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139. I thank R. Frostig, B. Lom, B. Alsina, and C. Colwell for discussions and comments; B. Hu for his contribution to Fig. 3; and D. Crandall for assistance with graphic art. S.C.C. has been funded by awards from the Alfred P. Sloan, Stein-Oppenheimer, and Arnold and Mabel Beckman Foundations and by the National Eye Institute.

REVIEW

Postsynaptic Signaling and Plasticity Mechanisms

Morgan Sheng* and Myung Jong Kim

In excitatory synapses of the brain, specific receptors in the postsynaptic membrane lie ready to respond to the release of the neurotransmitter glutamate from the presynaptic terminal. Upon stimulation, these glutamate receptors activate multiple biochemical pathways that transduce signals into the postsynaptic neuron. Different kinds of synaptic activity elicit different patterns of postsynaptic signals that lead to short- or long-lasting strengthening or weakening of synaptic transmission. The complex molecular mechanisms that underlie postsynaptic signaling and plasticity are beginning to emerge.

Excitatory synapses of the brain primarily use glutamate as their neurotransmitter. Different classes of glutamate receptors in the postsynaptic membrane transduce the glutamate signal released from the presynaptic terminal into electrical and biochemical events in the postsynaptic neuron. The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)–type glutamate receptor opens in response to glutamate binding and mediates most of the rapid excitatory postsynaptic current (EPSC). The *N*-methyl-D-aspartate (NMDA)–type glutamate receptor is calcium-permeable and opens in response to glutamate only when the postsynaptic membrane is concomitantly depolarized. Different patterns of activation of NMDA receptors (NMDARs) can lead to either long-term potentiation (LTP) or long-term depression (LTD) of synaptic strength. These long-lasting forms of synaptic plasticity are intensively studied because they may represent ways of encoding "memories" in the brain.

Changes in synaptic strength can occur by presynaptic mechanisms such as altered neurotransmitter release (*1*–*3*). Recent evidence, however, also points to a postsynaptic locus for the expression of plasticity, in which changing the activity and/or abundance of postsynaptic AMPA receptors (AMPARs) is the primary means of modulating synaptic transmission. Wherever the site of plasticity expression, there is general agreement that the initiating events ("induction") of LTP and

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LTD, as characterized in the CA1 region of the hippocampus, occur in the postsynaptic neuron and require calcium influx through NMDARs. Thus, it is important to understand postsynaptic signal transduction in general and calcium-dependent signaling mechanisms of NMDARs in particular. This review will cover recent advances in the molecular interactions and biochemical signals that underlie synaptic plasticity. The focus is on NMDAR-dependent plasticity in the CA3-CA1 synapse of the hippocampus (the most intensively studied connection for synaptic plasticity), but examples of plasticity at other synapses will be discussed. Although some mechanistic themes may apply to synapses in general, the molecular details of plasticity clearly differ between different synapses.

NMDAR Signaling Mechanisms

NMDARs are embedded in the postsynaptic density (PSD), a microscopic structure associated with the postsynaptic membrane that contains a variety of scaffolding and signaling proteins (*4*). The COOH-terminal cytoplasmic tails of NMDAR subunits bind to the PSD-95, Dlg, and ZO-1 Homology (PDZ) domains of the PSD-95–SAP90 family of scaffold proteins in the PSD (*5*–*7*). PSD-95, in turn, interacts with a host of cytoplasmic signaling molecules, including neuronal nitric oxide synthase and SynGAP, thereby connecting NMDARs to divergent signal transduction pathways (*4*, *7*–*9*) (Fig. 1). The cytoplasmic tails of NMDARs also bind directly to cytoskeletal and signaling proteins, including α-actinin and calmodulin (CaM) (*5*–*7*). In this way, NMDARs are integrated in the protein network of the PSD, the components of which likely mediate many aspects of postsynaptic signaling by NMDARs (*4*) (Fig. 1).

