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Dendritic spine formation and stabilization

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Formation, elimination and remodeling of excitatory synapses on dendritic spines represent a continuous process that shapes the organization of synaptic networks during development. The molecular mechanisms controlling dendritic spine formation and stabilization therefore critically determine the rules of network selectivity. Recent studies have identified new molecules, such as Ephrins and Telencephalin that regulate filopodia motility and their transformation into dendritic spines. Trans-synaptic signaling involving nitric oxide, protease, adhesion molecules and Rho GTPases further controls contact formation or the structural remodeling of spines and their stability. Evidence also suggests that activity and induction of plasticity participate to the selection of persistent spines. Together these new data provide a better understanding of the mechanisms, speed and steps leading to the establishment of a stable excitatory synapse.

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Introduction

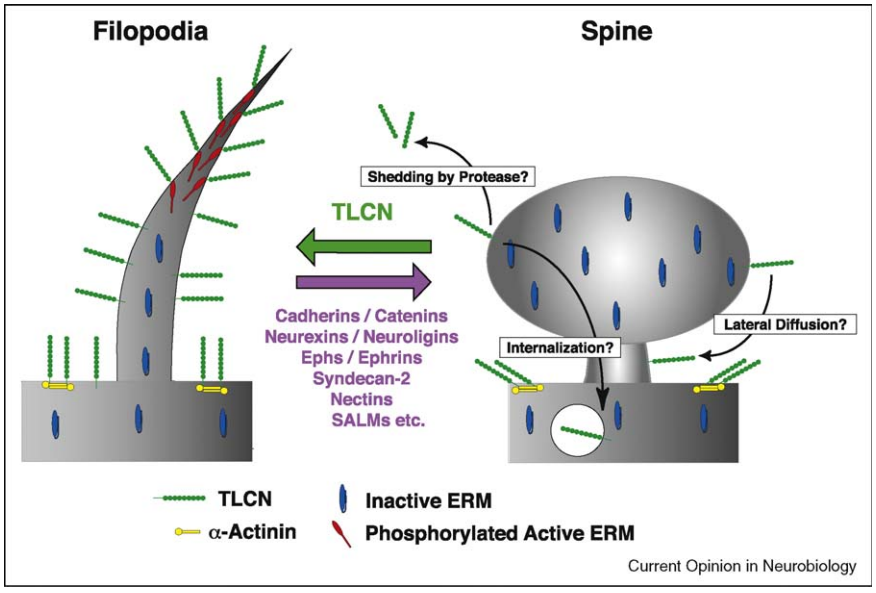
Morphological alteration of excitatory synapses is one of the most important and efficient cellular mechanisms underlying plasticity of neural functions [1,2]. In the central nervous system, the majority of glutamatergic excitatory inputs are received by dendritic spines of postsynaptic neurons. Spines are specialized protrusions emerging from neuronal dendrites, with characteristic bulbous enlargements at their tips (spine heads). Dendritic spines are first formed in early postnatal life, shaped up by the animal's experience, and maintained into adulthood. Time-lapse imaging of spine dynamics visualized with genetically engineered fluorescent proteins revealed that the spines are not static, but actively move, and alter their morphology continuously even in the adult brain, reflecting the plastic nature of synaptic connections [3–7]. Hippocampal synapses undergo structural changes in size and shape after long-term potentiation *in vitro* and experience *in vivo* [8–10]. Additionally new spines are formed, which become functional synapses and eventually replace non-activated ones [9,11,12•,13••,14]. Finally, abnormal spine structures are often associated with various neurological disorders such as Fragile X, Down, and Rett syndromes [15].

