hippocampus of mature rats, about a half of all spines contain some form of SER, which can take the form of vesicles, tubules, or a "spine apparatus" (Spacek and Hartmann, 1983; Spacek and Harris, 1997), a stack of SER cisternae and dense plates between the cisternae (Gray, 1959). Most mushroom spines, which also have perforated synapses, contain a spine apparatus, and their number of cisternae is positively correlated with the PSD area (Spacek and Harris, 1997). Although in the cortex and the hippocampus, the spine apparatus is common, it is rarely found in the cerebellum (Spacek, 1985).

As discussed above, the morphology of dendritic spines is highly variable. Importantly, it is becoming clear that the spine structure directly reflects the functional features of the spine synapses. The spine head volume, PSD area and the number of vesicles in presynaptic terminals are positively correlated in cerebellar Purkinje cells (Harris and Stevens, 1988) and CA1 pyramidal neurons (Harris and Stevens, 1989). Also, the active zone size, postsynaptic density size (PSD), the number of docked vesicles and spine head volume are positively correlated in the olfactory cortex (Schikorski and Stevens, 1999). The number of the docked vesicles corresponds to the readily releasable pool of vesicles, which, in turn, correlates with the release probability (Rosenmund and Stevens, 1996; Schikorski and Stevens, 2001). Therefore, the spine volume reflects the release probability of its synapse. Also, as discussed below, the PSD area is positively correlated with the number of AMPA receptors in the PSDs (Nusser et al., 1998). In addition, the spine neck length, which enables the calcium compartmentalization (Yuste and Denk, 1995), is directly correlated with the

degree of biochemical isolation (Majewska et al., 2000a,b). Therefore, morphological differences between stubby or long spines could actually reflect a significant difference in their biochemical function.

Taken together, these findings demonstrate that synaptic parameters such as spine head volume, PSD area and active zone area are directly correlated with synaptic strength, and the spine neck also is also correlated with the potential function of the spine to implement calcium-dependent learning rules (Yuste et al., 2000).

Molecular organization of dendritic spines

We will now review the different types of proteins present in dendritic spines.

Glutamate receptors

Being major sites of glutamatergic inputs in neurons, spines have all four types of glutamate receptors, NMDA, AMPA, kainate, and metabotropic glutamate receptors (Fig. 2). NMDA receptor (NMDAR) is thought to be a heterotetramer composed of NR1, NR2A-D, or NR3 and is invariably found in the PSD (Takumi et al., 1999). In addition to the binding of glutamate, NMDAR activation requires a postsynaptic depolarization to remove its blockade by Mg^{2+}. This property is important for coincidence detection of pre- and postsynaptic activation by the spines (Yuste and Denk, 1995; Yuste et al., 1999). Ca^{2+} influx through NMDAR is thought to be important in synaptic plasticity (Malenka and Nicoll, 1999). AMPA receptor (AMPAR) is also thought to be heterotetramer made from the GluR1-4 subunits. Most AMPAR is Ca^{2+}-impermeable, and this feature appears to be controlled by the incorporation of the GluR2 subunit. Whereas AMPAR comprising of GluR1, 3 and 4 shows Ca^{2+}-permeability, GluR2-containing AMPAR is not Ca^{2+}-permeable (Hollmann et al., 1991). This is attributed to RNA editing which converts a glutamine residue in the channel pore forming domain to a positively charged arginine (Hume et al., 1991; Sommer et al., 1991). A number of electrophysiological experiments have suggested that there is a large variability in the number of functional AMPAR from synapse to synapse, although every synapse might contain NMDAR (Malenka and Nicoll, 1997). Indeed, immunoelectron microscopy showed that the number of AMPAR in single synapses is variable from spine to spine and is correlated with the PSD area (Nusser et al., 1998). Also, no AMPAR was detected in ~15% of asymmetric synapses located on spines in CA1 (Nusser, 1998), consistent with the idea of silent synapses formed exclusively of NMDAR (Malenka and Nicoll, 1997). These electrophysiological and morphological findings are consistent with two-photon imaging of calcium accumulations in spines mediated by NMDAR and AMPAR (Yuste et al., 1999). Also, mapping of functional AMPAR by two-photon uncaging of caged
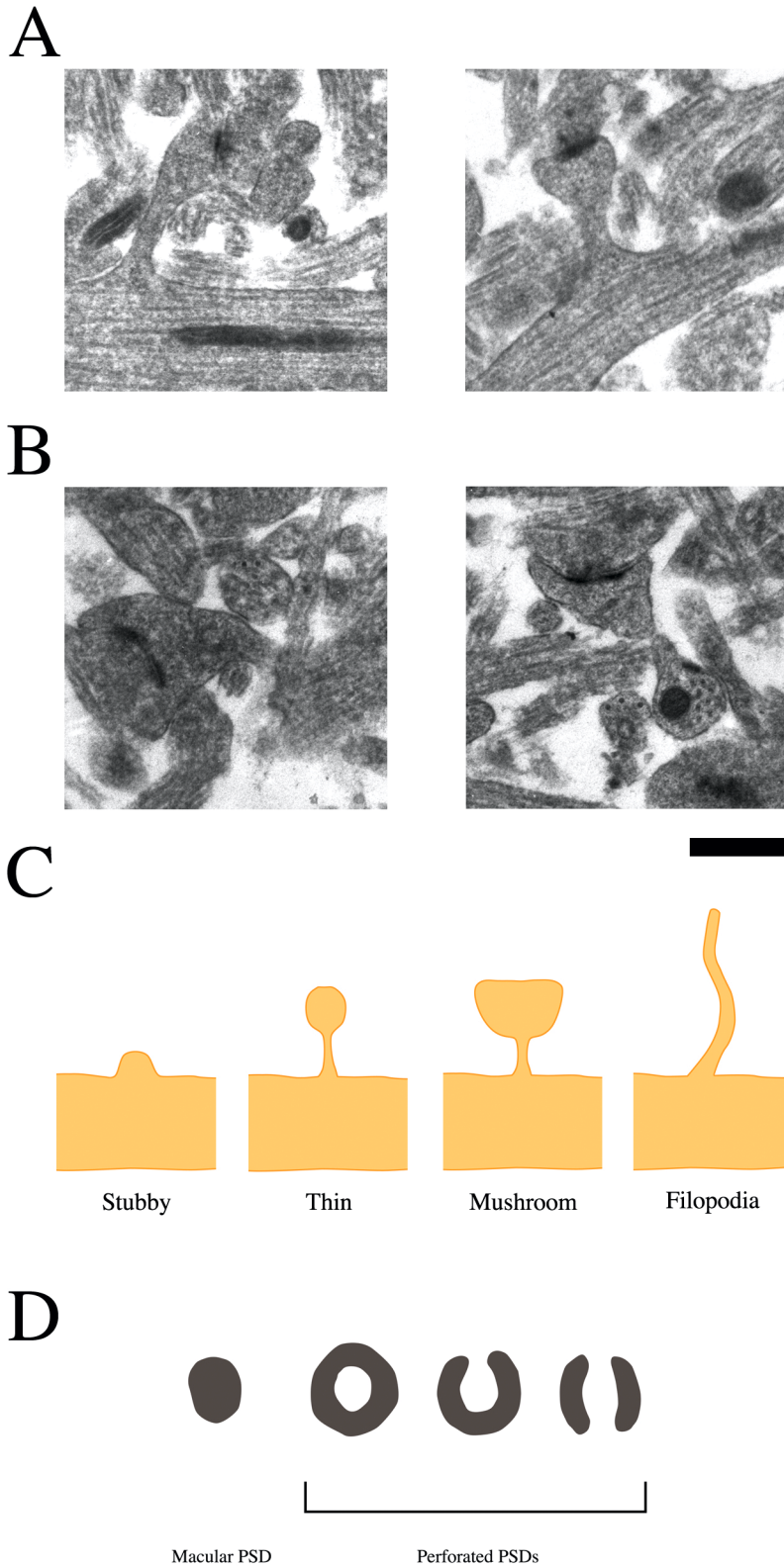
A

