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DYNAMISM OF POSTSYNAPTIC PROTEINS AS THE MECHANISM OF SYNAPTIC PLASTICITY

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1. OVERVIEW

At excitatory synapses of the vertebrate central nervous system, the neuronal information is transmitted via glutamate which is released from presynaptic terminals and opens postsynaptic cation channels integrated in glutamate receptor proteins. This transmission is not static, but rather is dynamically regulated in both a positive and negative manner by its own activity level as well as by interactions with other synaptic inputs. Such regulation is collectively called synaptic plasticity. After the discovery of synaptic plasticity, intensive work over the past three decades has shown that excitatory synapses in the central nervous system exhibit a remarkable degree of plasticity. Most importantly, growing evidence suggests that synaptic plasticity is the molecular/cellular basis of learning and memory.

The most thoroughly studied form of synaptic plasticity is long-term potentiation (LTP) in the hippocampal CA1 region. LTP was first described in 1970 by Bliss and co-workers[1]. In the 1980s, the requirement for postsynaptic depolarization coupled with presynaptic glutamate release and a resultant influx of Ca^{2+} through *N*-methyl-D-aspartate (NMDA) receptors was established. Thereafter, debate about whether LTP is expressed as an increase in transmitter release or as an increase in the 'sensitivity' of the postsynapse to released glutamate continued almost the entire decade of the 1990s. In the late 1990s, the new concept of a postsynaptically silent synapse and dynamic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors mostly put an end to this long-standing debate. These evidences concluded changes occurring postsynaptically, although presynaptic changes have not been entirely ruled out. Much research along this line has been conducted, boosted by the isolation of cDNAs for the glutamate receptors and other proteins as well as by advances in imaging technology. Such studies have drastically changed the view of postsynaptic receptor proteins as being rather static to being highly dynamic and regulated by synaptic activity.

In this chapter, we will briefly overview the history of LTP research and then summarize the current understanding of the activity-dependent regulation of postysynaptic protein localization in the context of hippocampal LTP.

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2. MOLECULAR MECHANISMS OF HIPPOCAMPAL CA1 LTP

2.1. LTP Induction

Glutamatergic excitatory synaptic transmission is mediated through two types of ionotropic glutamate receptors: AMPA receptors and NMDA receptors[2,3]. AMPA receptors are permeable only to monovalent cations (Na^+, K^+) and mediate synaptic transmission under low frequency synaptic inputs. In contrast, NMDA receptors are regulated by membrane potential as well as ligands and are permeable to Ca^{2+} as well as Na^+ and K^+. Because their dependence on membrane potentials is caused by an extracellular Mg^{2+} block of the channel pore to just under the resting membrane potential, the contribution of NMDA receptors to basal synaptic transmission is minimal. Thus, synaptic plasticity is expressed as AMPA receptor response. When the cell is depolarized by high frequency synaptic input stimulation, Mg^{2+} dissociates from the NMDA receptor allowing Ca^{2+} influx into the postsynapse. This increase in intracellular Ca^{2+} through NMDA receptors is essential for the induction of LTP[4]. Thus, it is well accepted that LTP is induced by postsynaptic machinery.

Intracellular Ca^{2+} influx causes the activation of multiple signaling molecules that triggers an increase in AMPA receptor-mediated transmission. The most well-characterized and critical molecule for triggering LTP is calcium/calmodulin-dependent protein kinase II (CaMKII). CaMKII is a serine/threonine protein kinase highly enriched in the brain (1-2% of total protein)[5]. Although Ca^{2+} is required for the initial activation of CaMKII, this kinase no longer requires Ca^{2+} once it is autophosphorylated at threonine 286[5]. From this feature, CaMKII has been suggested as a memory molecule that keeps enhanced transmission after the transient increase in synaptic activity. Indeed the autophosphorylated status of CaMKII increases and is sustained after the induction of LTP[6]. Drugs that inactivate CaMKII or genetic ablation of CaMKII block LTP[7-9]. Furthermore, the constitutively active form of CaMKII can potentiate AMPA receptor-mediated excitatory postsynaptic current (EPSC) without changing NMDA receptor mediated EPSC in a manner occluding electrophysiologically induced LTP[10,11]. The functional significance of CaMKII autophosphorylation has been also confirmed by genetic methods. Mice carrying a CaMKII point mutation at threonine 286 exhibit a loss of plasticity and are deficient in learning[12].

