Minireview

The role of protein phosphatase-1 in the modulation of synaptic and structural plasticity

Richard P. Munton, Sándor Vizi, Isabelle M. Mansuy*

Institute of Cell Biology, Department of Biology, ETH Hönggerberg HPM D24, CH-8093 Zurich, Switzerland

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Abstract Synaptic plasticity is a phenomenon contributing to changes in the efficacy of neuronal transmission. These changes are widely believed to be a major cellular basis for learning and memory. Protein phosphorylation is a key biochemical process involved in synaptic plasticity that operates through a tight balance between the action of protein kinases and protein phosphatases (PPs). Although the majority of research in this field has concentrated primarily on protein kinases, the significant role of PPs is becoming increasingly apparent. This review examines one such phosphatase, PP1, and highlights recent advances in the understanding of its intervention in synaptic and structural plasticity and the mechanisms of learning and memory.

Keywords: Protein phosphatase-1; Synaptic plasticity; Structural plasticity; LTP; LTD; Learning and memory

1. Introduction

Protein phosphorylation is one of the most important and widespread post-translational modifications in cells that drives rapid, reversible and activity-dependent signal transduction. The process of phosphorylation can lead to the activation of various types of targets, including membrane receptors, intracellular enzymes, or cytoskeletal proteins, and can regulate processes such as protein trafficking, protein–protein interactions, enzyme activity as well as a multitude of cellular processes from apoptosis to synaptogenesis. The role of protein phosphatases (PPs) in biological systems is often considered secondary to the role of protein kinases simply because kinases are the primary effectors of phosphorylation while phosphatases are the effectors of dephosphorylation. However, protein phosphorylation/dephosphorylation is a dynamic process that relies equally on protein kinases and PPs and more specifically, on a delicate and concerted balance between these proteins.

Four major PPs, able to specifically remove phosphate groups from serine (Ser) and threonine (Thr) residues, are present in the cytoplasm of animal cells [1]. PP1, PP2A, and PP2B are three of these enzymes encoded by the same gene family while PP2C arises from a distinct gene. PP1, PP2A, and PP2B are complexed to other proteins in vivo and have broad and overlapping specificities in vitro accounting for virtually all measurable Ser/Thr phosphatase activity in tissue extracts. PPs are highly abundant in the brain where, together with protein kinases, they contribute to the control of synaptic plasticity and memory. Synaptic plasticity is a property of neuronal connections, or synapses, which modifies the efficacy of synaptic transmission. Such modulation of transmission efficacy is driven by correlations in the firing activity of pre- and postsynaptic neurons, a concept first proposed by Donald Hebb [2]. Strengthening or weakening of synaptic efficacy is believed to be a cellular basis for learning and memory. Research in the last decade has implicated many protein kinases in short- and long-term modification of signal transmission between synapses, but recently the functional role of complementary PPs has also been revealed.

This review covers recent advances on PP1 and its involvement in protein phosphorylation in synaptic and structural plasticity, and ultimately in learning and memory. Recent data from a variety of studies utilizing pharmacological, behavioural, imaging and electrophysiological techniques suggest that PP1 not only functions to reverse kinase activity but to negatively regulate synaptic strength, and learning and memory.

2. PP1

The role of PP1 was initially described after discovery of its participation in the regulation of glycogen metabolism, but it was subsequently discovered to have an essential role in many other cellular processes such as cell division, muscle contractility, transcription, translation and apoptosis. The importance of PP1 in neural systems became was apparent after the discovery of high expression of several of its catalytic subunits in brain tissue, including α, β, γ1, and γ2. Multiple interactions

* Corresponding author. Fax: +41-1-633-10-69.
E-mail address: isabelle.mansuy@cell.biol.ethz.ch (I.M. Mansuy).

Abbreviations: AKAP, A-kinase anchoring protein; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid; BD, binding domain; CaMKII, calcium/calmodulin-dependent protein kinase type II; CREB, cyclic AMP-dependent response element binding; DA, dopamine; DARPP-32, dopamine and cAMP-regulated phosphoprotein Mr 32000; EM, electron microscopy; I1, inhibitor-1; LTD, long-term depression; LTP, long-term potentiation; mRNA, messenger RNA; NMDA, *N*-methyl-D-aspartate; p70S6K, p70S6 kinase; PDZ, postsynaptic-density-protein/disc-large/zo-1; PKA, cAMP-dependent protein kinase A; PKC, protein kinase C; PP1, protein phosphatase-1; PSD, postsynaptic density; SAM, sterile alpha motive; serine, Ser, serine; Thr, threonine; TNG38, *trans*-Golgi network binding protein 38 kDa

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with various regulatory proteins compartmentalize these several isoforms to varying but distinct subcellular locations. For example, the β isoform is found predominantly in the cell soma, whereas γ1 in addition to the soma is also found in large quantities in dendrites and presynaptic boutons where it co-localizes with abundant neuronal proteins such as calcium/calmodulin-dependent protein kinase type II (CaMKII) and synaptophysin [3].