B

C

Stubby   Thin   Mushroom   Filopodia

D

Macular PSD   Perforated PSDs

Fig. 1. Ultrastructure of Dendritic spines. Electron micrographs of dendritic spines in hippocampal slice culture. **A.** Thin spines with macular PSDs. **B.** Mushroom spines with perforated PSDs. **C.** Schematic drawing of spine morphologies in categories, as described by Peters and Kaiserman-Abramof (1970). **D.** Schematic drawing of macular and perforated PSDs. Scale bar: 500 nm.

glutamate has recently been done (Matsuzaki et al., 2001).

Kainate receptors (KAR) are composed of GluR5-7 or KA1-2 subunits (Chittajallu et al., 1999) and are also found in the PSDs (Huntley et al., 1993; Roche and Huganir, 1995). Like the GluR2 subunit of AMPAR, GluR5 and 6 subunits are subjected to RNA editing which regulates Ca^{2+}-permeability (Chittajallu et al., 1999).

Finally, metabotropic glutamate receptors (mGluR) are G-protein coupled receptors. Spines contain mGluR1 and 5 (Baude et al., 1993; Lujan et al., 1996), which are coupled to phospholipase C and phosphoinositide hydrolysis producing inositol triphosphate (IP3) (Finch and Augustine, 1998; Takechi et al., 1998). Interestingly, these mGluRs are found in the periphery, but not in the center, of the PSD (Baude et al., 1993; Lujan et al., 1996), suggesting the existence of functional subcompartments within the PSD or the spines.

Molecules related to Ca^{2+} homeostasis

In addition to Ca^{2+} influx through glutamate receptors, Ca^{2+} influx into spines occurs through
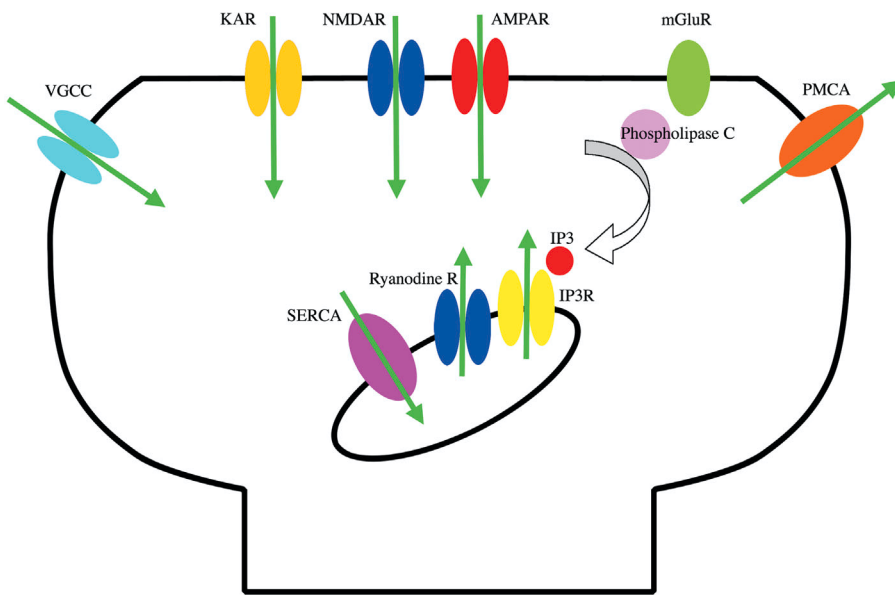

Fig. 2. Glutamate receptors and molecules related to Ca^{2+} homeostasis in dendritic spines. Green arrows indicate Ca^{2+} influx to and efflux from spine cytoplasm.
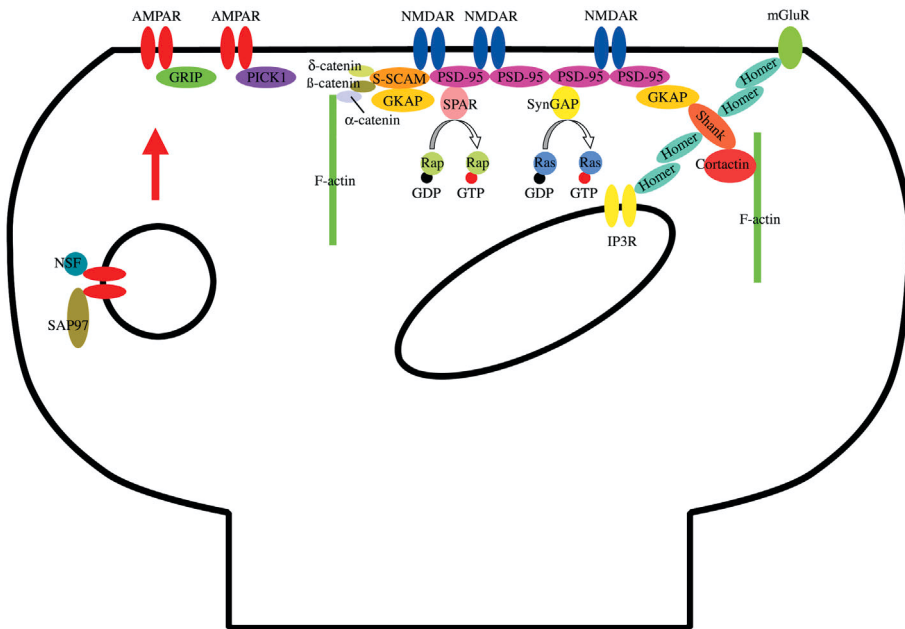

