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Molecular mechanisms of dendritic spine development and maintenance

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The large majority of excitatory synapses are located on dendritic spines which are discrete membrane protrusions present on neuronal dendrites. Interestingly the highly heterogeneous morphology of dendritic spines is thought to be the morphological basis for synaptic plasticity associated to learning and memory formation. Indeed dendritic spines structure is regulated by molecular mechanisms that are fine tuned and adjusted according to level and direction of synaptic activity, development, specific brain region, and different experimental behavioral conditions. This supports the idea that reciprocal changes between the structure and function of spines impact both local and global integration of signals within dendrites. An increasing number of proteins have been found to be morphogens for dendritic spines and provided new insights into the molecular mechanisms regulating spine formation and morphology. Thus determining the mechanisms that regulate spine formation and morphology is essential for understanding the cellular changes that underlie learning and memory in normal and pathological conditions.

Key words: excitatory synapses, postsynaptic density, mental retardation, long term potentiation, long term depression

INTRODUCTION

Dendritic spines are formed by small protruding pieces of membrane with a total volume ranging from less than 0.01 μm^3 to 0.8 μm^3 where about 90% of the excitatory synapses in mature brain are located (Harris 1999). Spines were first observed more than 100 years ago by the Spanish neuroscientist Ramon y Cajal (Ramon y Cajal 1891) using the Camillo Golgi "*reazione nera*" protocol (the "black reaction" or the Golgi staining). Since then, many scientists and neuroscientists have tried to understand their function also because their morphology has been found altered in individuals with severe neuropathology such as mental retardation and neurodegenerative diseases. Several studies suggest that dendritic spines function as useful morphological correlates of excitatory synapses and are possible repositories of long-term memory in the brain (Segal 2005).

Interestingly spines are present at the squid giant synapses (Young 1973) but are rarely found in other lower organisms (such as *Drosophila melanogaster* or *Caenorhabditis elegans*), this suggesting that they may have developed early in evolution in order to implement more complex nervous systems functions. This review is focused on cellular and molecular mechanisms that regulate spine shape and function. The knowledge on molecules that regulate spine morphology and plasticity are improving our understanding of their role in brain cognitive functions in normal and pathological conditions.

STRUCTURE OF DENDRITIC SPINES

Dendritic spines are essentially formed by a neck and head attached to the dendritic membrane. Most spines have constricted necks and are either mushroom shaped with heads exceeding 0.6 microns in diameter or thin shaped with smaller heads (Harris et al. 1992). Extensive electron microscopy studies of brain tissue have shown that spines can be also stubby, cup-shaped or branched protrusions with two or more heads, or single protrusions with multiple synapses along the head and neck (Harris and Kater 1994, Hering and Sheng 2001) and that these different shapes can be

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found at the same time on the same dendrites (Spacek and Harris 1998). This imperfect classification underlines the multiple forms and dimensions of the spine head and neck providing a way to functionally measure distinct shape categories. For example the classic mushroom-shaped spines have a large head and narrow neck; whereas thin spines have a smaller head and narrow neck while stubby spines show no obvious constriction between the head and its attachment to the dentritic shaft. However, this static view does not reflect the real *in vivo* situation because at least in developing neurons, the majority of the spines change their shape over periods of minutes or hours (Parnass et al. 2000).

Indeed spine motility is developmentally regulated and, in mature neurons, there are fewer transitions between categories (Dunaevsky et al. 1999, Parnass et al. 2000). In brain, spines and presynaptic buttons are surrounded by glial cells in such a way as to form an intercommunicating tripartite complex and at least half of the circumferences of about 57% of the synapses are covered by astrocyte processes (Ventura and Harris 1999). Typical mature spines have a single excitatory synapse located at the head, but the same spines can also have an inhibitory input (Knott et al. 2002). However, spines essentially represent the main unitary postsynaptic compartment for excitatory input.

Dendritic spines are different also in the intracellular composition which mainly consist of postsynaptic density (PSD) facing the presynaptic button and a cytoskeletal structure formed almost exclusively by F-actin.

PSD is an electron-dense thickening of the membrane usually found at the head of the spine, where the synaptic junction is located. The PSD contains hundreds of proteins including NMDA (N-methyl-d-aspartate), AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate), and metabotropic glutamate receptors; scaffolding proteins such as PSD-95; and signaling proteins such as calcium/calmodulin dependent kinase II (CamKII) (Kennedy 2000). The PSD surfaces vary from small discs to large irregular shapes that can be perforated by electron lucent regions. It usually occupies about 10% of the surface area exactly opposite with the presynaptic active zone. The PSD is probably the most complex spine organelle in which hundreds of components are associated each other in a complex based on a series of protein-protein interaction domains of which the PDZ domain is one of the most important (Sheng and Sala 2001). Mushroom spines have larger, more complex PSDs with a higher density of glutamate receptors (Matsuzaki et al. 2001, Nicholson et al. 2006).