3. Endogenous regulation of PP1 activity

The specificity and activity of PP1 is largely controlled by interacting proteins, the majority of which act as regulatory subunits modulating PP1 catalytic activity, or scaffolding proteins that compartmentalize PP1 to discrete subcellular locations in close proximity to target substrates. Many endogenous proteins have been shown to interact with PP1, leading to substantial changes in localization and activity (Table 1). Most of these interacting protein sequences include two highly conserved docking motifs, [RK]-x(0,1)-V-x-F and F-x-x-[RK]-x-[RK], expressed in standard ProSite format. Most regulatory accessory proteins have inhibitory actions on PP1 activity. An additional level of regulation occurs via phosphorylation of these inhibitory proteins. For example, inhibitor 1 (I1) is an ubiquitously expressed 28 kDa protein which upon phosphorylation by cAMP-dependent protein kinase A (PKA), results in potent inhibition of PP1. I1 messenger RNA (mRNA) is strongly expressed in the hippocampus [4] and I1 protein is found in the CA1 region of the hippocampus [5]. Other proteins such as G-substrate and dopamine and cAMP-regulated phosphoprotein Mr 32000 (DARPP-32), which exhibit considerable sequence overlap with I1, also inhibit PP1 [6]. Although the most thoroughly researched accessory proteins are inhibitory, two proteins which inhibit the related phosphatase PP2A, I1 (PP2A) and I2 (PP2A), actually increase the catalytic activity of PP1 in vitro [7]. In neural tissue, the sequestration of PP1 to structures such as the postsynaptic density (PSD), a multi-protein complex found at synapses and that contains hundreds of signal transduction molecules, is particularly important for the functioning of many signalling proteins. Yotiao is a neuromuscular junction protein member of the A-kinase anchoring protein (AKAP) family that sequesters both PKA and PP1 to *N*-methyl-D-aspartate (NMDA) type glutamate receptors [19]. Spinophilin is a binding protein containing a postsynaptic-density-protein/disc-large/zo-1 (PDZ) domain, sequestering PP1 to actin. Spinophilin is also responsible for the association of PP1 with other receptors such as the D2 dopamine (DA) receptor [20], alpha adrenergic receptor [21] and p70S6 kinase (p70S6K) [22]. The role of a selection of PP1 interacting proteins in synaptic activity is explored in the following sections.

4. Modulation of glutamatergic transmission

Synaptic transmission involves the release of a neurotransmitter from a presynaptic neuron and activation of signalling cascades via receptors on the postsynaptic membrane of another neuron. The nature and magnitude of the postsynaptic signal can be modulated by the actions of protein kinases and PPs, which often work via complex feedback loops and cross-talk between different signalling cascades resulting in tight control over communication between neurons. Changes in synaptic transmission can involve protein transcription and protein synthesis DNA translation although the following sections illustrate the importance of PP1 in modulation of glutamatergic activity at synapses. Glutamate is the major excitatory neurotransmitter in mammalian nervous system that mediates its effects in part through α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) and NMDA receptors. Both AMPA and NMDA receptors are members of ionotropic receptor superfamily, and upon binding of glutamate, allow entry of cations into the neuron. In neostriatal neurons AMPA receptor activity was shown to be regulated by PP1 by two distinct molecular mechanisms [23]. Firstly, control of AMPA receptor activity is regulated by the association of PP1 to spinophilin localizing PP1 to the PSD and in close proximity to AMPA receptors. Secondly, phosphorylated DARPP-32, a DA- and cAMP-regulated phosphoprotein highly enriched in the neostriatum, inhibits

Table 1
Proteins interacting with PP1

Protein	Location	Function	Reference
Inhibitors			
I1	Widely distributed, cytosol	Inhibition of PP1	[8]
DARPP-32	Brain, kidney, cytosol	Inhibition of PP1	[9]
I2	Widely distributed, cytosol and nucleus	Inhibition of PP1	[10]
G-substrate (cGMP-dependent protein kinase substrate)	Brain	Inhibition of PP1	[11]
I1 PP2A (PHAP1) and I2 PP2A (SET, PHAPII, TAF1b)	Widely distributed	Inhibition of PP2A but stimulation of PP1	[7]
Scaffolding proteins			
Neurabin I	Neuronal, plasma membrane and actin cytoskeleton	Neurite outgrowth	[12]
Spinophilin (neurabin II)	Plasma membrane and actin cytoskeleton	Glutamatergic transmission	[13]
NF-L (neurofilament-L)	Neuronal, plasma membrane and cytoskeleton	Synaptic transmission?	[14]
AKAP220	Brain, testis, peroxisomes/cytoskeleton	PKA and PP1 signalling	[15]
Yotiao (AKAP)	NMDA receptor	Synaptic transmission	[16]
BH-protocadherin	Neuronal membrane	Neuronal cell–cell interactions	[17]
Tau	Neuronal microtubules	Microtubule stability	[18]

Neuronal proteins interacting with PP1. The majority of PP1 interacting proteins are inhibitory and scaffolding proteins. Comprehensive information on binding motifs and a list of published interacting partners of PP1 can be found at http://pp1signature.pasteur.fr/.