Fig. 3. NMDA receptor-PSD95 complex and AMPAR binding proteins.

voltage-gated Ca^{2+} channels (VGCC) (Yuste et al., 2000; Fig. 2). VGCC was detected in spines using fluorescence-labeled ω-conotoxin, a selective blocker for VGCC (Mills et al., 1994). Moreover, with fluctuation analysis of Ca^{2+} transients induced by action potentials, the number of VGCC in single spines was estimated to be 1-20 (Sabatini and Svoboda, 2000).

Cytoplasmic Ca^{2+} can also be increased through release from internal stores. Inositol triphosphate receptors (IP3R) and ryanodine receptors (Fig. 2), involved in release of Ca^{2+} from internal stores, are found on smooth endoplasmic reticulum in spines (Walton et al., 1991; Sharp et al., 1993; Martone et al., 1997). However, they appear to exist differentially in spines from different brain regions. In rat hippocampus, ryanodine receptors, but not IP3 receptors, were found in spines (Sharp et al., 1993). On the other hand, both receptors were found in neostriatal spines (Martone et al., 1997). Finally, IP3 receptors, but not ryanodine receptors, were present in spines from avian cerebellum (Walton et al., 1991).

Molecules involved in exclusion of cytoplasmic Ca^{2+} are also found in spines (Fig. 2). Plasma membrane Ca^{2+}-ATPase (PMCA) pumps out cytoplasmic Ca^{2+}, and one of four isoforms of PMCA, PMCA2 was found in spines using immunoEM (Hillman et al., 1996; Stauffer et al., 1997). Also, two of the isoforms, PMCA 2b and 4b were shown to interact with PSD-95 family proteins (see below; DeMarco and Strehler, 2001). Cytoplasmic Ca^{2+} is also sequestered into the ER by sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA; Fig. 2). SERCA is also thought to be present in spines (Majewska et al., 2000a).

NMDAR-PSD-95 complex

In addition to glutamate receptors, various proteins are found in the PSD of asymmetric synapses (Fig. 3). PSD-95/SAP90, PSD-93/Chapsyn-110, SAP97 and SAP102 compose a PSD-95 subfamily of membrane-associated guanylate kinase (MAGUK) protein family, characterized by multiple protein-binding domains including 3 PDZ domains, a src homology 3 (SH) domain and a guanylate kinase-like (GK) domain (Sheng and Sala, 2001). Through these protein-binding regions, PSD-95 and other members of PSD-95 family interact with a variety of proteins and are thought to work as a scaffold in the PSD (Sheng and Sala, 2001). Specifically, the PDZ domains of PSD-95, PSD-93 and SAP102 bind to the C-terminal of NR2 subunit (Kornau et al., 1995; Kim et al., 1996; Muller et al., 1996a). The C-terminal sequence of NR2 may be required for the localization of NMDA receptors to the PSD (Mori et al., 1998; Steigerwald et al., 2000). However, the interaction

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Table 1. Proteins in NMDAR/PSD-95 complex

PSD-95/SAP90, PSD93/Chapsin-110, SAP97, SAP102
Members of PSD-95 family of MAGUK proteins. They contain 3 PDZ domains, 1 GK domain and 1 SH3 domain, and interact with a variety of membrane and cytoplasmic proteins. Binding partners of PSD-95 includes K^+ channels, kainate receptor subunits (GluR6 and KA1), neuroligin, neuronal NO synthase, PMCA4b and citron (Sheng and Sala, 2001).

GKAP/SAPAP
GKAP is consisted of 4 members, GKAP 1-4 (Sheng and Kim, 2000). The PDZ domain of GKAP binds to the GK domain of PSD-95 (Kim et al., 1997; Naisbitt et al., 1997; Satoh et al., 1997; Takeuchi et al., 1997) and of S-SCAM (Hirao et al., 1998). The C terminal of GKAP binds to the PDZ domain of shank (Naisbitt et al., 1999).

Shank
Characterized by a variety of protein-binding domains such as multiple ankyrin repeats, a SH domain, a PDZ domain, a proline-rich region and a SAM domains. Shank comprises three known members, Shank 1, Shank2 and Shank 3 (Sheng and Kim, 2000). Shank binds to Homer (Tu et al., 1999) and actin-binding protein, cortactin (Naisbitt et al., 1999).

Homer
Products of three genes, Homer 1, Homer 2 and Homer 3. Homer contains an EVH1 domain and a C-terminal coiled coil (CC) domain (Xiao et al., 2000). One of splice variants, Homer 1a, is a product of immediate early gene regulated by synaptic activity (Brakeman et al., 1997), whereas Homer 1b and c are constitutively expressed (Kato et al., 1998; Xiao et al., 1998). Homer 1b and 1c contains a CC domain and show self-multimerization, but Homer 1a lacks a CC domain (Kato et al., 1998; Xiao et al., 1998). Homer binds to mGluR, IP3R and Shank (Tu et al., 1998, 1999).

SynGAP
A Ras GTPase-activating protein (GAP). SynGAP binds to the PDZ domains of PSD-95, and its GAP activity is inhibited by CaMKII (Chen et al., 1998; Kim et al., 1998).

SPAR
A Rap GTPase-activating protein. SPAR binds to the GK domain of PSD-95 and may regulate spine morphology (Pak et al., 2001).

S-SCAM
S-SCAM contains 5 or 6 PDZ domains, a GK domain and two WW domains (Hirao et al., 1998). Three isoforms, S-SCAMα, ß and γ are found (Hirao et al., 2000b). S-SCAM binds to GKAP through the GK domain (Hirao et al., 1998), and PSD-95 (Hirao et al., 2000b), neuroligin, NMDAR (Hirao et al., 1998), ß- and δ-catenin (Ide et al., 1999; Nishimura et al., 2002) through the PDZ domains.