2.2. LTP Expression

How does the increased activity of CaMKII and likely other signal transduction molecules lead to the expression of LTP? Over the past few decades, significant attention was dedicated to one conceptually simple question: Is the expression of LTP due to presynaptic or postsynaptic modifications? Postsynaptic modification would be attributed to the change in postsynaptic AMPA receptor properties or number, whereas presynaptic modulation would be the enhancement of the glutamate release probability from the presynaptic nerve terminus. Much evidence in support of postsynaptic modification and against presynaptic modification has been generated. Most investigators report that AMPA receptor-mediated EPSCs increase significantly more than NMDA receptor-mediated EPSCs[13], and the sensitivity against exogenous AMPA is increased following LTP[14]. If LTP is caused by presynaptic modifications, the effect of these two components of EPSCs should be the same, although other explanations are also possible[15]. Experiments to de-

termine release probability by measurement of the paired-pulse facilitation (a presynaptically originated short-term plasticity)[16-18], glutamate receptors in out-side-out patch excised from neurons[19] or glial cell glutamate transporter currents as a indirect measure of synaptically released glutamate[20,21], and the use-dependent antagonists of NMDA or AMPA receptors[22,23] all showed no change in the release of glutamate from the presynaptic terminus before and after LTP.

On the other hand, the presynaptic theory relied heavily on results from quantal analysis that the failure rate of synaptic transmission changes after LTP, because the transmission failure was thought to result from a failure in neurotransmitter release[24]. However, this is not the case for glutamatergic synapses in the central nervous system. Several groups have reported that synaptic failures can be caused by a postsynaptic mechanism[25-27]. They identified synapses that have only NMDA receptors. Because NMDA receptors are blocked at a hyperpolarized membrane potential by extracellular Mg^{2+}, these synapses are functionally silent under basal synaptic activity. However, application of LTP induction procedures to such "silent" synapses causes rapid appearance of AMPA receptor-mediated EPSCs. Thus synaptic failure is attributable not only to transmitter release failure but also to postsynaptic failure in the response to released glutamate. Immunohistochemical studies at both the light and electromicroscopic levels also support the existence of silent synapses[28-32].

2.3. Activity-Dependent AMPA Receptor Synaptic Insertion

How are silent synapses 'unsilenced' by the induction of LTP? The simplest hypothesis is that there exists a delivery/exocytotic mechanism that introduces AMPA receptors into silent synapses. Evidence favoring this concept has accumulated over the last few years. LTP induction increases the surface binding of ^{3}H-glutamate[33]. Postsynaptic infusion of toxin that selectively inhibits the exocytotic process blocks LTP[34]. Prolonged FM1-43 staining revealed dendritic organella that cause exocytosis in a Ca^{2+} and CaMKII activity-dependent manner[35,36]. In these reports, however, the identity of the substance(s) subject to exocytosis was not known.

The first evidence for activity-dependent synaptic delivery of the AMPA receptor came from a study using the AMPA receptor subtype GluR1 tagged with green fluorescence protein (GFP) expressed in organotypic hippocampal slice cultures through a viral expression system[37]. Newly synthesized recombinant GluR1-GFP is diffusely distributed throughout and retained within the dendrite. However, high frequency stimulation induces the movement of GluR1-GFP into the spine. This movement is dependent on the activation of NMDA receptors and is sustained in the spine for at least 50 min. This effect can be mimicked by coexpression of GluR1-GFP with truncated CaMKII, a constitutively active form of the enzyme[38].

Furthermore, use of an electrophysiological tagging technique supported this conclusion[39]. Most endogenous AMPA receptors in hippocampal CA1 pyramidal cells contain the GluR2 subunit, and the current-voltage relationship is linear. However, GluR1 overexpressed from a strong viral promoter makes a homomeric receptor lacking GluR2. Such exogenous receptors exhibit inward rectification, allowing them to be distinguished from endogenous receptors. Using this approach, an insertion of exogenous AMPA receptors into the spine was monitored by determination of the ratio of EPSCs at negative and positive membrane potentials. This assay confirmed that LTP induction or coexpression of truncated CaMKII drives GluR1-containing receptors into the synapse in an organotypic