PP1 and thus maintains AMPA receptor currents. Accordingly, intracellular application of dephosphorylated DARPP-32 rapidly reduced AMPA receptor currents. Phosphorylation of DARPP-32 occurs after activation of PKA by stimulation of dopamine-D1 receptors, suggesting that PP1 is heavily involved in the interplay and cross-talk between multiple signalling cascades. DARPP-32 is also involved in PP1 regulation of NMDA receptor responses providing further evidence for cross-talk between glutamatergic and dopaminergic signalling pathways. NMDA receptor currents are known to be enhanced by phosphorylation as phosphatase inhibitors enhance NMDA receptor channel currents. Furthermore, dephosphorylation of NMDA receptors by PP1 and PP2A has been shown to decrease the probability of channel opening. DA, via activation of D1 receptors, enhances NMDA-evoked responses in striatal neurons via PKA phosphorylation of DARP-32 [24]. Mice lacking DARPP-32 exhibit reduced enhancement of NMDA receptor currents and, furthermore, pharmacological inhibition of PP1 by okadaic acid increased D1-induced NMDA receptor currents. These data suggest that constitutively active PP1 is responsible for attenuating NMDA receptor currents.

Another protein that interacts with PP1 could play an important role in the phosphorylation state of and therefore functioning of NMDA receptors [16]. The scaffolding protein yotiao binds both the regulatory subunit of PKA and PP1, sequestering the two enzymes directly to the C-terminus of the NMDA NR1 subunit. PP1 was found to be constitutively active and was able to limit NMDA currents when complexed with yotiao. This process is reversed by PKA-activation, which rapidly increased channel currents. The ability of yotiao to sequester a complimentary phosphatase and kinase could be a way to improve the speed and efficiency of activity-dependent modifications of the NMDA receptor.

5. Targets of PP1 in long-term potentiation (LTP) and long-term depression (LTD)

PP1 has also been implicated in long-term changes in glutamatergic transmission, which is explored in this section and summarized in Fig. 1. Bidirectional changes in the excitability of neurons are thought to be a major correlate for the strengthening and weakening of synapses, or synaptic plasticity. Although many types of synaptic plasticity have been described, typically changes in synaptic efficacy are a result of NMDA receptor activation followed by calcium entry, and activation of kinases and phosphatases (see [25] for review). PKA, protein kinase C (PKC), and CaMKII have all been shown to be involved in synaptic plasticity by modulating glutamate receptor function by phosphorylation of AMPA or NMDA receptor subunits [26]. LTP is a form of synaptic plasticity where synaptic efficacy is increased after high frequency stimulation. This stimulation induces transient NMDA receptor mediated calcium entry resulting in a sustained increase in CaMKII activation due to Thr-286 autophosphorylation. It occurs after transient calcium entry induced by stimulation of NMDA glutamate receptors followed by activation and persistent increase in CaMKII activity by autophosphorylation at Thr-286. The converse of LTP, LTD, is a decrease in synaptic efficacy induced by low frequency stimulation, initiated by cascades involving PP1 and calcineurin (PP2B). The loci of expression of LTP and LTD have not been determined precisely, but there is ample evidence for the involvement of a multitude of pre- and postsynaptic elements. For instance, LTP at the Schaffer collateral-CA1 synapse in the hippocampus involves interacting signalling components, including CaMKII and cAMP pathways (Fig. 1). The involvement of PP1 in CaMKII signalling is tightly regulated and involves phosphorylation of the inhibitory PP1-interacting