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between NMDAR and PSD-95 does not seem important for synaptic localization of NMDAR since the localization of NMDAR appears intact in PSD-95 knockout mice (Migaud et al., 1998). Also, when synaptic localization of PSD-95, PSD-93 and presumably other PSD-95 family proteins was disrupted by a mutant of CRIPT, a microtubule-binding protein found in spines (Niethammer et al., 1998), the synaptic clustering of NMDAR was still intact (Passafaro et al., 1999). Thus, the function of PSD-95 does not seem to be synaptic clustering of NMDAR but instead its link to intracellular signaling machinery (Migaud et al., 1998).

Through PSD-95, NMDAR makes a complex with a variety of proteins including GKAP, Shank, Homer, SynGAP, SPAR and S-SCAM (Fig. 3; see Table 1 for description of individual proteins). Through the PSD-95/GKAP/Shank/Homer complex, NMDAR is also linked to mGluR and IP3R (Tu et al., 1998, 1999). This linkage may enable functional interaction between these receptors, and their close association may allow Ca^{2+} influx through NMDAR and IP3 generated in mGluR-dependent manner to access IP3R and regulate its function (Tu et al., 1999). This coupling can be regulated by neuronal activity through activity-dependent expression of Homer 1a, which is thought to work as an endogenous dominant negative regulator for constitutive forms of Homer (Xiao et al., 1998). Also, the proximity of NMDAR to SynGAP (Kim et al., 1998) and SPAR (Pak et al., 2001) may enable synaptic activity to be coupled to Ras and Rap signaling pathways.

AMPAR-binding proteins

Synaptic expression of AMPAR is thought to be dynamically regulated by neuronal activity (Malinow and Malenka, 2002). Several proteins interact with specific subunits of AMPAR and may mediate this dynamic regulation of trafficking and stabilization of AMPAR. AMPAR subunits have PDZ-binding motif in its C terminal and, through this motif, bind to PDZ proteins (Sheng and Sala, 2001). Whereas GluR1 binds to SAP97 (Leonard et al., 1998), GluR2 and GluR3 bind to PDZ-containing proteins, GRIP/ABP (Dong et al., 1997; Srivastava et al., 1998) and PICK1 (Xia et al., 1999). Disruption of these interaction prevents synaptic localization of AMPAR (Hayashi et al., 2000; Osten et al., 2000). Other than PDZ proteins, GluR2 also binds to NSF, an ATPase which has a role in membrane fusion

Table 2. Actin-binding proteins found in dendritic spines.

Myosin (Miller et al., 1992; Cheng et al., 2000; Walikonis et al., 2000)
An actin-based motor protein. Two isoforms of myosin heavy chain subunits, myosin IIB (Miller et al., 1992; Cheng et al., 2000) and V (Walikonis et al., 2000) are found in spines. Myosin IIB makes a complex with actin, drebrin, and gelsolin (Hayashi et al., 1996).

DLC (Naisbitt et al., 2000)
A light chain subunit of microtubule-based motor, dynein, and myosin V. DLC interacts with GKAP (Naisbitt et al., 2000).

Drebrin (Hayashi et al., 1996)
Drebrin interacts with gelsolin and myosin IIB and modulates actomyosin interaction in vitro (Hayashi et al., 1996). Overexpression of drebrin makes spines longer (Hayashi and Shirao, 1999).

Gelsolin
A Ca^{2+}-sensitive actin-severing and capping protein (Kwiatkowski, 1999)

α-actinin, Spectrin and Fodrin (Wyszynski et al., 1997; Ursitti et al., 2001).
Actin-crosslinking proteins in spectrin family (Matsudaira, 1991). Ca^{2+} inhibits actin-crosslinking ability. α-actinin binds to the C terminal of both NR1 and NR2B subunits, and this binding may be inhibited by Ca^{2+}/calmodulin (Wyszynski et al., 1997). α-fodrin binds to ankyrin repeats of Shank (Bockers et al., 2001).

Protein 4.1N (Walensky et al., 1999)
A brain isoform of protein 4.1 which contains spectrin-actin–binding domains (Hoover and Bryant, 2000). 4.1N binds to a membrane proximal region of the GluR1 C-terminal (Shen et al., 2000) and to a MAGUK protein, CASK (Biederer and Sudhof, 2001).

Adducin (Seidel et al., 1995)
Adducin binds to spectrin and recruits spectrin to the fast-growing end of F-actin (Matsuoka et al., 2000). This actin-related activity is regulated by Ca^{2+}/calmodulin and phosphorylation by PKA, PKC and Rho kinase (Matsuoka et al., 1996, 1998; Fukata et al., 1999).

Spinophilin/Neurabin II (Allen et al., 1997)
An actin binding protein with a PDZ domain. Spinophilin binds to PP1 and regulates PP1 activity (Allen et al., 1997; Satoh et al., 1998).

Synaptopodin (Mundel et al., 1997)
Synaptopodin is enriched in dendrites and spines, specifically around the spine apparatus in the spine neck (Deller et al., 2000).

Cortactin (Naisbitt et al., 1999)
An actin binding protein which has a role in cortical actin assembly and membrane dynamics (Weed and Parsons, 2001). Cortactin binds to Shank (Naisbitt et al., 1999).

(Nishimune et al., 1998; Osten et al., 1998; Song et al., 1998). In addition, GluR1 binds to an actin binding protein, protein 4.1N (Shen et al., 2000). Disruption of these bindings also reduces the surface expression of AMPAR (Noel et al., 1999; Shen et al., 2000).

Actin and associated proteins

Electron micrographs of spines show that spine heads are filled with a "fluffy" indistinct material, and indeed this feature is used as a way to distinguish spine profiles from surrounding neuropil and particularly from dendrites, which are instead rich in microtubules (Peters et al., 1991). This material represents actin filaments probably broken during fixation or staining procedure (Fifkova and Morales, 1992). The organization of actin filaments can be preserved when they are decorated by myosin subfragments. With this technique, it was shown that actin filaments form a mesh-like network in spine heads and interact with plasma membrane and the PSD by their barbed ends, whereas, in spine necks, actin filaments form long bundles (Fifkova and Delay, 1982).