Other organelles are localized at spines: about 50% of the spines on hippocampal CA1 cells and virtually all Purkinje cell spines also have a smooth endoplasmic reticulum (SER) (Spacek and Harris 1997), and some pyramidal cell spines contain the spine apparatus, an organelle formed by two or more disks of SER separated by an electron-dense material (Westrum et al. 1980). Large spines usually have a proportionally large synapse and contain different types of organelles. It has been observed that both SER and spine apparatus are usually associated with larger spines and are formally absent in small spines (Spacek and Harris 1997). As SER is known to play a role in Ca^{2+} handling (Burgoyne et al. 1983, Andrews et al. 1988) differently sized spines may have different ways of controlling calcium homeostasis.

Larger spines are more likely to contain polyribosomes (Ostroff et al. 2002) and endosomal compartments (Cooney et al. 2002, Park et al. 2006), also perisynaptic astroglia is more often found associated to bigger spines (Witcher et al. 2007).

All these features suggest that larger spines are functionally stronger in their response to glutamate, local regulation of intracellular calcium, endosomal recycling, protein translation and degradation, and interaction with astroglia. Smaller spines may be more flexible, rapidly enlarging or shrinking in response to subsequent activation (Segal 2005).

Three immunogold labeling studies of the hippocampus (Nusser et al. 1998, Takumi et al. 1999, Racca et al. 2000) have shown that number of AMPA and NMDA receptors is proportional to the PSD area and spine volume because their density is constant within the PSD. The PSD and spine volume are also proportional to the area of the active zone, which is itself proportional to the number of docked vesicles (Schikorski and Stevens 1997), which in turn correlates with the amount of neurotransmitter release per action potential (Murthy et al. 1997). All of these data suggest that large spines represent stronger synapses for both presynaptic and postsynaptic properties, and that the growth of the spine head during development probably correlates with a strengthening of synaptic transmission.

DEVELOPMENT OF DENDRITIC SPINES

How are mature spines formed? Early spines are often very long and have frequent filopodia-like shape but, later during development, their mean length decreases and the number of filopodia is greatly reduced. Three major changes can be observed during the maturation process: an increase in spine density, a decrease in overall length and a decrease in the number of dendritic filopodia with a simultaneous decrease in spine motility (Dunaevsky et al. 1999, Nimchinsky et al. 2001) (Fig. 1). Several studies have shown that filopodia rapidly protrude and retract from dendrites, especially during the early stages of synaptogenesis (Dailey and Smith 1996, Ziv and Smith 1996, Fiala et al. 1998), and it is widely believed that dendritic filopodia are the precursors of dendritic spines, although various hypotheses as to how the transition from filopodia to spines takes place have been put forward.

Filopodium might actively seek out synaptic partners in the developing dendrites and when it makes contact with an axon, it becomes shorter and draws the axonal terminal closer to the dendrite shaft. Subsequently, a fully mature synapse is formed on the spine head, spine motility gradually decreases and the structure is stabilized (Dailey and Smith 1996, Ziv and Smith 1996, Fiala et al. 1998). Some filopodia become spines with synapses, whereas others withdraw into the dendrite to form synapses on the dendritic shaft

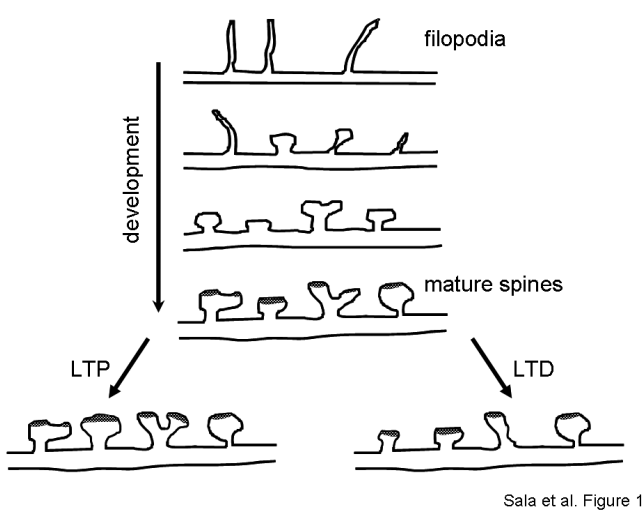

Fig. 1. The figure shows a schematic representation of morphological changes of dendritic spines during development and after LTP and LTD

(Marrs et al. 2001). These shaft synapses either reemerge as spines or are preferentially eliminated later in life (Bourne and Harris 2007).

Stabilization of hippocampal spines requires assembly of pre- and postsynaptic elements, although the timing of these events may vary (Nagerl et al. 2007). From the pre-synaptic side dense core vesicles containing piccolo and bassoon (called PTVs from Piccolo-Bassoon transport vesicles) appear early in axonal processes and travel until they stop where new synapses will form, suggesting that presynaptic active zones are prepackaged (Zhai et al. 2001, Shapira et al. 2003). On the post-synaptic site PSD-95, the major PSD scaffold protein, is necessary to stabilize the spine and synapses, as evidenced by RNAi knockdowns that cause spine and excitatory synapses loss (Gerrow et al. 2006, Ehrlich et al. 2007). Assembly of PSD-95 is spatially and temporally correlated with spine morphogenesis and the clustering of presynaptic vesicle proteins (Marrs et al. 2001, Okabe et al. 2001). Stabilization of dendritic spines is also facilitated by the insertion and activation of glutamate receptors because AMPA receptor activation decreases spine motility and stabilizes spine shape (Fischer et al. 2000).