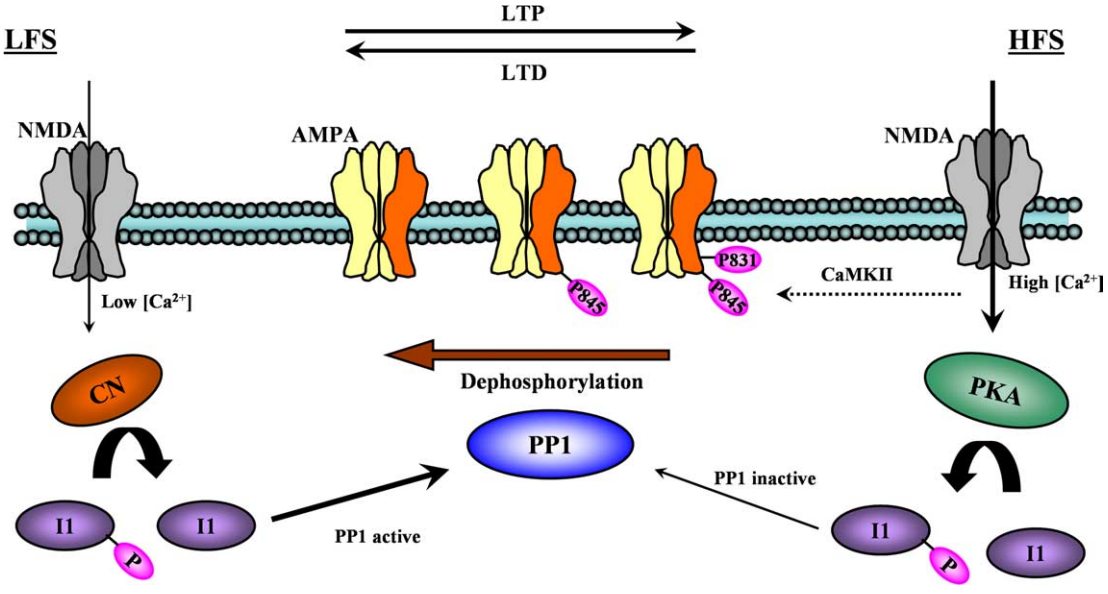

Fig. 1. PP1 activity on GluR1 subunit of AMPA receptors plays a pivotal role in bidirectional synaptic plasticity. High frequency stimulation leads to PKA-dependent phosphorylation of I1 and subsequent inactivation of PP1. This reduces the amount of Ser-845 dephosphorylation, then in turn LTD. Additionally, HFS activates CaMKII which phosphorylates Ser-831 leading to LTP. Conversely, low frequency stimulation activates a calcineurin cascade that leads to I1 dephosphorylation and relief of PP1 inhibition. PP1 then dephosphorylates Ser-845 leading to the expression of LTD. LFS given to a previously potentiated synapse (depotentiation) initiates dephosphorylation of Ser-831. HFS delivered to previously depressed synapses (de-depression) phosphorylates Ser-845 by overcoming phosphatase activity.

protein I1 by PKA. Stimulation that induces LTP triggers cAMP-dependent phosphorylation of endogenous I1 resulting in a decreased PP1 activity. This same stimulation also results in increased phosphorylation of CaMKII at Thr-286, indicating that the cAMP pathway uses PP1 to gate CaMKII signalling in LTP [5]. Additionally, dephosphorylation of CaMKII occurs specifically by the PP1 held in PSD by scaffolding proteins [13,27–31], although another phosphatase, PP2A, which dephosphorylates soluble cytoplasmic CaMKII is unable to dephosphorylate PSD bound CaMKII [32]. Together, these data illustrate the complexity of the mechanisms of control of CaMKII activation and in turn of LTP by PP1.

Three decades of research have implicated a multitude of mechanisms in the induction of LTP and LTD, and a large body of evidence suggests that both LTP and LTD are associated with a change in the phosphorylation state of the GluR1 receptor subunit of AMPA receptor channels. GluR1 receptor channels can be phosphorylated at two positions, Ser-831 and Ser-845. Basal phosphorylation of Ser-845 by PKA enhances GluR1 receptor currents [33], and during LTP, CaMKII phosphorylates GluR1 subunits at Ser-831 which leads to increased channel conductance in some, but not all neurons. During LTD, calcineurin activation following low frequency stimulation dephosphorylates I1, preventing PP1 inhibition, which in turn dephosphorylates AMPA receptors at Ser-845 decreasing AMPA receptor currents. Additionally, both PP1 and calcineurin have been implicated in activity-dependent endocytotic internalization of AMPA receptors, an additional process thought to decrease the efficacy of glutamatergic transmission during LTD, with dephosphorylation of Ser-845 a pre-requisite for endocytosis [34–36]. Electrophysiological studies demonstrate that disruption of PP1 binding to synaptic targeting proteins blocks the induction of LTD by low frequency stimulation, but has no effect on basal synaptic currents mediated by AMPA or NMDA receptors [37]. Directly loading PP1 into neurons also had no effect on basal transmission but enhanced the expression of LTD. Additionally, synaptic activation of NMDA receptors leads to increased PP1 activity at synaptic locations. These results demonstrate that PP1 does not regulate basal transmission but is involved in activity-dependent regulation of LTD.