Careful electron microscopic observations uncovered the fine structures and subcellular organelles of dendritic spines and identified a unique dense thickening, the so-called postsynaptic density (PSD), under the surface membrane of spine heads. The PSD is a postsynaptic specialization usually apposed to synaptic vesicle-containing presynaptic boutons. A number of biochemical and molecular biological studies have been performed to elucidate molecular compositions of spines, especially in the PSD, and provided comprehensive lists of functional molecules including cell recognition/adhesion molecules, neurotransmitter receptors, ion channels, intracellular adaptor proteins, cytoskeletal proteins, kinases/phosphatases, GTP-binding proteins and extracellular proteases [16]. Gain- and loss-of-function analyses revealed that many of these molecules are involved in various aspects of spine development and functions. This is particularly the case of cell adhesion molecules, such as cadherin/catenin, neurexin/neuroligin, Eph/ephrin, nectins, SALMs, SynCAMs, which play roles in the formation, maturation, and stabilization of spine synapses [17] (Figure 1). Some of these molecules are also present in another type of dendritic protrusions called dendritic filopodia. We review here some recent findings regarding the mechanisms of synaptogenesis, focusing on two types of dendritic protrusions: filopodia and spines (Figure 2).

Dendritic filopodia

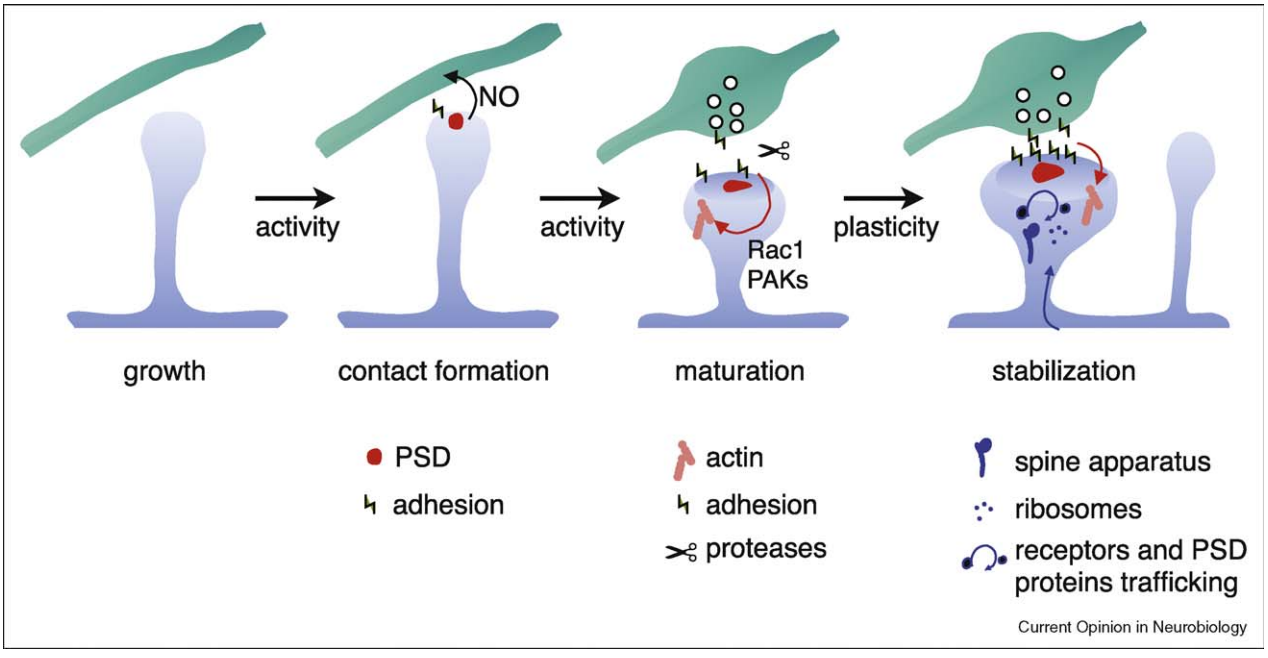
Dendritic filopodia are long, thin, headless, and most often PSD-free protrusions abundantly present in developing neurons. They can still be found later in the adult brain, but mainly under specific conditions such as induction of plasticity, following ischemia or during regeneration after neuronal injury [18–20]. Dendritic filopodia are highly motile and flexible structures, with an average lifetime in the range of minutes to hours [6,21,22]. Due to their high motility, they are ideally suited to explore the space around the dendrites searching for appropriate binding partners. Filopodia repeatedly make transient contacts with axons, however only a selected subset of these contacts get stabilized, over a range of

Figure 1



A schematic diagram of functional molecules involved in the dendritic filopodia-spine transition. An adhesion molecule TLCN (green) is abundantly found in dendritic filopodia but mostly excluded from spines. In filopodia, the cytoplasmic region of TLCN binds to phosphorylated active forms of ERM family actin-binding proteins (red), but not to their inactive forms (blue). TLCN also binds to another actin-binding protein, α-actinin (yellow), in dendritic shafts. Many cell surface molecules (purple) induce filopodia-to-spine transition and synapse maturation, whereas TLCN plays a unique role as a brake of synaptogenesis that enhances filopodia formation/maintenance and also spine-to-filopodia reversion.