Although pharmacological block of NMDA receptor signaling does not affect the emergence or density of spines during development (Kirov et al. 2004, Alvarez et al. 2007), knocking down NMDA receptors through RNA interference (RNAi) results in increased spine motility and eventual elimination (Alvarez et al. 2007). If filopodia are essential for synaptogenesis, filopodia should be maintained long enough to find appropriate presynaptic partners. Three molecules such as Telencephalin, SynGAP and Paralemmin-1 have been suggested to play a major role in filopodia formation.

Telencephalin is an adhesion molecule of the Ig superfamily and SynGAP is a ras-GTPase activating protein; both of these proteins maintain filopodia in a dynamic state during synaptogenesis, and mice deficient in either protein show accelerated spine development and larger spine heads (Vazquez et al. 2004, Matsuno et al. 2006, Furutani et al. 2007). Interestingly when filopodia become spines, telencephalin relocates to the dendritic shaft and is replaced with adhesion molecules, N-cadherin and α-catenin, which stabilize the new spine (Togashi et al. 2002, Abe et al. 2004). SynGAP interacts with the PDZ domains of PSD-95

and links NMDA receptor activation to Ras signaling pathways (Chen et al. 1998, Kim et al. 1998).

Paralemmin-1 is a membrane associated protein shown to induce cell expansion and process formation. When localized to dendritic membranes paralemmin-1 is able to induce filopodia and recruit synaptic elements to contact sites. Knockdown of paralemmin-1 in developing neurons reduces the number of filopodia and spines (Arstikaitis et al. 2008).

In adult hippocampal CA1 pyramidal and granule cells, dendritic spine density ranges from two to four spines per micrometer of dendrite (Harris et al. 1992, Sorra and Harris 1998), whereas it is more than ten spines per micrometer in Purkinje cells (Harris and Stevens 1988, Napper and Harvey 1988). Also the density of spines is not homogeneous throughout the dendritic tree but increases at each layer and varies across cortical areas, thus suggesting that the afferent system independently regulates different parts of the dendritic tree. In macaque monkeys and humans the density on basal dendrites in the cortical areas of the frontal pole and orbitofrontal cortex is generally higher than in neurons of the primary visual and somatosensory cortices (Elston 2000, Jacobs et al. 2001), suggesting a link between spine density and overall number and level of cortical processing in these regions. Thus the "higher" order areas involved in a greater degree of convergent processing might need for more synapses and therefore more spines.

MORPHOGENIC SPINE MOLECULES

Considerable efforts have been made to characterize the molecular mechanisms controlling spine morphology, formation and plasticity. The majority of morphogenetic spines molecules can be classified in four categories: acting binding-proteins, small GTPase and associated proteins, cell surface receptors and postsynaptic scaffold proteins.

Acting binding-proteins

Filamentous actin (F-actin) is the main constituent of the cytoskeleton of dendritic spines and is remarkably dynamic. Actin-binding proteins are likely to be involved in spine formation and shape (Sekino et al. 2007). A number of actin-binding proteins are highly localized to spines: drebrin, profilin II, N-catenin, spinophilin/neurabin II, synaptopodin, and myosin II and VI.

One of these is drebrin an F-actin-binding protein, which is mainly expressed in neurons. Drebrin overexpression in cortical neurons increases the length of dendritic spines (Hayashi and Shirao 1999), and its activity may be mediated by its binding to F-actin and other actin-regulatory proteins, such as profilin, myosin and gelsolin. Drebrin promotes actin assembly and the synaptic clustering of PSD-95 in the PSD (Takahashi et al. 2003) and it's redistributed to dendritic spines, together with an increase in F-actin content, after the induction of LTP in the dentate gyrus (Fukazawa et al. 2003). Thus drebrin is important in inducing F-actin polymerisation and/or stabilization.

Like drebrin, profilin II and N-catenin are recruited to spines by general synaptic activation and by NMDA receptor activation or LTP induction. The accumulation of profilin II in dendritic spines persists for hours beyond the initiating stimulus, depends on an increase in postsynaptic Ca^{2+} levels and, probably, on the association of profilin II with Ena/VASP family proteins. Profilin II stabilises spine morphology in a mature state, and suppresses dendritic spine motility by reducing actin dynamics (Ackermann and Matus 2003). More recently it has been shown that in the lateral amygdala fear conditioning leads to the movement of profilin into dendritic spines with associated enlargement of PSD and this might contribute to the enhancement of associatively induced synaptic responses following fear learning (Lamprecht et al. 2006). These results suggest that profilin II plays a role in linking the activation of glutamate receptors with the actin-based stabilisation of synapse morphology.