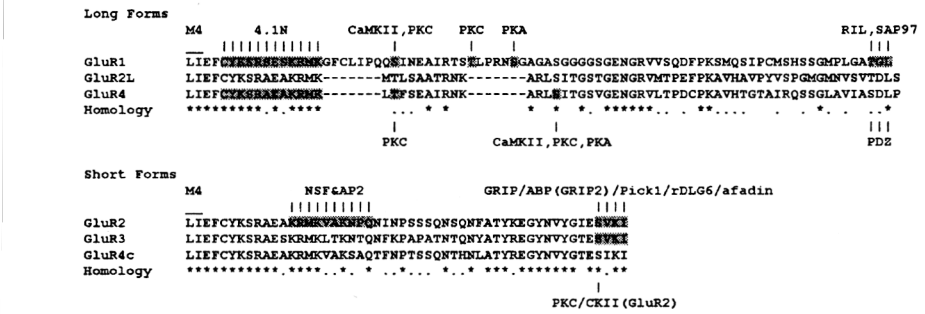

Fig. 1. Homology alignment of intracellular carboxyl tails of AMPA receptor subunits. Identified interacting proteins and corresponding regions on receptor (shaded) are shown. See text for detail. M4, 4th membrane associated region; 4.1N, band 4.1 neuronal subtype; CaMKII, Ca^{2+}/calmodulin dependent protein kinase type II; PKC, protein kinase C; PKA protein kinase A; RIL, reversion-induced LIM domain gene product; SAP97, synapse associated protein 97; NSF, *N*-ethylmaleimide sensitive factor; AP2, adapter protein 2; GRIP, glutamate receptor interacting protein; ABP, AMPA receptor binding protein; Pick1, protein interacting with C kinase 1; rDLG6, rat disc large.

hippocampal slice culture[39]. This activity-dependent synaptic insertion of GluR1-GFP is also observed *in vivo* in the rodent barrel cortex, indicating that the same mechanism works at *in vivo* cortical synapses[40]. In support of this hypothesis, mice lacking GluR1 cannot induce LTP[41], an effect that can be rescued by transgenic expression of GluR1-GFP[42].

2.4. Interplay between AMPA Receptor Phosphorylation and Synaptic Delivery

What triggers the synaptic delivery of AMPA receptors? There are three phosphorylation sites on the carboxyl terminus of GluR1[43,44] and two of them are well-characterized (Figure 1). Serine 831 of GluR1 is the CaMKII phosphorylation site[43,45,46]. Phosphorylation at this site increases the single channel conductance[47]. LTP induction increases this phosphorylation[45,48] as well as the AMPA receptor conductance[49]. Thus, CaMKII phosphorylation of GluR1 may be a mechanism for the enhancement of AMPA receptor responsiveness through the modulation of channel conductance. However, this phosphorylation does not appear to be necessary for delivery of GluR1 to the synapse since a mutant receptor that cannot be phosphorylated by CaMKII at this site was still delivered to the synapse by coexpression of a truncated CaMKII[39].

In contrast, phosphorylation at serine 845 is necessary for CaMKII-induced synaptic delivery of GluR1. A mutant GluR1 which has mutation at this site is not delivered to the synapse by the coexpression with truncated CaMKII[50]. This site is phosphorylated by protein kinase A (PKA) but not by CaMKII itself. Interestingly, the application of forskolin, an activator of adenylate cyclase, cannot induce the AMPA receptor delivery although it can drastically enhance the serine 845 phosphorylation. It has been considered that the cAMP pathway works as a 'gating mechanism' that can modulate LTP through the modulation of protein phosphatase and inhibitor-1[51,52].Thus this PKA phosphorylation of GluR1 may act as the second gating mechanism. Consistent with this importance of GluR1 phosphorylation in the regulation of AMPA receptor delivery, mutant knock-in mice lacking serines 831 and 845 exhibited impaired synaptic plasticity[53].

Neither of these phosphorylation sites on GluR1 is the direct substrate of CaMKII necessary for synaptic delivery of GluR1. This implies that there must be another CaMKII substrate that acts for the synaptic delivery of GluR1. Although there are many CaMKII substrates in the postsynaptic density (PSD) fraction[54,55], only two proteins, other than CaMKII itself, are known to contribute to the activity-dependent synaptic delivery of GluR1. SynGAP [synaptic Ras GTPase-activating protein (GAP)] is a negative regulator

of Ras and interacts with PSD-95 and SAP102[56,57]. SynGAP phosphorylation by CaMKII inactivates Ras GAP activity thereby relieving Ras from inhibition and increasing its activity. Activation of Ras is important for inducing LTP and synaptic delivery of GluR1 through a CaMKII-dependent signal cascade[58].