The number and variety of NMDAR- and PSD-associated proteins has exploded in the past few years, thanks to advances in mass spectrometric identification of polypeptides (*9*). However, little is known about the stoichiometry of specific proteins in the PSD or about the temporal and spatial sequence of specific protein-protein interactions. It should be emphasized that the PSD is a dynamic structure, in which protein interactions may be transient and stoichiometries variable. Underscoring this point, PDZ-containing scaffold proteins are important in the trafficking of glutamate receptors through the secretory pathway (*10*–*12*), as well as in the stabilization of receptors at synaptic sites (*13*). Understanding the temporal dynamics and three-dimensional organization of the PSD will be critical to elucidating the mechanisms of synaptic regulation.

Calcium-CaM-dependent protein kinase II (CaMKII). CaMKII has received much attention because it is persistently activated after NMDAR stimulation and is essential for NMDAR-dependent LTP (*14*) (Fig. 2). The activation of CaMKII stimulates its binding to the cytoplasmic domain of the NMDAR subunit NR2B. By interfering with autoinhibitory interactions within CaMKII, binding to NR2B locks CaMKII in an activated state that cannot be reversed by phosphatases (*15*). Such a mechanism could prolong the local activation of CaMKII in a synapse-specific manner.

Because CaMKII is abundant in the PSD (*4*), there has been speculation that CaMKII plays a structural (nonenzymatic) role in synapses. In keeping with this, CaMKII has been found to bind to α-actinin (*16*), an actin-binding protein enriched in PSD, thus providing a link to the actin cytoskeleton. The AMPAR-binding proteins SAP-97 (*17*) and protein 4.1N (*18*) also interact with F-actin. On the basis of these interactions, it has been hypothesized that activated CaMKII, which is recruited to the PSD by binding to NMDARs and possibly other targets, may increase anchoring sites for AMPARs at the synapse, thereby enhancing synaptic transmission (*14*).

The most direct evidence, however, indicates that CaMKII enhances synaptic transmission by increasing the activity and/or synaptic delivery of AMPARs. Whether CaMKII acts as a scaffold or as an enzyme, the direct involvement of NMDARs in the activation and binding of CaMKII can explain the recruitment of activated CaMKII to stimulated synapses.