Neurons express two isoforms of actin, ß- and γ-actin, both of which are enriched in dendritic spines (Kaech et al., 1997). Polymerized actin (F-actin) in spines is more stable in comparison to F-actin from other cellular compartments. For example, an actin polymerization blocker, cytochalasin D, does not effectively depolymerize F-actin in spines even after 24 hours of treatment, whereas it readily disrupts F-actin in the cell body and dendritic shaft (Allison et al., 1998). However, another polymerization blocker, latrunculin A can depolymerize F-actin effectively in spines (Allison et al., 1998).

Dendritic spines also contain a variety of actin-associated proteins, including myosin, drebrin, α-actinin, fodrin, spectrin, adducin, spinophilin, synaptopodin and cortactin (Fig. 4; see Table 2 for description of individual proteins). Together with actin filaments, these proteins could play a role in functions such as vesicle and protein transport, regulation of spine morphology or anchoring of membrane proteins.

Two types of myosin heavy chain subunits, Myosin IIB (Miller et al., 1992; Cheng et al., 2000) and V (Walikonis et al., 2000), have been found in spines. Myosin V interacts with GKAP through a light chain subunit of Myosin V, dynein light chain (DLC), suggesting that GKAP/DLC interaction may be involved in trafficking of proteins in the PSD-95 complex (Naisbitt et al., 2000). Myosin IIB can make a complex with actin, drebrin, and gelsolin, and drebrin was shown to regulate actomyosin interaction (Hayashi et al., 1996).

Two of these actin-binding proteins, α-actinin and protein 4.1N, may directly couple glutamate receptors to the actin cytoskeleton. α-actinin binds to actin filaments and the cytoplasmic tails of both NR1 and NR2B subunits (Wyszynski et al., 1997). This binding may be regulated by synaptic activity since NR1/α-actinin binding is inhibited by Ca^{2+}/calmodulin (Wyszynski et al., 1997). Protein 4.1N contains spectrin-actin–binding domains (Walensky et al., 1999) and binds to a membrane proximal region of the GluR1 C-terminal (Shen et al., 2000). Two other actin-binding proteins, cortactin (Naisbitt et al., 1999) and α-fodrin (Bockers et al., 2001), binds to Shank and may connect NMDAR and mGluR to the actin cytoskeleton through the PSD-95/GKAP/Shank/Homer complex.

As suggested from multiple links between actin and glutamate receptors, the actin cytoskeleton is thought to have important roles in anchoring glutamate receptors. Complete depolymerization of F-actin in spines by latrunculin A led to a 40% decrease the number of spines with NMDAR clusters or AMPAR clusters (Allison et al., 1998). Although the synaptic localization of NMDAR clusters was disrupted, NMDAR remained clustered in nonsynaptic sites. On the other hand, AMPAR dispersed, suggesting that synaptic clustering of NMDAR and AMPAR is dependent on F-actin in different ways (Allison et al., 1998). Also, GluR1 is extractable with TritonX 100, but NR1 is not, suggesting that NMDAR tightly associates with cytoskeleton, but AMPAR associates weakly (Allison et al., 1998). The association of NMDAR with PSD-95 and GKAP is not dependent on F-actin, whereas this protein complex is tightly associated with F-actin (Allison et al., 1998, 2000). The clustering of α-actinin, drebrin and CaMKIIa (see below) in spines is F-actin-dependent, and these proteins are weakly associated with F-actin (Allison et al., 1998, 2000).

Many of these actin-binding proteins can be regulated by Ca^{2+}. For example, gelsolin is a Ca^{2+}-sensitive actin-severing and capping protein (Kwiatkowski, 1999). Also, the actin-related activity of adducin is regulated by Ca^{2+}/calmodulin (Matsuoka et al., 1996). In addition, fodrin and spectrin can be degraded by a Ca^{2+}-dependent protease, calpain (Siman et al., 1984). These Ca^{2+}-dependent properties of actin-binding proteins might be important in the regulation of actin dynamics in spines. For instance, recently gelsolin is suggested to have an important role in activity-dependent regulation of actin turnover in spines (Star et al., 2002).

Finally, actin-binding proteins may regulate formation and morphogenesis of spines. For example, in spinophilin knockout mice, the spine density of neurons was abnormally high during development (Feng et al., 2000). Also, overexpresion of drebrin causes elongation of spines (Hayashi and Shirao, 1999).

Other cytoskeleton proteins

The existence of microtubules in spines is controversial. Using microtubule-preserving technique including albumin-pretreatment, it was proposed that microtubules connect the spine apparatus to the PSD (Westrum et al., 1980). However, ß-tubulin was detected only in the PSD but not in the spine cytoplasm with immunoEM (Caceres et al., 1983). Whether a

microtubule-associated protein, MAP2, is present in spines is also controversial. Caceres et al. (1983) found strong immunoreactivity to MAP2 in spine cytoplasm, whereas Beernhardt and Matus (1984) (Bernhardt and Matus, 1984) did not detect any immunoreactivity in spines and the PSD. Also, Kaech et al. (1997) showed confinement of MAP2 in dendritic shaft using transfection of GFP-tagged MAP2.

Although intermediate filaments in spines have not been thoroughly studied, several neurofilament proteins were found in the PSD (Walsh and Kuruc, 1992) and interact with GKAP (Hirao et al., 2000a). Also, a type IV intermediate filament protein, α-internexin is found in spines (Benson et al., 1996; Suzuki et al., 1997).

Adhesion molecules

Dendritic spines also contain a variety of cell adhesion molecules, including N- and E-cadherin, cadherin-related neuronal receptor (CNR), integrins, neural cell adhesion molecule (NCAM), densin-180, neuroligin1 and syndecan-2 (Fig. 5; See Table 3 for description of individual proteins).

Like glutamate receptors, these adhesion molecules are linked to the actin cytoskeleton. Cadherins interact with actin filaments through ß-catenin and α-catenin (Takeichi, 1995), and integrins (Geiger et al., 2001) and densin-180 (Walikonis et al., 2001) binds to α-actinin.

Syndecan-2 (Hsueh et al., 1998) and neuroligin 1 (Irie et al., 1997; Hirao et al., 1998) are linked to actin filaments by binding to PDZ proteins, CASK (syndecan-2), PSD-95 and S-SCAM (neuroligin 1).

Interestingly, neuroligin 1 may have a central role in presynaptic differentiation since neuroligin ,1 even expressed in non-neuronal cells, triggers the formation of presynaptic structures on contacting axons (Scheiffele et al., 2000).