Another candidate is PSD-95, a major PSD protein that binds directly to the NMDA receptor[59]. Overexpression of PSD-95 in hippocampal neurons enhanced AMPA receptor-mediated EPSC without changing NMDA receptor mediated EPSC[60,61] in a manner occluding with LTP. This enhancement is mediated by the synaptic delivery of GluR1 receptor[60,61] likely through an interaction with stargazin, an AMPA receptor binding transmembrane protein[62-64] . Interestingly, the neuronal activity induced by eye opening can increase the amount of PSD-95 in the synaptoneurosome fraction prepared from the superior colliculus and visual cortex without changing the total amount of PSD-95[65]. The Drosophila homologue of PSD-95, *dlg*, changes its localization at the synapse by CaMKII phosphorylation[66]. Thus similar activity-dependent translocation of PSD-95 may take place in CA1 neurons.

In addition to a role as a signal transduction molecule, CaMKII may have structural role as well. It has been shown that CaMKII is translocated to the synapse by synaptic activity (see section 3.2. for detail). Inspired by this, Lisman established a model in which CaMKII has a structural role[5]. In his model, CaMKII, when activated, moves to the postsynapse thereby triggering the assembly of other postsynaptic proteins through direct or indirect interactions with molecules such as actinin-2, band 4.1, SAP97, and eventually AMPA receptors[5]. In fact, the amount of CaMKII is very high in the postsynapse, almost comparable with actin and well beyond the levels of other signal transduction components, which may be consistent with a structural role of CaMKII in the postsynapse.

In addition, the PDZ-protein binding motif on the very end of GluR1 is also important for GluR1 synaptic insertion[39,67]. SAP-97, a PDZ domain containing membrane associated guanylate kinase (MAGUKs) family protein similar to PSD-95, is known to bind to this site[68]. However, mutant mice lacking the last 7 amino acids of GluR1 including the PDZ protein binding domain showed normal LTP[69]. Therefore, the contribution of the PDZ binding domain to the delivery is still controversial. These results imply that the GluR1 delivery mechanism cannot be explained by single protein-protein interaction. Multiple protein interactions taking place in parallel are likely to be necessary for this delivery.

2.5. Subunit-Specific Rules of AMPA Receptor Delivery to the Synapse

Two-hybrid screening boosted the identification of glutamate receptor-interacting proteins. Several independent groups noticed that GluR2 binds to *N*-ethylmaleimid-sensitive factor (NSF), a protein involved in the membrane fusion process[70-72]. Intracellular infusion of partial peptide corresponding to the NSF-binding site of GluR2 induced a rapid but partial suppression of AMPA receptor-mediated current. Viral expression of this peptide reduced the surface amount of the receptor as detected by surface immunostaining[73]. A recent study showed that clathrin adapter protein type 2 (AP2) binds to an overlapping sequence, an event that is necessary for NMDA-receptor-induced internalization of AMPA receptor and essential for long-term depression (LTD)[74].

The rapid reduction in synaptic response and surface GluR2 by the infusion of the peptide indicates that constitutive recycling of GluR2 protein occurs between the synaptic and extrasynaptic pools. In this way, GluR2 is thought to play a maintenance role in keeping a certain synaptic strength through its own recycling. At a glance, this sounds

contradictory to results indicating that synaptic delivery of the AMPA receptor requires synaptic activity. However, the one critical difference is that these studies used GluR2 whereas activity-dependent delivery was studied with the GluR1 subunit.

When we compare amino acid sequences among different subunits of the AMPA receptor, extracellular and transmembrane regions are relatively conserved, whereas the intracellular cytoplasmic tails of each subunit, which bind to intracellular scaffolding proteins and are involved in determining receptor trafficking, diverge into two groups[75,76]. GluR1, GluR4, and an alternative splice form of GluR2 (GluR2L) have longer cytoplasmic tails whereas GluR2, GluR3, and an alternative splice form of GluR4 (GluR4c) have shorter cytoplasmic tails. A side-by-side comparison of GluR1-GFP and GluR2-GFP revealed that these two forms show very different behaviors: Whereas GluR1 was retained within dendritic shafts, GluR2 was constitutively delivered to the synapses in a manner not requiring synaptic activity as determined by both imaging and electrophysiological tagging[76]. These results are consistent with the data obtained for the peptide interfering with NSF interaction. Surface immunostaining in dissociated cultures of GluR1 and GluR2 epitope tagged at the extracellular domain led to an essentially similar conclusion[67].