αN-catenin is a cadherin-associated protein and, together with β-catenin, links the adhesion molecules to the cytoskeleton and actin. The overexpression of αN-catenin accelerates dendritic spine maturation and decreases spine motility, thus suggesting that it promotes spine morphogenesis and stabilisation (Abe et al. 2004). Conversely, in the absence of αN-catenin, dendritic spines are more motile and their filopodia rapidly protrude and retract from the spine heads, a sign of unstable synaptic contacts. As it appears to accumulate in activated synapses, αN-catenin may mediate neural activity-dependent signals that stabilize synapses by suppressing motility and turnover (Abe et al. 2004). One possible bidirectional interaction between the profilin/actin and cadherin/αN-catenin systems has been proposed in which neural activity contributes to changes in spine shape by modifying

any of these molecular interactions (Abe et al. 2004).

Spinophilin (or neurabin II) and neurabin I are two related F-actin-binding proteins with similar domain structures containing a PDZ and a coiled-coil domain that form homo- and heterodimers. *Via* its actin-binding domain, spinophilin (as its name suggests) is predominantly localised on the dendritic spines of pyramidal neurons; it seems to be required for the correct maturation of dendritic spines because knockout mice have more filopodia and spines early in development and altered glutamatergic transmission (Feng et al. 2000). On the contrary, neurabin I overexpression induces the formation of dendritic filopodia in immature cultured hippocampal neurons, and promotes the enlargement of dendritic spines in older neurons (Oliver et al. 2002). Also neurabin I amino-terminal fragment alone, which contains the actin-binding domain, increases the density and length of dendritic spines by increasing actin polymerisation and spine motility (Zito et al. 2004). It is not clear how spinophilin and neurabin I have opposite effect on spines, although they both bind a similar set of proteins in synapses, such as Lfc, a Rho GEF that regulates the Rho-dependent organisation of F-actin in spines (Ryan et al. 2005).

Another actin-associated protein is Synaptopodin that is localized in the spine apparatus. Synaptopodin regulates spine structure because mice lacking synaptopodin also lack a spine apparatus and display deficits in synaptic plasticity (Deller et al. 2007).

It has been recently demonstrated that actin cytoskeleton in spine can also be regulated by myosins, molecular motors that translocate along and/or produce tension in actin filaments. Two Myosin, myosin II and myosin VI, are enriched in PSD preparations and regulate spine shape. Neurons from mice lacking myosin VI exhibit abnormally short dendritic spines and reduced synapse number (Osterweil et al. 2005), and inhibition of myosin II destabilizes mushroom spines (Ryu et al. 2006).

Small GTPase and associated proteins

Most of the extracellular and intracellular signals, affecting the organization of F-actins in cells, converge on small GTPase of the Rho family. These GTPases act as molecular switches existing in the active guanosine triphosphate (GTP)-bound and inactive guanosine diphosphate (GDP)-bound states and modify actin binding proteins activity thus promoting or inhibiting actin filament polymerization.

Indeed in neurons, morphological changes in the peripheral region of dendritic spines resemble the actin-based ruffling motion of lamellipodia, a typical actin-based motility in many cell types. This suggests that small GTPases induce morphological changes of dendritic spines by remodeling dendritic spines F-actin filaments.

Among the Rho family of small GTPases, RhoA, Rac1 and Cdc42 have profound influence on spine morphogenesis by regulating the actin cytoskeleton (Newey et al. 2005). It's generally demonstrated that RhoA inhibits, whereas Rac and Cdc42 promote, the growth and/or stability of dendritic spines.

Among the upstream and downstream regulators of Rho family GTPases an expanding number of guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) appear to control Rho family GTPases postsynaptically. αPIX (α-p21-activated kinase [PAK] interacting exchange factor) or ARHGEF6 is a GEF for Rac and can locally activate Rac1/Cdc42 in dendritic spines and its gene has been found mutate in patients affected by a form of X-linked mental retardation (Kutsche et al. 2000, Zhang et al. 2005). βPIX, highly homologous to αPIX, has a unique C-terminal sequence that is able to interact with the PSD protein Shank (see later) and to be recruited to synapses by Shank (Park et al. 2003).

Also implicated in dendrite and spine development is another Rac-GEF called Tiam1, which is associated with, and regulated by, NMDA receptors and mediates EphB receptor-dependent dendritic spine development (see later) (Tolias et al. 2005, 2007). GEFT is a newly identified GEF for Rho family GTPases, widely expressed in the brain and promotes neurite outgrowth and dendritic spine enlargement, probably *via* the Rac and Cdc42-PAK pathways (Bryan et al. 2004). As mentioned before the Rho-specific GEF Lfc interacts with neurabin and spinophilin and appears to be important for Rho-dependent organization of F-actin in spines. Lfc translocates to spines upon neuronal depolarization, and its overexpression reduces spine size (Ryan et al. 2005).