Figure 2



A schematic diagram of putative molecular steps leading from spine growth to its stabilization. Current evidence suggests that newly formed spines initially grow without a PSD and a presynaptic partner. They might however rapidly express functional excitatory receptors, which, upon activation by ambient glutamate, could promote the formation of a PSD and through expression of adhesion molecules and retrograde signals such as NO promote presynaptic differentiation and contact formation. This process occurs within minutes to hours depending upon developmental stage or level of activity in the local environment. Functionality of the new synapse then ensures its maturation and enlargement through activation of multiple signaling pathways targeting Rho GTPases and regulation of the actin cytoskeleton. Long-term persistence of the spine might however require induction of synaptic plasticity, additional enlargement and acquisition of the machinery for protein synthesis.

minutes, through the generation of calcium transients [23••]. Interestingly, this process of stable contact formation is not sensitive to glutamate receptor antagonists, which precludes a role for transmitter release in these mechanisms. Filopodia, however, are able to discriminate between partners, as they never make stabilized contacts with inhibitory axons [23••]. This suggests therefore that filopodia are able to recognize potential partners possibly through expression of recognition or signaling molecules. Whether these stable contacts between filopodia and axons represent future sites of PSD formation in spines is yet unclear.

Once the proper choice has been made, dendritic filopodia can be morphologically and functionally transformed into spines [5,6,18,22,24]. This process however may not be always very effective. Imaging studies in living, young mice or in hippocampal slice cultures suggests that only 10–20% of filopodia may actually be transformed into spines and that most of these spines disappear within subsequent days [6,22]. The situation might however be different during early phases of development, when the probability to reach a partner is lower and the motility of filopodia might represent a major advantage. A recent work shows that filopodia motility plays a critical role for synapse formation during development. Elimination of EphBs was found to decrease filopodia motility without affecting spine motility, and this resulted in a reduced rate of synaptogenesis, an effect prominent in early, but not late development [25]. During this period, filopodia-mediated synapse formation also depended on trans-synaptic interactions, involving namely the Rho GTPase effector PAK. Thus filopodia appear to be particularly important structures as precursors of spines during early development.

Molecular control of filopodia fate

In contrast to a wealth of knowledge on dendritic spines, little is known about the molecular and cellular mechanisms underlying the formation and maintenance of dendritic filopodia, the transformation from filopodia to spines, and the physiological significance of dendritic filopodia. However, several functional molecules have been recently identified, which regulate the formation of dendritic filopodia (Table 1). These molecules can be classified into two categories: 'accelerators' and 'brakes'. The accelerators include calcium/calmodulin-dependent protein kinase II (CaMKII) [19], syndecan-2 [26,27] and paralemmin-1 [28], which enhance filopodia formation and further accelerate spine maturation. In striking contrast, the brakes are molecules that not only induce but also maintain dendritic filopodia, thus slowing spine maturation and sometimes even causing spine-to-filopodia reversion [29–31,32••,33]. Among the brake molecules, telencephalin (TLCN) is of special interest and importance, which is a dendrite-associated adhesion molecule specifically expressed by spiny neurons in the mammalian telencephalon.

Telencephalin: a brake of spine maturation

TLCN is a cell adhesion molecule belonging to the immunoglobulin (Ig) superfamily. TLCN gene is present only in mammalian species. The Ig-like domains of TLCN most closely resemble those of intercellular adhesion molecules (ICAMs) that serve various important functions in cell–cell interactions of the immune system such as the formation of immunological synapses between T lymphocytes and antigen-presenting cells [34]. TLCN is the only neuronal member of the ICAM family and thus also called ICAM-5. TLCN displays four unique features with respect to its expression and localization. (1)

Table 1

Molecules involved in dendritic filopodia formation.