What, then, will happen if these two subunits are combined? In fact, most AMPA receptors in hippocampal CA1 synapses contain GluR2. They make two distinct populations: those composed of GluR1 and GluR2 or those composed of GluR2 and GluR3[77]. Coexpression experiments indicated that a heteromeric AMPA receptor including GluR1 and GluR2 behaved like GluR1 whereas a GluR2 and GluR3 complex had properties similar to GluR2. Thus GluR1/2 acts as activity-dependent receptor, and GluR2/3 maintains normal synaptic transmission.

2.6. Developmental Switch of Activity-Dependent AMPA Receptor Subunits

In both GluR1 knock-out mice and GluR1 phosphorylation site mutant knock-in mice, whereas adult mice exhibit impairments in LTP, juvenile mice show normal LTP[42,53]. These results suggest that different AMPA receptor subunits may participate in activity-dependent delivery throughout development, although other explanations such as presynaptic mechanisms are still possible. GluR4 and GluR2L, which have sequences similar to GluR1, may contribute to activity-dependent AMPA receptor trafficking at different developmental stages. The immature hippocampus (<P10) expresses GluR4 that complexes with GluR2[78]. Studies of activity-dependent GluR4 delivery using electrophysiological tagging indicate that this subunit mediates activity-dependent delivery during the immature period. As the expression of GluR4 disappears by postnatal day 10, GluR2L may contribute to the activity-dependent delivery of AMPA receptor during the following juvenile period[79]. GluR2L is expressed mainly in pyramidal neurons and dentate gyrus granule cells in the hippocampus. At both the mRNA and protein level, GluR2L expression in the hippocampus peaks approximately 2 weeks after birth. At this stage, GluR2L is assembled with ~20% of the GluR1 and GluR3 subunit populations.

How is the delivery of these receptors controlled? GluR4 is phosphorylated by PKA at serine 842, which is also conserved in GluR2L, and this phosphorylation is sufficient for delivery of GluR4 to the synapse. Unlike for GluR1, CaMKII activity is not necessary[50,78]. Consistent with this, the kinase requirement of LTP developmentally switches from PKA at younger age to CaMKII at older age[80].

3. ACTIVITY-DEPENDENT DYNAMICS OF POSTSYNAPTIC PROTEINS

AMPA receptors do not exist by themselves. They bind to various interacting proteins that can regulate the AMPA receptor. Such proteins often have multiple protein-interaction interfaces and, in turn, bind to other binding partners, eventually making a dense network of protein interactions at the postsynapse that include NMDA receptor and other signaling and structural components. This network of proteins comprises the electron-dense structure, which has been recognized as postsynaptic density. Therefore, it would not be hard to imagine that such postsynaptic proteins also respond to neuronal activity and change their properties such as conformation, posttranslational modifications, and interacting partners, thereby changing their localization. Now, accumulating evidence suggests that acute activity-dependent delivery to the synapse or removal from the synapse occurs not only for the AMPA receptor, but also for many other postsynaptic proteins.

3.1. Activity-Dependent Delivery of NMDA Receptors

Although the synaptic insertion or removal of the NMDA receptor by chronic activity modulation has been reported[81], generally, the mobility of the NMDA receptor during acute synaptic plasticity, such as LTP, has been considered to be lower than that of the AMPA receptor. Only a few studies have noticed that the NMDA receptor also rapidly changes its location under certain conditions of activity.

In slices obtained from adult animals, 20%-40% of the NMDA receptor resides within the cells. LTP induction induces surface delivery of the NMDA receptor as determined by biotinylation and chymotrypsinization of receptors on the surface[82]. During synaptogenesis, a membranous packet containing the NMDA receptor is rapidly recruited to nascent synaptic contacts[83]. Activation of type I metabotropic glutamate receptors (mGluRs) in cortical slices induces internalization of NMDA receptors[84].