Syndecan-2 may also have an important role in spine development. Syndecan-2 accumulates on spines during the period of the morphological maturation of spines from filopodia, and exogenous expression of syndecan-2 in immature neurons causes accelerated spine formation (Ethell and Yamaguchi, 1999)

Also, N-cadherins (Tang et al., 1998), integrins (Bahr et al., 1997) and PSA-NCAM (Muller et al., 1996b) have been suggested to be involved in synaptic plasticity. This is not surprising if one considers their roles in the interaction with presynaptic or extracellular components at the synaptic junctions. For example, N-cadherin seems localized in the adhesive structure surrounding the PSD in synaptic junctions (Beesley et al., 1995; Fannon and Colman, 1996; Uchida et al., 1996; Benson and Tanaka, 1998). ß-catenin shows activity-dependent redistribution (Murase et al., 2002), and the resulting change in cadherin adhesion may regulate synaptic function and spine morphology

Table 3. Adhesion molecules in dendritic spines

N- and E-cadherins (Beesley et al., 1995; Fannon and Colman, 1996; Uchida et al., 1996; Benson and Tanaka, 1998)
Ca^{2+}-dependent homophilic cell adhesion molecules. Their cytoplasmic regions are linked to actin filaments through ß- and α-catenin (Takeichi, 1995).

CNR (Kohmura et al., 1998)
CNRs are products of 14 or 15 genes in mammal, but the proteins show a huge variety, which might be produced by somatic DNA rearrangement or trans-splicing (Hamada and Yagi, 2001).

Integrin (Einheber et al., 1996; Nishimura et al., 1998; Schuster et al., 2001)
Integrins are heterodimers composed of combinations of α- and ß-subunits and bind to the extracellular matrix. Integrins are linked to actin filaments through their binding to α-actinin and other actin-binding proteins (Geiger et al., 2001). Three subunits, α8 (Einheber et al., 1996), ß8 (Nishimura et al., 1998) and ß1 (Schuster et al., 2001) integrins are found in spines.

NCAM (Persohn and Schachner, 1987, 1990)
A Ca^{2+}-dependent homophilic adhesion molecule, which has three splice variants, NCAM 120, 140 and 180. The extracellular region of NCAM can be glycosylated by polysialic acid (PSA), and the degree of polysialation has an effect on the adhesion property of NCAM and other adhesion molecules (Walsh and Doherty, 1997).

Densin-180 (Apperson et al., 1996)
Densin-180 is a transmembrane protein containing a PDZ domain (Apperson et al., 1996), which binds to α-actinin (Walikonis et al., 2001). Densin-180 also binds to CaMKII (Strack et al., 2000b), and densin-180, CaMKII and α-actinin make a ternary complex (Walikonis et al., 2001). Densin-180 also binds to δ-catenin, which in turn binds to N-cadherin. δ-catenin is enriched in spines (Izawa et al., 2002).

Neuroligin 1 (Song et al., 1999)
A ligand of ß-neurexin (Ichtchenko et al., 1995). Neuroligin 1 and b-neurexin function as cell adhesion molecules in excitatory synapses (Song et al., 1999). Neuroligin1 also intracellularly binds to PSD-95 (Irie et al., 1997) and S-SCAM (Hirao et al., 1998).

Syndecan-2 (Hsueh et al., 1998)
A cell surface heparan sulfate proteoglycan. Its C-terminal binds to the PDZ domain of CASK (Hsueh et al., 1998). CASK co-localizes with syndecan-2 in the PSD (Hsueh et al., 1998) and interacts with protein 4.1 and links syndecan-2 to the actin cytoskeleton (Biederer and Sudhof, 2001). The C terminal of syndecan-2 binds to synbindin, which is structurally related to yeast proteins involved in vesicle transport (Ethell et al., 2000).

(Togashi et al., 2002).

Kinases

Ca^{2+}/Calmodulin dependent kinase II (CaMKII) is a major constituent of the PSD in spines (Fig. 6; Kennedy et al., 1983; Kelly et al., 1984; Ouimet et al., 1984), where it contributes >10% of the protein mass (Kelly and Cotman, 1978). CaMKII is a serine/threonine protein kinase and has two dominant isoforms, α and ß

in the brain (Soderling et al., 2001). Binding of Ca^{2+}/Calmodulin activates kinase activity and autophosphorylates Thr286 (Soderling et al., 2001). This Ca^{2+}-dependent activation of CaMKII induces targeting of CaMKII to the PSD through binding of CaMKII to NMDAR (Strack and Colbran, 1998; Leonard et al., 1999; Shen and Meyer, 1999). CaMKII phosphorylates NR2B (Omkumar et al., 1996), and this phosphorylation at Ser 1303 inhibits the binding between CaMKII and NR2B (Strack et al., 2000a). CaMKIIß binds directly to
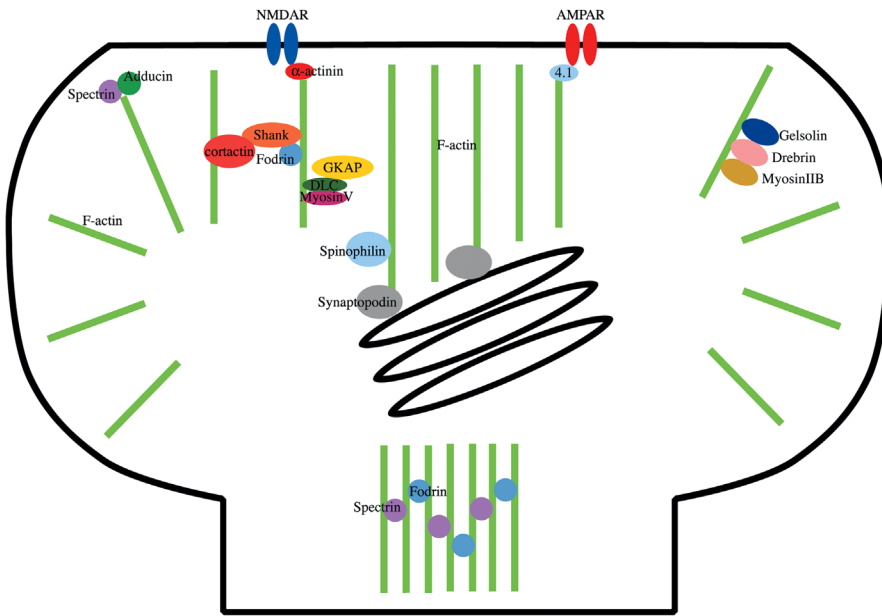