The p21-activated kinases PAK1 and PAK3 are the two major synaptic Rac effectors to promote formation and/or growth of spines. In hippocampal organotypic slice cultures RNAi-mediated suppression of PAK3 or expression of dominant-negative PAK3 induces abnor-

mally long dendritic spines that lack synapses and a loss of mature spine synapses (Boda et al. 2004, Node-Langlois et al. 2006). Although PAK3 knockout mice showed no detectable spine abnormalities they exhibited impaired long-term synaptic plasticity and reduced memory consolidation (Meng et al. 2005) and in human PAK3 mutation causes mental retardation (Allen et al. 1998). Finally, similar to PAK3 knockout mice, transgenic mice expressing dominant negative PAK in the forebrain showed reduced spine density in cortical but not hippocampal neurons (Hayashi et al. 2004). Downstream to PAK and Rho-associated kinase ROCK is the Lim-kinase, which inactivates ADF/cofilin through phosphorylation. Lim-kinase knockout mice show abnormal spine morphology, abnormal synaptic plasticity and impaired spatial learning (Meng et al. 2002).

A scaffold proteins involved in Rho GTPase signaling that is implicated in spine regulation is GIT1 (G-protein-coupled receptor kinase-interacting protein 1). GIT1 contains an ADP-ribosylation factor (Arf) GAP domain that interacts with PAK3 and PIX (Zhang et al. 2005). Thus, GIT1, PIX, Rac and PAK might function in a signaling complex to regulate dendritic spine development. Insulin receptor substrate 53 (IRSp53), an adaptor protein that connects Rac1 to WAVE2, is implicated in filopodia and lamellipodia formation in non-neural cells. IRSp53 is enriched in the PSD by binding to Shank and PSD-95 family scaffolds and its overexpression in cultured neurons increases the density of dendritic spines, while its siRNA-mediated knockdown reduces spine density, length and width. The overexpression of IRSp53 with a point mutation in the SH3 domain (where WAVE2 binds) reduces spine density and size, thus suggesting that IRSp53 plays a role in linking PSD-95 to activated Rac1/Cdc42 and downstream effectors of actin regulation in spines (Choi et al. 2005).

The Abl-interactor (Abi) adaptor proteins, so-named because they bind to the Abl tyrosine kinases, also play a part in Rac GTPase signaling and actin regulation. Abi2 is highly expressed in brain and Abi-2-deficient mice show reduced spine density and a decrease in the relative proportion of mushroom spines, which is associated with deficits in short- and long-term memory (Grove et al. 2004). It has been recently shown that Abi-1 interacts with ProSAP2/Shank3, a PSD scaffold protein (see below), and translocates upon NMDA application from PSDs to nuclei where interacts with Myc/Max proteins and enhances E-box-regulated gene transcription. In hippocampal neurons downregulation of Abi-1 may result in excessive dendrite branching, immature spine and synapse morphology and a reduction of synapses, whereas overexpression of Abi-1 has the opposite effect. Thus Abi-1 can act as a specific synapto-nuclear messenger and is essentially involved in dendrite and synapse formation (Proepper et al. 2007).

As mentioned before components of Rho GTPase signaling pathways are highly represented among the genes mutated in human non-syndromic mental retardation, such as αPIX and PAK3 mutations (Newey et al. 2005). Another gene mutated in a form of syndromic X-linked mental retardation is oligophrenin that encodes a Rho-GAP. Oligophrenin suppression, both by RNAi in neuronal culture and gene knockout *in vivo*, decreases spine length, mimicking the effect of active RhoA (Govek et al. 2004, Khelfaoui et al. 2007).

The involvement of Rac1 in spine formation has been further supported by the role of Kalirin-7, a guanine nucleotide exchange factor (GEF) for Rac1, which overexpression in cultured cortical neurons increased the number and size of spine-like protrusions. This effect is dependent on GEF activity because the overexpression of a Kalirin-7 mutant lacking GEF activity reduced the number of spines (Penzes et al. 2001, Xie et al. 2007).

In addition to Rho, Rac and Cdc42, the Ras family of GTPases and their downstream MAP kinase signaling pathways also regulate spine morphogenesis as demonstrated by the observation that transgenic mice expressing active Ha-Ras show increased spine density in cortical pyramidal neurons (Gartner et al. 2005). Indeed filopodia can be induced in cultured neurons by means of multiple depolarizing stimuli and this effect depends on activation of the Ras/MAPK pathway (Wu et al. 2001).

Rap GTPases are closely related to Ras, but appear to play opposing roles in synaptic plasticity. Whereas Ras promotes LTP, Rap1 and Rap2 mediate LTD and depotentiation, respectively (Zhu et al. 2005). In contrast to the RasGAP SynGAP, a postsynaptic RapGAP SPAR, but not SPAR2, binds actin and promotes the growth of spines (Pak et al. 2001, Pak and Sheng 2003, Spilker et al. 2008). All these data suggest that Ras and Rap might act antagonistically in the postsynaptic compartment to regulate synapse strength and spine morphology.

Cell surface receptors

An important role in stabilizing the nascent spines and their synapses is played by the cell-adhesion molecules, such as N-cadherins, catenins, neurexins, and neuroligins, and Ephs and ephrins.

The cadherin-based homophilic cell adhesion system helps to maintain pre- and post-synaptic apposition and is important for spine morphogenesis (Salinas and Price 2005).