Molecule	Description	Approach	Filopodia formation	Spine formation	Reference
Brakes of spine maturation					
Telencephalin (TLCN; ICAM-5)	Ig superfamily cell adhesion molecule that interacts with ERM proteins	Overexpression Gene knockout	Up Down	Down Up	Matsuno *et al.* [32••]
Ezrin/Radixin/Moesin (ERM)	Actin-binding proteins that interact with TLCN	Constitutive active Gene silencing	Up Down	Not analyzed Up	Furutani *et al.* [33]
SynGAP	Ras GTPase-activating proteins	Gene knockout	Down	Up	Vazquez *et al.* [30]
Ras-PI3K-Akt-mTOR	Small GTPase and its downstream signaling molecules	Constitutive active Dominant negative	Up Down	Down Up	Kumar *et al.* [31]
Polo-like kinase 2 (Plk-2; SNK)	Ser/Thr kinase that phosphorylates and destabilizes spine-associated RapGAP (SPAR)	Overexpression	Up	Down	Pak and Sheng [29]
Accelerators of spine maturation					
CaMKII	Calcium/calmodulin-dependent Ser/Thr kinase	Constitutive active	Up	Up	Jourdain *et al.* [19]
Syndecan-2	Heparan sulfate proteoglycan linked to neurofibromin-PKA-Ena/VASP pathway	Overexpression Gene silencing	Up Down	Up Down	Ethell and Yamaguchi [26] Lin et al. [27]
Paralemmin-1	Lipid-anchoring phosphoprotein	Overexpression Gene silencing	Up Down	Up Not analyzed	Arstikaitis *et al.* [28]

Telencephalon-specific expression: TLCN is specifically expressed in the mammalian telencephalon, the most rostral brain segment including the cerebral neocortex, hippocampus, striatum, amygdala, olfactory cortex, and olfactory bulb. The 1.1-kb 5'-flanking region of the mouse TLCN gene is necessary and sufficient as a telencephalon-specific transcriptional enhancer [35]. (2) Spiny neuron-specific expression: In the telencephalon, TLCN is present only in spiny neurons. In the olfactory bulb, for example, TLCN is present in the spine-bearing granule cells, but not in the aspiny mitral cells [36]. (3) Dendrite-specific localization: In the telencephalic spiny neurons, TLCN protein is localized to the somatodendritic compartment, but not to the axon. The carboxyl-terminal 17 amino-acid sequence in the cytoplasmic region of TLCN protein directs its dendrite-specific localization [37]. (4) Postnatal appearance correlated with dendritic development: the ontogenic expression of TLCN parallels the dendritic elongation, branching, spine formation, and synaptogenesis in each region of the telencephalon during the postnatal development [38–40].

TLCN plays a unique role in synaptogenesis [32••]. In cultured hippocampal neurons, TLCN is abundantly present in dendritic filopodia, but is mostly excluded from mature spines. When TLCN is overexpressed in these neurons, the number of dendritic filopodia is markedly increased with a concomitant decrease of the number of spines. The TLCN-induced morphological changes of dendritic protrusions include the formation of new filopodia from dendritic shafts, the preservation of pre-existing filopodia, and the reversion from spines to filopodia. On the contrary, TLCN deficiency causes the acceleration of spine maturation with decreased number of dendritic filopodia in developing hippocampal neurons both *in vitro* and *in vivo*. Recently, it has been demonstrated that TLCN induces dendritic filopodia formation through the cytoplasmic interaction with ERM (ezrin/radixin/moesin) family actin-binding proteins [33] and that the extracellular region of TLCN is proteolytically cleaved by matrix metalloproteinase (MMP)-2 and -9 [41]. These results indicate that TLCN is a key molecule in synaptogenesis, which slows spine maturation by preserving the flexible state of dendritic filopodia. Filopodia-to-spine transition might be triggered by the exclusion of TLCN from the filopodial plasma membrane though proteolytic shedding, internalization, or lateral diffusion (Figure 1). In addition, the persistence of TLCN expression in the perisynaptic region of adult neurons and its possible involvement in LTP [42,43] suggest a putative role as a softener of synapses, which maintains structural dynamics and functional plasticity also in the mature brain.