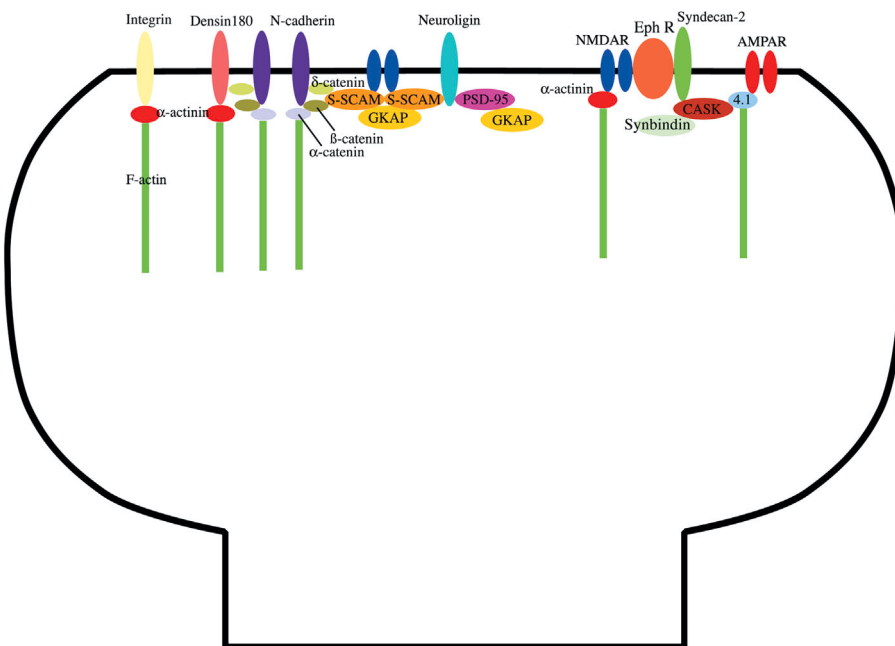**Fig. 4.** Actin (green filaments) and actin-binding proteins in dendritic spines.

Fig. 5. Cell adhesion molecules in dendritic spines.

F-actin, and may target CaMKIIα to synapses (Shen et al., 1998). Additionally, AMPAR may also be regulated by CaMKII, since CaMKII phosphorylates GluR1 subunits and enhances their single channel conductance (Barria et al., 1997; Derkach et al., 1999). Other CaMKII substrates include PSD95, SAP90, dynamin, and α-internexin (Yoshimura et al., 2000).

Protein kinase C (PKC) is a Ca^{2+}-dependent serine/threonine kinase present in the PSD (Fig. 6; Wolf et al., 1986). Specifically, α (Xia et al., 1999) and γ (Kose et al., 1990) isoforms of PKC were found in spines. PKC binds to PICK1 and this complex may be transported to spines (Perez et al., 2001). Phosphorylation of GluR2 by PKC leads to dissociation of GluR2 with GRIP/ABP and binding of PICK1 to GluR2 as described above (Matsuda et al., 1999; Chung et al., 2000). PKC also phosphorylates adducin and regulates its actin-capping ability (Matsuoka et al., 1996, 1998). In addition, PKC may regulate the distribution of NMDAR (Lan et al., 2001; Fong et al., 2002).

IP3 3-kinase A phosphorylates IP3 into inositol 1,3,4,5-tetrakisphosphate (IP4), which enhances Ca^{2+} entry from voltage-gated Ca^{2+} channels and Ca^{2+} internal release from IP3 receptors (Irvine and Schell, 2001). IP3 3-kinase A is activated by interaction with Ca^{2+}/calmodulin and through phosphorylation by CaMKII (Communi et al., 1997; Woodring and Garrison, 1997). IP3 3-kinase A binds to F-actin through N-terminal 66-amino acids, and this binding localizes the kinase to spines (Fig. 6; Schell et al., 2001).

Finally, Eph receptors are a family of receptor tyrosine kinases that were found in the PSD (Fig. 6; Torres et al., 1998; Buchert et al., 1999). Ligand binding

to a subtype of Eph, EphB receptors, induces a direct interaction of EphB with NMDAR in a kinase-independent manner, whereas EphB tyrosine kinase activity appears to be required for synapse formation (Dalva et al., 2000). EphB receptors phosphorylate syndecan-2, and this phosphorylation leads to an interaction between syndecan-2 and EphB receptors. This phosphorylation seems crucial for syndecan-2 clustering and spine maturation (Ethell et al., 2001).

Phosphatases

Several members of serine/threonine protein phosphatases have roles in synaptic transmission and plasticity (Winder and Sweatt, 2001). Among them, two members, protein phosphatase 1 (PP1) and calcineurin/protein phosphatase 2B, are found in dendritic spines (Fig. 6; Goto et al., 1986; Ouimet et al., 1995). α and γ1 isoforms of PP1 are highly concentrated in spines (Ouimet et al., 1995). PP1 binds to spinophilin, and this binding regulates PP1 activity (Allen et al., 1997). PP1 also binds to a scaffold protein, Yotiao (Westphal et al., 1999). Yotiao in turn binds to the NR1 subunit of NMDAR (Lin et al., 1998) and protein kinase A (PKA) (Westphal et al., 1999). This binding may enable PP1 and PKA to regulate NMDA receptor channel activity (Westphal et al., 1999). PP1 dephosphorylates CaMKII in the PSD (Shields et al., 1985; Strack et al., 1997), and this dephosphorylation may lead to dissociation of CaMKII from the PSD (Yoshimura et al., 1999).