The major neuronal cadherin (N-cadherin) undergoes activity-dependent conformational change and redistribution at synapses, and its activity is required for depolarization-induced spine enlargement (Okamura et al. 2004). N-cadherin also regulates spine morphology *via* its binding proteins, α- and β- catenin, which interact with the actin cytoskeleton. In a proposed model β-catenin is recruited to cadherin and spines by tyrosine dephosphorylation mediated by receptor tyrosine phosphatases of the LAR (leukocyte common antigen-related) family, which are required for spine and synapse development (Dunah et al. 2005).

Remarkably, even AMPA receptors themselves (specifically the extracellular N-terminal domain of subunit GluR2, called NTD domain) can induce spine enlargement (Passafaro et al. 2003). The first N-terminal 92 amino acids of the extracellular domain are necessary and sufficient for GluR2's spine-promoting activity. This part of the GluR2 can interact directly with N-cadherin, in cis or in trans and form a synaptic complex that stimulates presynaptic development and function as well as promoting dendritic spine formation (Saglietti et al. 2007).

Eph receptor–ephrin binding results in multimeric clusters that bridge juxtaposed cell surfaces and mediate cell-cell adhesion and bidirectional signaling. Trans-endocytosis of the eph-ephrin complex loosens the adhesion between the pre- and postsynaptic elements, which may permit structural synaptic plasticity. EphB receptors directly associate with NMDA receptors at synapses, and ephrinB induced activation of EphB receptors causes NMDA receptor clustering (Dalva et al. 2000). EphB2 also associates with the GTP exchange factors intersectin and kalirin (Penzes et al. 2003). The intersectin-Cdc42- Wasp-actin and kalirin-Rac-Pak-actin pathways may regulate the EphB receptor–mediated morphogenesis and maturation of dendritic spines in cultured hippocampal and cortical neurons. Perhaps the interaction of presynaptic ephrins with postsynaptic Eph receptors coordinates the establishment of the well-known correlation between presynaptic vesicle number and postsynaptic size during structural synaptic plasticity.

Synapse formation is thought to be regulated by bidirectional signaling between pre- and postsynaptic cells and recently a number of synaptic specific adhesion molecules (Neuroligin, SALM and netrin-G ligand) have been identified to regulate synapses and spines formation at least *in vitro*. Interestingly all these molecules bind to PSD-95 (see later). Neuroligin1 overexpression promotes postsynaptic differentiation and spine morphogenesis in cultured rat hippocampal neurons and controls the balance between excitatory and inhibitory synapses number (Chih et al. 2004).

Overexpression of postsynaptic netrin-G ligand 2 (NGL-2) in cultured rat neurons increased the number of PSD-95-positive dendritic protrusions, while competitive inhibition by soluble NGL-2 or NGL-2 knockdown by siRNA reduced the number of excitatory synapses but not inhibitory (Kim et al. 2006).

Similarly SALM2, a SALM isoform, overexpression increases the number of excitatory synapses and dendritic spines while in absence of SLAM2 the number of excitatory synapses and dendritic spines is reduced (Ko et al. 2006). These results suggest that both NGL-2 and SALM2 regulates differentiation of excitatory synapses and conseguentily of the dendritic spines.

Postsynaptic scaffold proteins

Among the scaffold proteins present at synapses two major families has been implicated in spine morphogenesis: the PSD-95 family and the Shank family.

The PSD-95 family is encoded by four genes, PSD-95/SAP90 (synapse-associated protein 90), PSD-93/chapsyn-110, SAP102, and SAP97. The structure of these proteins is characterized by the assembly of three PDZ domains, an SRC homology (SH3) domain and a guanylate kinase-like (GK) domain. SAP97 also has a LIN2/LIN7 (L27) domain at the N-terminal. The PSD-95 family belongs to a protein superfamily called membrane-associated guanylate kinase (MAGUK), which is characterized by the presence of at least one PDZ and one GK domain.

As mentioned before, it has been demonstrated that PSD-95 probably plays an important role in synapse

and dendritic spines formation by means of its interaction with neuroligin, NGL and SALM, all postsynaptic membrane proteins that interact trans-synaptically with specific pre-synaptic membrane proteins. For example, the trans-synaptic neuroligin-neurexin interaction seems to be important in inducing pre- and postsynaptic differentiation (Graf et al. 2004, Prange et al. 2004, Chih et al. 2005). Actually the amount of PSD-95 regulates the balance between the number of inhibitory and excitatory synapses (Prange et al. 2004).

PSD-95 interacts also with a wide variety of cytoplasmic signalling molecules and thus, by physically bringing together cytoplasmic signal-transducing enzymes and surface receptors, may facilitate signal coupling in the PSD. For example, the overexpression of PSD-95 in hippocampal neurons increases the number of dendritic spines and the maturation of excitatory synapses (El-Husseini et al. 2000), which may be related to the recruitment of transmembrane proteins but also of intracellular signalling proteins. Some of these proteins are directly involved in spine formation, including kalirin-7 (Penzes et al. 2003), SPAR (Pak et al. 2001), and IRSp53 (Choi et al. 2005).