Another type of synaptic depression, depotentiation, has been described in the hippocampus and also involves PP1. Depotentiation is the reversal of LTP, reflecting a reduction of the potentiated state of synapses to basal levels, and is a potential mechanism for preventing the saturation of the storage capacity of a neuronal network [38]. Specific electrophysiological protocols utilize low frequency stimulation following LTP, which activates NMDA receptors and phosphatase cascades resulting in dephosphorylation of Ser-residues on GluR1 subunits. Pharmacological agents inhibiting PP1, but not other phosphatases such as PP2A or calcineurin have been shown to specifically prevent depotentiation. In agreement with previous studies that implicate phosphorylation of Ser-831 by CaMKII in the induction of LTP, a decrease in phosphorylation at this site has been observed along with a reduction in Thr-286 phosphorylation of CaMKII. However, Jouvenceau and colleagues [39] concluded that LTD and depotentiation are indeed separate processes involving different phosphatase cascades demonstrating that depotentiation requires PP1, PP2A and calcineurin, but LTD only requires PP1 and PP2A. Although, further research is required to delineate the exact functions of different phosphatases in depotentiation, it is clear that dephosphorylation of AMPA receptors by PP1 in depotentiation is an activity-dependent mechanism.

To summarize, a large body of research has indicated that PP1 plays an important role in the modulation of synaptic activity and particularly in the regulation of glutamate receptors in bidirectional plasticity. Such function occurs via a multitude of mechanisms, involving multiple kinase and phosphatase cascades. The role of phosphorylation on the modulation of neuronal activity is activity-dependent and reversible, which further illustrates that phosphatase mechanisms not only serve to limit kinase activity in complex signalling pathways but have their own functions.

6. Structural plasticity

6.1. Structural plasticity at the synapse: historical aspects

Besides controlling the above-mentioned molecular events of synaptic transmission, PP1 is also involved in regulatory pathways responsible for establishing the structural basis for long-term memory. Though it is widely accepted that learning induces not only molecular alterations but also structural changes, the nature and degree of these changes are not well known. Even then, it is recognized that the primary substrates of structural plasticity are dendritic spines, neuronal structures which hold most synapses in the brain. Spines were described more than a century ago as small protrusions at the dendritic surface [40]. These micrometer-sized structures are specialized for receiving synaptic afferents and accordingly, they cover the dendrites of many neurons at a high density. Major advances in understanding the significance of dendritic spines are linked to the development of techniques allowing visualization of these tiny structures. The first method for rendering spines visible was the silver impregnation technique introduced by Camillo Golgi. Spines revealed by Golgi staining have immediately captivated neuroscientists and gained a pivotal role in theories of neural function. As early as 1899, Ramon y Cajal postulated that spines are motile structures that can have a role in the regulation of neuronal activity. It took more than half a century to prove unambiguously with electron microscopy (EM) analysis that spines indeed bear synapses and therefore are directly involved in neuronal communication. At that time, however, dendritic protrusions were generally considered as immobile elements of the "hardwired" brain with little capacity for plasticity. Intriguingly, the pioneering studies which mobilized the field presented only indirect clues for spine motility by detecting actin in dendritic spines [41]. Several lines of neurobiology research have now provided direct evidence for dynamic changes in spine morphology and for the importance of motility at dendritic protrusions in neuronal development and synaptic plasticity.

6.2. Dendritic motility in the developing nervous system

Spines were initially described in the mature brain where they carry the vast majority of excitatory synapses. Recent in vitro studies, however, revealed that developing neurons also extend highly dynamic dendritic processes, termed filopodia [42]. These structures are usually more elongated than dendritic spines and can rapidly appear, extend, and retract (in tens of minutes) [43]. In addition, they can develop dendritic growth cones. The movements of these structures have been

visualized by high-resolution confocal microscopy in time-lapse imaging studies. In these experiments, the density of filopodia on mature neurons was found to be much smaller than on developing cells, leading to the suggestion that filopodia establishing a stable synaptic contact with a presynaptic partner can develop into dendritic spines. However, only 25% of synapses have been localized on filopodia in the early postnatal brain while the rest were found on dendritic shafts [44]. Filopodia might therefore not be major players in developmental spinogenesis. Instead, they may have another specific role as they have been detected on neurons that later do not develop spines [45,46]. Now it seems that they are mainly dendritic precursors, as filopodia have been shown to form new dendritic branches in vivo [47]. In the optic tectum of zebrafish larvae, filopodia on non-spiny dendritic arbors were stabilized and formed new dendritic segments only if they had previously established a stable synaptic contact. These results support a "synaptotrophic" model for dendritic development, where synapse formation on filopodia plays an active role in shaping the dendritic arbor.