Phosphorylation, at least in part, may regulate these processes. Application of phorbol ester, an activator of protein kinase C or intracellular infusion of the active form of protein kinase C increased NMDA-evoked current in dissociated hippocampal neurons[85]. In contrast, application of purified serine/threonine protein phosphatases decreased NMDA receptor-mediated current[86]. Phorbol ester treatment changes subcellular distribution and increases the surface amount of the NMDA receptor in both heterologous systems (Xenopus oocytes) and hippocampal neurons[82,87-89], though it may also explained by modulation of channel activity. The NMDA receptor has been shown to be phosphorylated by CaMKII and PKC at the carboxyl tail, and the actual sites of phosphorylation have been identified[90,91]. The NR1 subunit of the NMDA receptor has an ER retention signal regulated by an adjacent PKC phosphorylation site and PDZ domain protein binding[92,93]. However, the phorbol ester still showed an effect, even in a mutant where all known PKC phosphorylation sites carboxyl termini are eliminated[94], leaving much ambiguity as to the precise mechanism of this process.

3.2. Ca^{2+}/Calmodulin-Dependent Protein Kinase Type II (CaMKII)

It had been noticed that the amount of CaMKII in the postsynaptic density differed depending on the way animal brain tissue is prepared[95]. This observation was the first indication that CaMKII acutely change its localization depending on cellular conditions. The effect was reproduced in vitro with isolated crude PSD-like fractions. This in vitro

study found that CaMKII translocation is dependent on its autophosphorylation[96]. An elegant series of experiments by Shen and Meyer using GFP-labeled CaMKII demonstrated activity-dependent translocation in living neurons. This delivery of α-CaMKII requires its autophosphorylation but those at threonine 286, which renders α-CaMKII constitutively active, does not define the binding[97]. Rather, autophosphorylation of the regulatory domain at threonine 305/threonine 306, which regulates calmodulin binding, also regulates localization. β-CaMKII has a distinct mechanism for its subcellular localization. It has a unique filamentous actin (F-actin)-binding domain, and, upon activation, is liberated and relocated to the PSD[98]. Furthermore, recent in vivo studies also showed that the translocation of endogenous CaMKII is driven by synaptic activity[99].

What provides the binding sites for α-CaMKII at the synapse? It has been suggested that the carboxyl tail of the NR2B subtype of the NMDA receptor binds to α-CaMKII[100,101]. This binding keeps α-CaMKII in the constitutively active form independent of calmodulin binding[102]. This process may act as another mechanism for keeping the constitutive activity of this enzyme independent of phosphorylation at threonine 286. However, NR2B cannot be the only binding site for α-CaMKII. Whereas α-CaMKII is a highly abundant protein constituting up to 10% of the protein at the postsynaptic density, the number of NMDA receptors on a single synaptic contact will be much less considering single channel conductance and the size of the synaptic response. In fact, pharmacological activation of protein kinase C with phorbol ester delivers α-CaMKII to synapses while rapidly dispersing NMDA receptor from the synapse[89]. These mismatches suggest the presence of another mechanism that provides a binding site for α-CaMKII. Another CaMKII-binding protein enriched in the postsynapse, densin-180[103,104] and F-actin[98], may underlie such a mechanism.

Other kinases such as protein kinase C family members have also been shown to rapidly change their distribution in a nonneuronal cells in studies with GFP-fusion proteins. It would be interesting to know whether activity-dependent redistribution is a general phenomenon among various kinase molecules.

3.3. Other Postsynaptic Scaffolding and Cytoskeletal Proteins

Activity-dependent delivery of PSD-95 and homer1C (PSD-Zip45), binding proteins for the NMDA receptor and mGluR, respectively, were determined by time-lapse imaging of GFP-fusion proteins[105]. This study first indicated that, whereas PSD-95 is relatively stable, Homer1C rapidly redistributes in response to high extracellular potassium and glutamate application. Further, the turnover rates of these proteins were estimated by use of a fluorescent recovery after photobleaching (FRAP) assay. In this assay, GFP signals in the spine head were photobleached by repeated local scanning. The recovery time course of the fluorescence in the spine head by influx from the dendritic shaft is measured as the turnover rate. The fluorescence level of homer1C returns to 50% of the original within 5 minutes[105]. A protein with an even faster rate is actin, which recovers within 1 minutes, while GFP itself takes only 1 second[106]. PSD-95 showed slower kinetics with recovery of only 20% of PSD-95-GFP taking more than 30 minutes. This assay demonstrated that postsynaptic scaffolding proteins show unexpectedly fast turnover rates even in mature hippocampal neurons.

Differential stimulation protocols were found to alter the direction of homer1C assembly-disassembly[105]. Transient increases in intracellular Ca^{2+} by voltage-dependent Ca^{2+}

channel activation induced homer1C clustering. In contrast, NMDA receptor-dependent Ca^{2+} influx resulted in the disassembly of PSD-Zip45 clusters. In contrast, PSD-95 distribution was relatively stable under these stimulation[105]. Thus, neuronal activity differentially redistributes a specific subset of PSD proteins, which are important for localization of both surface receptors and intracellular signaling complexes.