Synapse formation through spine growth

In more mature tissue, time-lapse imaging has shown that new protrusions may also directly appear as spines [5,11]. This process, which occurs within minutes, probably accounts for about half of all protrusions formed in young (1–3 weeks old) hippocampal slice cultures [11,19,22]. Typically, these new spines have long necks and small heads, which sometimes makes them difficult to distinguish from filopodia, except that they are less motile. In young neurons, new spines and filopodia are produced at a high rate and seemingly in a random fashion. Also most of them are essentially transient and tend to disappear within hours [7,22]. The reasons for this are still unknown, but could be linked to a failure to stabilize due to a lack of activity, lack of expression of a postsynaptic density or lack of induction of plasticity [44]. Three-dimensional electron microscopic (EM) reconstruction of newly formed spines *in vitro* and *in vivo* or following LTP inducing protocols in slices has revealed that they do not seem to initially express a PSD [12,22,45]. Consistent with this, EM analyses in the cortex or hippocampus have shown the existence of a small population of spines devoid of a PSD or even without presynaptic partner, suggesting that spine growth could precede synapse formation [22,45,46]. In these experiments, morphologically mature synapses identified at the EM level on new spines were reported only after a delay of 10–24 h, while analyses of PSD-95-EGFP expression in slice cultures detected the formation of new puncta after about 5 hours. A recent study suggests that this might even be faster [47••]. In hippocampal slice cultures, new spines stimulated through glutamate uncaging become functional within 10 min and show evidence of morphologically mature synapses already after 1.5 hour. While it is not clear how to account for these temporal variations, one possible parameter could relate to activity. For example, De Roo *et al.* observed that acquisition of PSD-95-EGFP puncta in newly formed spines was markedly reduced and delayed by blockade of glutamate receptors, suggesting that the expression of the PSD could be under the control of activity [22]. Thus, accumulation of receptors and other PSD components might occur at a different speed depending on the level of activity detected by the new spine. A main conclusion from these studies however is that formation of functional synapses can be extremely fast.

If new spines grow before establishing a synaptic contact, then an intriguing question is to understand how they select their partner and stimulate their differentiation into a presynaptic terminal. While adhesion molecules are likely candidates to contribute to this process [48•,49], a recent work also points at an interesting role of nitric oxide (NO). NO is produced at excitatory synapses by neuronal nitric oxide synthase (nNOS) which is brought to the synapse through its interaction with the second PDZ domain of PSD-95. Upon overexpression of PSD-95, nNOS expression also increases and leads to the formation of spines that become innervated by multiple presynaptic partners [50]. The same situation is also

found when NO is increased in the tissue through application of a NO donor. Spines contacted by 4–6 terminals are then regularly found. Conversely, blockade of nNOS interferes with synapse formation and actually results in a loss of spines. NO produced by expression of the PSD in the newly formed spine could thus represent a retrograde signal stimulating nearby axons to differentiate and form a presynaptic terminal [50]. This could also help newly formed protrusions to target existing terminals and compete with other spines. The formation of a synapse on spines that are produced randomly, at high rate, might thus rely on an intricate set of signals, mediated by adhesion molecules, the released transmitter, but also retrograde messengers in order to orchestrate the establishment of a structurally defined contact zone.