6.3. Dynamic spines in the mature brain

The traditional approach for studying spines of CNS neurons with high resolution is EM. The presence of synapses on dendritic spines contacted by other neural elements can still only be confirmed by this technique and exact quantitative characterization of morphological parameters of spines via 3D reconstruction also requires serial EM. The EM technique, however, is not amenable to follow the spontaneous dynamics of dendritic spines. Thus, the introduction of live confocal imaging is a crucial technical advance that allows the visualization of mature neurons in isolated neural systems and the investigation of the relationship between structure and function of dendritic spines with high temporal resolution. Rapid changes in the morphology of dendritic protrusions have been detected in cultured neural cells [48], neurons in slice cultures [49], and ganglion cells of the isolated retina [50]. Recently, further refinement with time-lapse imaging and correlated ultrastructural analysis led to the demonstration that motile spines form synaptic contacts. It was shown in cerebellar slice cultures that Purkinje cell spines with synapses are able to move [51]. The degree of spine motility appeared to closely correlate with the space available for moving spines, suggesting that spatial constraint imposed by surrounding neural and glial elements may reduce spine motility. The question of whether moving Purkinje cell spines detached from their presynaptic partner or just morphed around the intact synaptic contact still remains to be answered.

More recently, the advent of two-photon in vivo confocal microscopy led to the demonstration that spines are motile under certain conditions in the intact living brain [52]. Further improvement of this imaging technique is, however, needed for the visualization of synapses on motile spines. Thus, in a recently published elegant study [53] assessing long-term in vivo spine dynamics in the mouse brain, a combinatorial approach was employed: the apical dendritic segments of mouse barrel cortex neurons were first visualized in vivo, then identified spines were later reconstructed using serial EM. Two of the four spines which emerged from the reconstructed dendritic segments during the last day of imaging had clearly identifiable synapses on their head, providing firm evidence that new spines can form synapses in the adult brain. Further, results of the study support the view that these synapses were newly formed (as opposed to the possibility of raising pre-existing shaft synapses by the emerging new spines), suggesting an active role for dendritic spines in synaptogenesis. Kinetic properties of spine motility were also measured in these experiments: in the barrel cortex of adult mice 83% of spines were stable for over a day, 77% of spines were stable over 2–3 days, 60% of spines were stable over at least 8 days, and ~50% of spines were stable over 1 month. These data suggest a remarkably high turnover of dendritic spines in the adult brain, with no obvious change in spine density over the dendritic segments. Accordingly, the mean lifetime of stable spines has been estimated to be as short as ~4 months. The authors also confirmed a correlation between spine stability and morphology, with large mushroom shaped spines being the most stable. The plasticity of spine dynamics was also demonstrated: the contralateral whiskers were trimmed in a chessboard pattern to induce unilateral remodelling of whisker representation in the barrel cortex. Chessboard deprivation boosted spine turnover 2–3 days later in the corresponding barrel cortex by increasing the number of transient spines and decreasing the number of stable spines. Apparently, these two processes counterbalanced each other resulting in unchanged overall spine density.

Interestingly, another study employing transcranial in vivo imaging to visualize apical dendrites of pyramidal cells in the primary visual cortex of mice reported a much lower rate of spine dynamics [54]. In young animals, only ~1% of the visible dendritic protrusions extended or retracted within 4 days. Accordingly, 94% of spines remained stable over 3 days, 82% were stable over 2 weeks, and 73% of spines persisted for over 1 month. Filopodia that represented around 10% of the protrusions in young mice were much more dynamic; 90% of them extended or retracted within 4 days and only rarely (<1%) developed into spines. In adults, an even higher level of spine stability was noticed. More than 99% of dendritic protrusions were spines and no change in their number or location was observed in 99% of them within 3 days, in 97% of them over 2 weeks, or in 92% over 2 months. Even when neurons were repeatedly imaged over 4 months, 80% of the spines remained stable and the new spines appearing during this time constituted only 10% of total spine number. The estimated half-life of dendritic spines in adult mice was over 13 months, implying that dendritic spines remain unchanged for extended periods during the lifetime of the animal. Accordingly, the authors argued that the observed high level of structural stability in the adult nervous system could serve as a structural basis for long term storage of memory. The significant changes in spine morphology observed in periods of 3 days and 1 month in adult mice were, in turn, suggested to be associated with plastic changes in synaptic efficacy underlying new memory.

The strikingly different kinetic properties of cortical spines described in the above reports could be explained by the inherently different rates of spine dynamics in various regions of the cerebral cortex. Though, this hypothesis is not supported by observations describing similar kinetic parameters for spine turnover in diverse regions of the monkey brain [55,56]. Remarkably similar conclusions were drawn, however, in these two studies regarding the plasticity of axonal and dendritic branches. Both groups suggested that these neural processes are highly stable in the adult brain, leaving the role of establishing and disrupting physical connections between pre- and postsynaptic partners for the dendritic spines.