4. A GENERAL MECHANISM FOR ACTIVITY-DEPENDENT DELIVERY OF POSTSYNAPTIC PROTEINS

Are there any general mechanisms that regulate protein delivery to the postsynapse? We may be able to classify such mechanisms into three general categories (Figure 2).

For the first mechanism, there may be a structure that actively delivers proteins to the postsynapse, analogous to transport of proteins along dendrites (Figure 2A). The efficiency of this transport mechanism may be regulated by synaptic activity.

For the second mechanism, molecules may not undergo active transport, rather they move in and out of the postsynaptic site by simple passive diffusion, at a rate largely determined by shape and molecular weight. However, once they enter the postsynapse, they are trapped by putative binding sites. The capacity of these binding sites may be modulated by synaptic activity (Figure 2B). Binding of β-CaMKII to F-actin or post-translational modifications, such as myristoylation of PSD-95, would also fall into this category. Consistent with this notion, prevention of PSD-95 myristoylation blocks its delivery to the synapse[107].

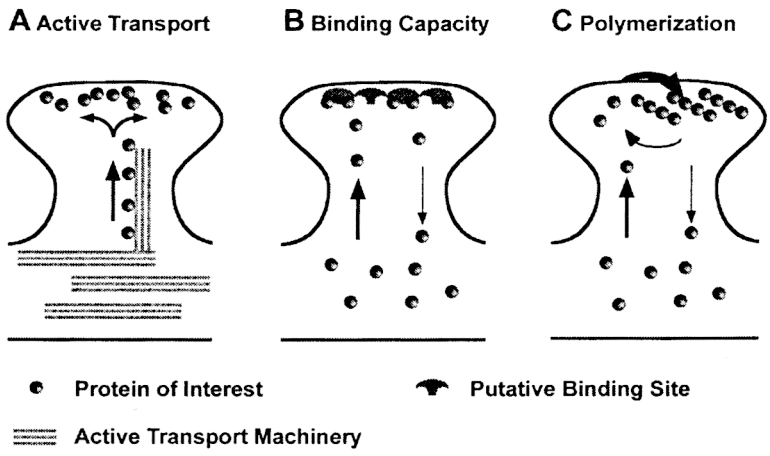

Fig. 2. Possible mechanisms for activity dependent delivery of synaptic proteins to postsynapse. **A** A modulation of active transport machinery. **B** A modulation of binding capacity at the postsynapse. **C** A modulation of polymerization status at the postsynapse. In **B** and **C**, the molecules enter the postsynaptic site by passive diffusion and do not require active transport mechanism.

For the third mechanism, the molecule can form a polymer, and the equilibration

between polymerization and depolymerization may be regulated by synaptic activity (Figure 2C). Once the monomers diffuse to the synaptic site, they will be trapped as polymer. One example of a protein that is regulated in this manner is actin. Actin exist in an equilibration between monomer (globular or G-actin) and polymer (F-actin) forms and F-actin predominantly exists in the postsynapse[105,106]. Its polymerization is indispensable for the formation of dendritic spines as well as for synaptic plasticity[105,108]. Importantly, various signal transduction mechanisms regulate the polymerization/depolymerization of actin. This increases or decreases in the F-actin may modulate binding sites for other actin-binding proteins such as cortactin, β-CaMKII, and various types of myosins. In this sense, F-actin would serve as the binding site described in the second mechanism of delivery and can be a "master regulator" of synaptic delivery for various postsynaptic proteins. Other proteins that exist as both monomers and polymers may also be regulated in this fashion. These include homer1B/C, PSD-95, and glutamate receptor-interacting proteins (GRIPs).

5. CONCLUDING REMARKS

Within the last few years, the view of postsynaptic proteins has completely changed from being associated with each other in a stable and fixed protein complexes to being highly dynamic components of the synaptic architecture. Not only that, this dynamism of postsynaptic proteins is the one of the major site of synaptic plasticity. Many questions still need to be addressed: How is it maintained at constant level? What is the exact mechanism of its regulation? How is the specificity to individual synapses are maintained? Fortunately, the field is rapidly advancing, promising imminent answers to these and other important issues in learning and memory.

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