Spine maturation and synaptic plasticity

Once a contact is made, the challenge of the new synapse is to become stabilized, a process that is likely to be regulated by neural activity [44]. Newly formed spines are usually thin and elongated and in general have a small head. They have often been referred to as learning spines in opposition to classical mushroom-shape spines that are representing more stable structures [2]. During this early phase of stabilization, when newly formed spines acquire a PSD, their spine head enlarges, a phenomenon that probably shows similarities with the spine head enlargement associated with LTP induction [8,10,22,44,51]. Increases in spine volume closely correlate with the accumulation of additional AMPA receptors [47••] and reorganization of the actin cytoskeleton [52•]. The mechanisms regulating these size changes start to be unraveled. Following induction of plasticity at single spines, enlargement was linked to a destabilization of the PSD, an increased dynamics of PSD proteins such as PSD-95 and SHANK2, and a contribution through phosphorylation of CaMKII [53]. This enlargement process however also involves a structural reorganization controlled by several signaling systems, including proteolysis of the extracellular matrix by proteases, and signaling to the cytoskeleton through adhesion molecules. Proteolytic activity of tissue plasminogen activator (tPA), released by dendritic spines, has been implicated in the pruning of synapses induced by a brief monocular deprivation in the developing visual cortex [54–56]. More recently, evidence was provided that MMP-9 activity is required for both LTP expression and spine enlargement and that this may involve signaling through a bêta1-integrin receptor, cofilin phosphorylation and actin polymerization [57•]. MMP-9 has however also been shown to cleave telencephalin, enhancing in this way spine maturation [41]. Additionally, in hippocampal cell cultures, a signaling pathway mediated by N-cadherin and involving the scaffold protein AF6/afadin, the Rho GTPase exchange factor Kalirin-7, Rac1, p21-activated kinases (PAKs) and the cytoskeleton has been proposed to regulate the size of the spine head [58••]. This pathway could allow synaptic adhesion molecules to rapidly coordinate spine remodeling associated with synapse maturation and plasticity. Rho GTPases such as Rac1 or Cdc42 appear to play central roles in this process and could actually be regulated by several different signaling complexes including protein kinases such as CaMKK and CaMKI associated with the GTPase exchange factor beta-Pix [59•] or the Calcium/calmodulin serine protein kinase CASK through a mechanism of SUMOylation [60]. A link between activity and these Rho GTPases could in this way not only regulate spine maturation and enlargement, but also activity-dependent spine formation. This central role of GTPases could thus account for the association of mutations of several of their partners with mental retardation [61] and defects in spine morphology and/or synaptic plasticity [62].

How this activity-dependent structural remodeling of spines confers them stability is still however unclear. The close correlation existing between spine head size, PSD size, receptor number and stability suggests that the phenomenon could be non-specifically related to the amount of receptors and proteins accumulated at the synapse: larger spines with larger PSDs express more adhesion and cross-linking molecules and are more stable. However, spine enlargement might be only transient and results suggest that spine head size actually shows continuous fluctuations over time [7,13••,63], while plasticity induced stabilization might be more lasting [13••]. It could be therefore, as suggested by some morphological experiments, that stabilization is provided through other mechanisms such as acquisition by the potentiated spines of the machinery for mRNA translation that would allow local regulation of protein synthesis and trafficking and thus confer independence and stability to the spine [64–66]. Additionally, this could be associated with the expression at the synapse of specific proteins able to stabilize the cytoskeleton or anchor pre- and postsynaptic structures. This would account for the dependence of synaptic plasticity on protein synthesis, although evidence for such a 'memory' protein or stability marker is still missing. Finally it is interesting that spine stabilization may be further associated with other local regulations that expand the phenomenon by favoring induction of plasticity on neighbor spines [10,67] or the growth of new functional synapses close to potentiated spines [13••], thus potentially creating clusters of stable synapses.

Conclusion

Dendritic spine formation and stabilization as a functional excitatory synapse remains a mystery with complex mechanisms involving a multiplicity of steps, regulations and molecules. Our current understanding suggests the existence of two parallel tracks, one based on the growth of filopodia and predominantly active in early phases of development, where regulation of motility by molecules

such as Ephrins and Telancephalin plays a critical role for the transformation into spine synapses, and a second one, mostly observed in later development and mature brain, where protrusions grow directly as spines, most likely without initial PSD or partner, and for which activity and forms of plasticity such as LTP are probably key factors leading through trans-synaptic signaling, NO, adhesion molecules, Rho GTPases and certainly yet unrecognized partners, to the maturation and persistence of the new synapse. The complexity of these mechanisms, while reflecting their importance, also reveals their fragility and the numerous possibilities of dysfunctions that appear to link genetic defects affecting synaptic proteins to a great number of cognitive and developmental neuropsychiatric disorders.

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