Shank is a large scaffold protein whose multidomain organization consists of an ankyrin repeat near the N-terminal, followed by a SH3, a PDZ domain, a long proline-rich region, and a sterile alpha motif domain (SAM) at the C terminus (Sheng and Kim 2000). Shank proteins (codified by three genes, *Shank1-3*) molecularly link two glutamate receptor subtypes: NMDA receptors and type-I metabotropic GluRs (mGluRs). The Shank PDZ domain binds to the C-terminal of SAPAP/GKAP, another PSD scaffold protein family, which is associated with the GK domain of PSD-95. The Homer interaction at the proline-rich domain ensures the association of Shank with type-I mGluRs.

Homer proteins are encoded by three genes (*Homer 1-3*), and typically consist of an N-terminal EVH1 domain followed by a coiled-coil domain that mediates dimerisation with other Homer proteins. The Ena/VASP homology 1 (EVH1) domain of Homer1 binds to a PPXXF or a very similar sequence motif present in Shank, mGluR1/5, inositol-1,4,5-trisphosphate (IP_3) receptor, ryanodine receptor, and to different members of the TRPC family of ion channels (Xiao et al. 2000, Yuan et al. 2003). Through their ability to self-associate, Homer isoforms containing the coiled-coil domain (termed "CC-Homer" or Homer1b for the Homer1 gene) can physically and functionally link the proteins and receptors that bind to the EVH1 domain. Homer1a is a short-splice variant of Homer1 that contains the EVH1 domain but lacks the coiled-coil domain. Importantly, Homer1a expression is induced at mRNA level by synaptic activity and, because it cannot dimerize, it functions as a natural dominant negative of the constitutively expressed Homer1b splice variant (Xiao et al. 2000).

The overexpression of Shank1 and Homer1b in hippocampal neurons accelerates the maturation of filopodial-like protrusions in mature spines, and promotes the enlargement of mature spines (which acquire the classical mushroom shape) without increasing their number. Shank and Homer also cooperate to promote the accumulation of postsynaptic density proteins in dendritic spines such as GKAP and NR1, and increase the F-actin content of spines (Sala et al. 2001).

Similarly overexpression of Shank3 in cerebellum granule cells induces dendritic spine and synapse formation by recruiting different subtypes of glutamate receptors, whereas the inhibition of Shank3 expression in hippocampal neurons reduces the number of dendritic spines. One sign of the global effect of Shank on synapse maturation is that its overexpression also induces the maturation of the presynaptic compartment (Sala et al. 2001, Roussignol et al. 2005).

It is therefore not surprising that, like PSD-95, Shank and Homer interact with a number of actin-binding proteins. Shank binds to cortactin, Abp1 (Qualmann et al. 2004), the Rac1 and Cdc42 exchange factor αPIX (Park et al. 2003) and Cdc42-binding protein IRSp53 (Soltau et al. 2002); and Homer binds to Rho GTPase-activating protein oligophrenin-1 (Govek et al. 2004). Interestingly, the interaction of Shank1 with Homer seems to be essential for inducing spine maturation, and the interaction with cortactin seems to be equally important for Shank3 (Roussignol et al. 2005). Finally, Shank1 and Homer1b can also recruit the entire ER compartment to dendritic spines, which may contribute to their enlargement effect (Sala et al. 2005).

The ability of Shank1 and Homer1 to promote spine morphogenesis depends on their ability to form a complex with each other, and correlates with their accumulation in spines (Sala et al. 2001, Sala et al. 2003). Homer 1a disrupts the interaction between full-length Homer1b and Shank, and inhibits the synaptic target-

ing of both proteins (Sala et al. 2003). As a consequence, the overexpression of Homer1a destabilises synapses and decreases the number and size of dendritic spines, also reducing the synapse number of both AMPA and NMDA receptors. In this case, the actions of Homer1a may contribute to the global activity-dependent loss of spines in a neuron, and the negative regulation of unstimulated synapses (Sala et al. 2003).

Interestingly, deletions or mutations in the human Shank3 gene have been linked to a severe form of mental retardation (Bonaglia et al. 2001, 2006) or to autism spectrum disorders (ASDs) (Durand et al. 2007), suggesting that Shank family proteins may play an important role in human cognitive development.

To support this hypothesis the Shank1 knockout mice showed altered protein composition of the PSD and smaller dendritic spines and synapses, which correlated with a weakening of basal synaptic transmission suggesting that Shank1 is important *in vivo* for regulating dendritic spine morphology and synaptic strength. Remarkably, Shank1-deficient mice displayed enhanced performance in a spatial learning task but their long-term memory retention in this task was impaired. These data suggest that Shank1 promotes the maturation of smaller, more plastic spines into larger, more stable spines, a cellular process required for normal cognitive development and a feature that may be relevant to human autism spectrum disorders (Hung et al. 2008).

DENDRITIC SPINES MOTILITY AND MORPHOLOGICAL MODIFICATION

Using time-lapse imaging and fluorescent proteins, considerable spine motility over a timescale of seconds to minutes has been documented in dissociated cultures (Fischer et al. 1998, 2000, Colicos et al. 2001), brain slices (Dunaevsky et al. 1999) and *in vivo* (Lendvai et al. 2000). Spine motility is actin-dependent, involves the remodelling of the actin cytoskeleton in the spine (Fischer et al. 1998, Dunaevsky et al. 1999).