6.4. PP1 is involved in the regulation of spines

Motility of dendritic spines is regulated by a network of interacting signalling pathways recognized to involve Rho GTPases [57,58], protein kinases and PPs, in particular PP1. These signalling pathways mainly exert their effects by directing the dynamic reorganization of the actin microfilament system in spines [48], a process intimately linked to synaptic plasticity [59,60]. As already mentioned before, the action of PP1 can be both restricted and facilitated by various regulatory subunits, which appear to be critical for the regulation of spine motility. Major regulatory subunits include neurabins that target the catalytic subunit of PP1 to dendritic spines. In vertebrates, two neurabin isoforms exhibiting 48% homology in their amino acid sequences have been identified: neurabin I and neurabin II (also named spinophilin). Neurabin I was isolated as an actin-binding protein that is present in neural growth cones and dendritic spines [30], while spinophilin was identified as a binding partner of PP1 enriched in spine heads in the hippocampus [13]. Both proteins can be isolated from the brain in the same PP1 complex [32], in accord with biochemical data suggesting homo- and heterodimerization for both neurabins.

Structurally, both neurabin I and spinophilin are large scaffolding proteins that carry a series of protein interacting domains (Fig. 2). Both proteins have an NH_2-terminal F-actin binding domain (BD), a central PP1 binding "RVXF" module, a PDZ domain, and a COOH-terminal putative "coiled coil" domain. The F-actin BD of spinophilin has been shown to be necessary and sufficient for targeting the protein to dendritic spines [61]. Also, this region in spinophilin is sufficient to bundle actin filaments. When phosphorylated by PKA on Ser-177, spinophilin still binds F-actin with high affinity but with reduced stoichiometry [62]. In consequence, PKA phosphorylation of spinophilin on Ser-177 releases spinophilin from F-actin. In contrast, activation of Rac1 GTPase can target PP1 to the cytoskeleton by promoting the F-actin binding of neurabin I.

The COOH terminal "coiled coil" domains in neurabins can serve to stabilize homo- and heterodimers of neurabin molecules. Binding of the *trans*-Golgi network membrane protein 38 kDa (TNG38) to this region is stronger in neurabin dimers, providing an additional way for stabilizing the association of neurabins. TNG38, an integral membrane protein, also directly attaches TNG38-containing membranes to the actin filament network. Besides F-actin binding, spinophilin can also target PP1 to several G-protein coupled neurotransmitter receptors by binding to their third intracellular loop. These receptors include the α_{2A}-, α_{2B}-, and α_{2C}-adrenergic and D2 dopaminergic receptors [21], as mentioned earlier. Finally, spinophilin has also been reported to bind ryanodine receptors and proposed to mediate the association of PP1 with them.

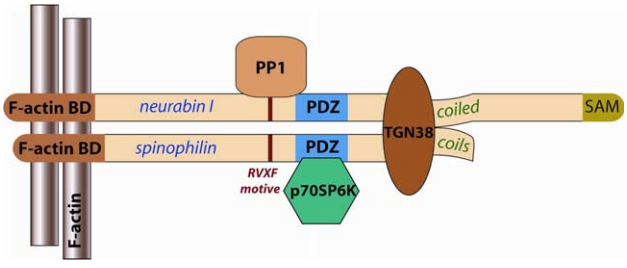

Fig. 2. Structure of a spinophilin/neurabin I heterodimer with associated proteins. PP1 and p70S6K can only bind to neurabins in a mutually exclusive manner (see text).

Neurabins are phosphorylated by PKA at Ser-461 and this event leads to the dissociation of PP1 from neurabin I. PP1 binds neurabins via the "RVXF" module and each of its catalytic subunits has a different affinity for that module ($PP1\gamma > PP1\alpha > PP1\beta$). The binding site of PP1 on neurabins overlaps with the binding site of the p70S6K, a mitogen activated protein kinase. Accordingly, the binding of the PP1 and p70S6K to neurabins is mutually exclusive. It has been shown, however, that neurabin I prefers to bind PP1 as opposed to p70S6K implying that the dissociation of PP1 from neurabin I is a pre-requisite for the binding of the kinase. It is worth noting that p70S6K plays an antagonistic role in the regulation of cellular morphology as compared to PP1. Active p70S6K induces the assembly of stress fibres while, in contrast, neurabin associated PP1 can initiate their disassembly and the formation of extended filopodia [63].