Calcineurin is composed of a catalytic subunit and a regulatory subunit, and binding of Ca^{2+}/calmodulin on
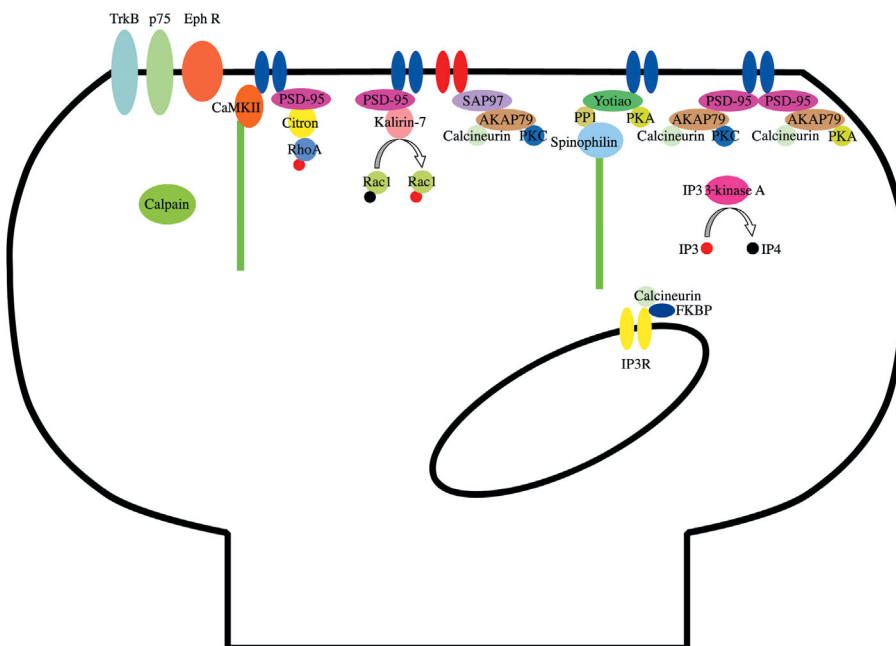

Fig. 6. Kinases, phosphatases and other proteins in dendritic spines.

the catalytic subunit activates phosphatase activity (Klee et al., 1998). Calcineurin binds to AKAP79 (Coghlan et al., 1995), which also binds to PKA (Ndubuka et al., 1993) and PKC (Klauck et al., 1996). AKAP79 binds to PSD-95 and SAP97, and may target these kinases and phosphatase to glutamate receptor complexes (Colledge et al., 2000). AKAP79 is found in the spines, but not in the PSD (Fig. 6; Sik et al., 2000). Calcineurin also binds to an immunophilin, FKBP12. Calcineurin is targeted to ryanodine and IP3 receptors through its association with FKBP, and may regulate Ca^{2+} release from internal stores (Fig. 6; Snyder et al., 1998).

Neurotrophin receptors

Dendritic spines also contain neurotrophin receptors. At least, two types of receptors, TrkB and p75, are found in the PSD (Fig. 6; Wu et al., 1996; Dougherty and Milner, 1999; Drake et al., 1999; Aoki et al., 2000). BDNF is also found in the PSD (Aoki et al., 2000). TrkB is primarily a receptor for BDNF and NT-4 whereas p75NTR binds to all neurotrophins with similar affinity (Huang and Reichardt, 2001). Neurotrophins mediate synaptic plasticity in spine synapses (Kovalchuk et al., 2002). Also, neurotrophin signaling modulates the morphology of dendritic spines. In cerebellar Purkinje cells, signaling through TrkB modulates spine density and morphology without any apparent effect on the parent dendrites (Shimada et al., 1998), whereas BDNF destabilizes spines and dendrites in cortical neurons (Horch et al., 1999).

Rho GTPases and related proteins

Rho GTPases are a family of proteins known to regulate the actin cytoskeleton in a variety of cell types (Hall, 1998). RhoA and Rac1, most well-characterized members of Rho GTPase, were shown to regulate spine density and morphology (Nakayama et al., 2000; Tashiro et al., 2000). The existence of proteins interacting with RhoA and Rac1 in spines suggests that RhoA and Rac1 must exist in spines (Fig. 6), although this has not been directly demonstrated. Kalirin-7 is a GDP/GTP exchange factor which activates Rac1. Kalirin-7 interacts with PSD-95, is localized in spines and regulates spine morphogenesis (Penzes et al., 2001). Also, citron, a downstream effector of RhoA, was found to bind PSD-95 and to be localized in spines in the thalamus and the cortex (Furuyashiki et al., 1999; Zhang et al., 1999).

Proteases

A Ca^{2+}-dependent protease, calpain, is enriched in spines and the PSD (Fig. 6; Perlmutter et al., 1988). Calpain can cleave a variety of proteins which exist in spines, including actin, spectrin, cortactin, NCAM, integrin, Ca^{2+} channel, ryanodine receptor and NMDAR (Chan and Mattson, 1999). Activation of NMDAR activates calpain and causes proteolysis of spectrins (Siman and Noszek, 1988). In addition, calpain degrades PSD-95 (Lu et al., 2000; Vinade et al., 2001) and GRIP (Lu et al., 2001) and may alter the structure of the PSD (Dosemeci and Reese, 1995; Lu et al., 2000; Vinade et al., 2001).

Summary and future perspectives: Spine as a nanomachine

Spines therefore appear to contain an large diversity of molecular cascades. Glutamate receptors and their associated proteins, actin and actin binding proteins, calcium homeostasis machinery, adhesion molecules, kinases, phosphatases, neurotrophin receptors, Rho GTPases, proteases, plus a host of other molecular families, some of which may not have been described yet, are present in spines. Among them, several proteins such as AMPAR (Nusser et al., 1998), Homer (Okabe et al., 2001) and ß-catenin (Murase et al., 2002) are known to differentially exist from spine to spine and show activity-dependent redistribution into and out of spines. As discussed before, spine morphology and synaptic function are also variable. These variety might be the reflection of the molecular diversity in spines. What is the purpose of this molecular richness? Although at this point we can only speculate about it, obviously this molecular diversity must be key for the function and plasticity of the synapse and may endow it with unusual biochemical flexibility.

Since spines are small (~1 fl), it is difficult to imagine such a crowded and more complex environment. Spines appear as one of the ultimate examples of miniaturization in Biology, and could represent a good testing ground for biological nanotechnology (Mehta et al., 1999). Indeed, there is already some evidence for both small number of molecules and extreme precision in their location. As explained above, the number of NMDAR or AMPAR on a given spine can be very small (Nusser et al., 1998). Also, the number of VGCC present on spines has been estimated to be as low as 1 (Sabatini and Svoboda, 2000). Moreover, even the position of these channels and receptors, particularly with respect to other molecular components of the spine, appears determined with extreme precision, like in the case of mGluR (Lujan et al., 1996). It seems to us that spines are built with great molecular sophistication, and that future studies to understand their structure and function must operate at an equally high level of experimental precision. It does not appear exaggerated to argue that the understanding of the function of the biochemical pathways present in spines may require single-molecule techniques (Mehta et al., 1999), in combination with detailed computational modeling of the spatio-temporal dynamics and kinetics of these molecules (Kennedy, 2000).

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