There is evidence that actin-based motility is controlled by synaptic activity (Fischer et al. 2000, Colicos et al. 2001). The activation of either AMPA or NMDA receptors greatly inhibits spine actin dynamics and actin-based protrusive activity from the spine head, and the spine becomes more rounded and regular (Fischer et al. 2000). In this case, the inhibition of spine motility by AMPA receptors was dependent on postsynaptic membrane depolarization and calcium influx through voltage-activated channels (Fischer et al. 2000).

But the main question concerning spine plasticity is whether LTP or LTD are the expression of morphological modifications in spine structure (Yuste and Bonhoeffer 2001) and indeed mounting evidence indicates that synaptic plasticity is associated with changes in spine morphology.

Actually the first report of the effect of LTP on spine morphology was published several years ago in 1975 by Van Harrefeld and Fifkova (Van Harreveld and Fifkova 1975) who used a similar experimental protocol to that used a few years before by Bliss and Lømo when they discovered LTP in the dentate gyrus (Bliss and Lomo 1973). The authors describe a significant increase in spine area and volume from two minutes to 23 hours after LTP induction with a peak at 10-60 minutes. Together with the observation that the spine neck becomes wider and shorter, these data suggest that tetanization is able to increase synapse size by increasing spine volume and recruiting an actin cytoskeleton, and that these changes last for hours (Fifkova and Anderson 1981, Fifkova 1985).

More recent experiments have shown that in the immature hippocampus, a major effect of LTP is to increase spine head size (Lang et al. 2004, Kopec et al. 2006, Park et al. 2006), which is followed by an accumulation of AMPA receptors at the synapse (Kopec et al. 2006). Both large and small spines undergo the same absolute increase in head volume and surface area (Lang et al. 2004, Kopec et al. 2006). These morphological changes of spines depend on NMDA receptor activation and might contribute to activity dependent formation and elimination of synaptic connections.

Enlargement of dendritic spines is not the only morphological modification observed after LTP. For example mobilization of recycling endosomes and vesicles and amorphous vesicular clumps into spines have been observed within minutes after the induction of LTP (Park et al. 2006). This vesicular accumulation might provide a source of plasma membrane for spine enlargement and probably transport of AMPA receptors. Interestingly also polyribosomes redistribute into the heads of dendritic spines that have enlarged synapses two hours after the induction of LTP (Ostroff et al. 2002). Sustained spine enlargement is accompanied

by an increase in F-actin levels (Lin et al. 2005, Kramar et al. 2006).

Synaptic stimulation can also drive the translocation of mitochondria into spines and the morphogenesis of dendritic spines depends reciprocally on the quantity and function of mitochondria in dendrites (Li et al. 2004).

The proportion of perforated and complex PSDs is increased one hour after induction of LTP (Popov et al. 2004). Electron microscopy analysis has shown that the size of the PSD is perfectly correlated with the size of the presynaptic bouton and the number of vesicles it contains (Harris et al. 1992), thus PSD enlargement induced by LTP should induce an enlargement of the presynaptic active zone and increase in the number of presynaptic vesicles.

While LTP-inducing stimuli cause formation of new spines and enlargement of existing spines, on the contrary long-term depression (LTD)-inducing stimulation is associated with shrinkage and/or retraction of spines associated with a depolymerization of actin (Chen et al. 2004, Nagerl et al. 2004, Okamoto et al. 2004, Zhou et al. 2004). Interestingly the observation that thin spines are mostly responsive to increases and decreases in synaptic activity has led to suggest that they are 'learning spines', whereas the stability of mushroom spines suggests that they are 'memory spines' (Fig. 1). Thus, the conversion of large 'memory spines' back into smaller 'learning spines' after LTD, resets the plasticity potential of the dendrite. However, although there seems to be a consolidated correlation between LTP/LTD and spine morphological modifications, there is no direct proof that these morphological changes contribute to synaptic strength as well as to the generation of potential new contact sites that will modify neuronal communication. Whether changes in the spine shape and size significantly impact synaptic signals is not obvious also because morphological changes are not required for all forms of synaptic plasticity.

CONCLUSIONS

In the past few years molecular biology, electrophysiology, and imaging studies have provided many insights into the mechanisms of the morphological modifications undergone by dendritic spines during development and synaptic plasticity in normal and pathological conditions.

Nevertheless, fundamental structural and functional questions remain open. One question is to determine the relationship between spine shape and function in detail as this perhaps means that neurons are able to adjust spine morphology in order to work or communicate better. However, we also need to know what determines the extrinsic factors that regulate connectivity along dendrites and axons and the intrinsic factors that regulate the availability of subcellular structures required to build and maintain dendritic spines and synapses. This is necessary to formulate a comprehensive understanding of neural circuits that underlie development of perception, memory, and cognition in humans.

A number of cognitive disorders are associated with subtle spine malformations, such as changes in spine length, distribution, number or morphology, but still a detailed morphological, physiological and biochemical analysis has to be made in order to correlate clinical phenotypes, molecular defects and spine abnormalities. All of these key questions and others will be addressed in the near future.

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