The significance of neurabins in shaping neural processes has been verified by eliminating neurabins from cells by genetic or molecular manipulations. Spinophilin knockout mice totally lacking spinophilin display a reduced brain size and a higher number of dendritic spines, accompanied by altered NMDA and AMPA receptor functions, reduced LTD, and a higher resistance to kainite-induced seizures and apoptosis [33]. Thus, it seems that hampered anchoring of PP1 in dendritic spines profoundly affects neural development, morphology and functions. Intriguingly, neurabin I antisense oligonucleotides hindered the formation of neurites in hippocampal cells [30], in contrast to the higher spine density observed in spinophilin knockout mice. Clearly, further investigations are needed to resolve this discrepancy and clarify the role of PP1 targeting neurabins in the structural plasticity of neural processes.

7. Learning, memory and forgetting

Several recent studies have investigated the role of PP1 in learning and memory by taking advantage of transgenic methodologies in mice. The genetic inhibition of PP1 by temporally controlled expression of the endogenous PP1 inhibitor I1 in forebrain neurons demonstrated a connection between repetitive learning and PP1 [64]. Repetitive learning is required for the formation and storage of accurate and long-lasting memory, with repetition being most effective when widely distributed over time rather than closely spaced or massed. When PP1 was genetically inhibited in both the hippocampus and cortical regions during learning, shorter intervals between training episodes were necessary for optimal performance in novel object recognition and Morris water maze tests. The observed learning enhancement correlated with increased phosphorylation of several synaptic and nuclear targets of PP1 including GluR1, CaMKII, and cyclic AMP-dependent response element binding (CREB) protein. For GluR1, phosphorylation levels of both Ser-845 and Ser-831 (unpublished) sites were found to be higher due to PP1 inhibition possibly reflecting an increase in the basal level of glutamatergic transmission. This shift in the overall balance of glutamate receptor phosphorylation towards a more excited state may

induce changes in the processes leading to LTD. Inhibition of PP1 additionally prolonged memory when induced only after learning, suggesting that PP1 also promotes forgetting. This property may account for ageing-related cognitive decay, as old mutant animals, in which PP1 was inhibited during and after learning, had preserved memory. These findings emphasize the physiological importance of PP1 as a suppressor of learning and memory, and as a potential mediator of cognitive decline during ageing. Such a PP1-mediated constraint upon memory could provide a molecular mechanism to erase erroneous memories. Continued activation of neural pathways is thought to maintain memories, whereas disuse will lead to active erosion and prevent the aggregation of superfluous neural activity.

Additional studies employing genetic or pharmacological approaches demonstrated a relationship between PP1 and learning and memory. Mice lacking two of the endogenous inhibitors of PP1, I1 and DARPP-32, were found to exhibit reduced cocaine place preference test performance; a behavioural test which measures the ability of the animal to associate a specific location with a rewarding drug [65]. These results support a role of PP1 in the mediation of changes in neuronal signalling that occurs following activation of the dopaminergic system. Pharmacological inhibition of PP1 in vivo also induces deficits in memory retention. Bennett et al. [66,67] found that the inhibition of PP1 or PP2A in chick brain by various concentrations of okadaic acid produced temporally specific deficits in memory retention. This suggested that different phosphatase enzymes may contribute to different stages of the enzymatic cascade believed to underlie memory formation.

The role of PP1 in memory and learning could also have relevance to clinical conditions. In addition to a possible involvement in age-related cognitive decline [64], a link between PP1 and impairment of spatial memory in Alzheimer's disease has been suggested. Hyperphosphorylation of Tau, a protein that sequesters PP1 to microtubules, leads to the formation of paired helical filament/neurofibrillary tangles, a hallmark for Alzheimer's pathology. It was found that rats injected with the PP1 and PP2A inhibitor, calyculin A, develop deficits in spatial memory retention in the Morris water maze test [68]. These results could pave the way for future research into the specific pathways of PP1 and lead to the development of treatments for memory and learning disorders in humans.

8. Conclusion

It is becoming increasingly clear that the actions of phosphatases regulate synaptic transmission in a highly specific and regulated manner. PP1 regulation can be activity-dependent and involve cross-talk between different signalling pathways from the level of molecular interactions to co-operation between different neurotransmitter systems. Additionally, an important role has been implicated for PP1 and interacting proteins in structural plasticity. This review explored only a few of the known PP1 targets at synapses and undoubtedly there are many more to be discovered. Delineating the roles of specific phosphatases in specific brain regions and subcellular locations, and identifying novel target substrates provide challenges for the future. The current advances in the analysis of protein networks in the post-genomic era look promising and novel proteomic methodologies could be of benefit to this field. Additionally, continuing improvements to transgenic techniques, will aid generation of animals which express or knock out proteins with increased spatial and temporal accuracy. This could help the analysis of specific pathways in specific brain regions, as currently studies in memory and learning are difficult due to the heterogeneity and complexity of signalling in the brain at all levels.

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