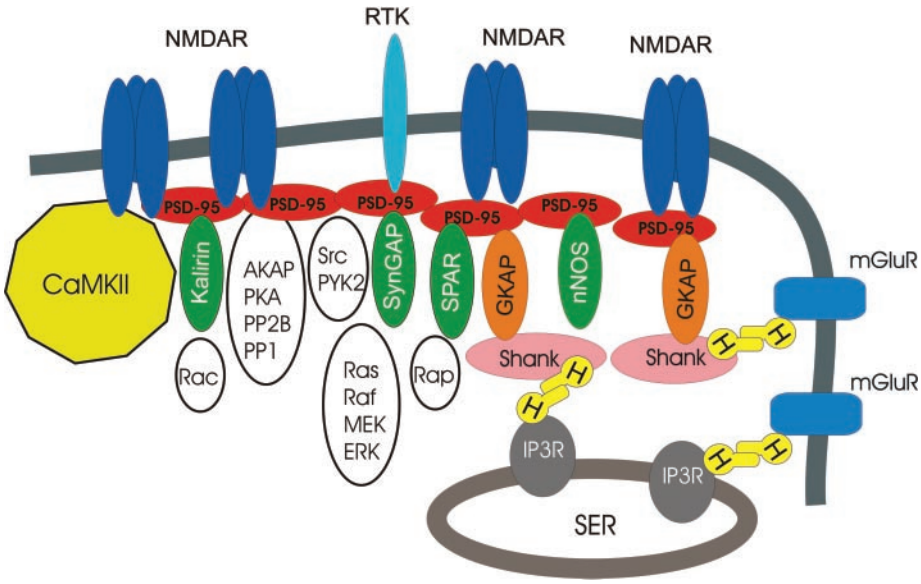

Fig. 1. A schematic of the NMDAR-associated protein complex. Major individual proteins of the PSD are shown as colored shapes, and their interactions are indicated by overlapping shapes (see text for details). Some specific sets of interacting proteins (e.g., the Ras-MAPK pathway) are grouped together for simplicity. The proteins GKAP, Shank, and Homer (H) link together the NMDAR complex, metabotropic glutamate receptors (mGluR), and IP_3 receptors (IP3Rs). It should be emphasized that the PSD is a dynamic structure, in which protein interactions may be transient and stoichiometries variable. nNOS, neuronal nitric oxide synthase; RTK, receptor tyrosine kinase; SER, smooth endoplasmic reticulum.

Ras–mitogen-activated protein kinase (MAPK). Although CaMKII is important, other signaling pathways are triggered by NMDARs. The Ras-MAPK pathway is a major postsynaptic signaling mechanism in synaptic plasticity (*19*).

First, Ras activity is stimulated by NMDAR activation (*20*), though the underlying mechanism is unclear. Elevated Ca^{2+} (from calcium-CaM) is known to activate Ras–guanine-nucleotide releasing factor (Ras-GRF), a guanine nucleotide exchange factor (GEF) for Ras, thereby stimulating Ras activity (*21*) (Fig. 2). Several additional Ca^{2+}-regulated Ras-GEFs are expressed in the brain, including the Ras guanyl nucleotide–releasing protein (Ras-GRP)–calcium- and diacylglycerol-related guanine nucleotide exchange factor (CalDAG)–GEF family, which are regulated not only by Ca^{2+} but also by diacylglycerol that is generated along with inositol trisphosphate (IP_3) by phospholipase C (PLC) (*21*). The calcium- and protein kinase C (PKC)–activated tyrosine kinase PYK2 (also known as CAKβ) can recruit the Grb2–SOS RasGEF complex, leading to stimulation of Ras.

Second, Ras and its regulators [Ras-specific GEFs and guanosine triphosphatase (GTPase)–activating proteins (GAPs)] are prominent components of the NMDAR complex (*9*). Examples include SynGAP, an abundant RasGAP that binds to PSD-95 (*22*,

23), PYK2, and neurofibromin (the RasGAP that is mutated in human neurofibromatosis type 1).

Third, Ras signal transduction is critical for synaptic plasticity and/or learning and memory. Knockout mice deficient in RasGRF1 show loss of LTP in the amygdala (*24*). Heterozygous mutant mice in neurofibromin are impaired in spatial memory (*25*). In hippocampal tissue, Ras activation seems to be required for NMDAR-dependent LTP (*26*); however, LTP is enhanced in H-Ras knockout mice (*27*).

Activated GTP-bound small heterotrimeric GTP–binding proteins (G proteins) act by binding to and stimulating specific effectors. Multiple effector pathways have been characterized for Ras, including MAPK and phosphoinositide 3′-kinase (PI3K) (*21*). The MAPK pathway is probably a major output for postsynaptic Ras signaling. The sequential protein kinases of the MAPK pathway [Raf, MAPK-ERK kinase (MEK) 1 and MEK2, and extracellular signal–regulated kinase (ERK) 1 and ERK2) are present in the NMDAR complex (*9*). LTP-inducing stimuli activate MAPK in hippocampus, and inhibitors of MEK, the upstream activator of MAPK, impair LTP (*19*). Sustained activity of the Ras-MAPK pathway has also been implicated in the formation of dendritic spines, a morphological correlate of synapse growth (*28*). By regulating various transcription factors such as the cyclic adenosine 5′-monophosphate (cAMP) response element–binding protein (CREB), the Ras-MAPK pathway also controls gene expression (*19*). Thus, Ras-MAPK signaling is likely to be important for both short- and long-term synaptic plasticity.

PI3K. Another major effector of Ras is PI3K, which phosphorylates the 3′ position of phosphoinositides. PI3K activity increases during hippocampal NMDAR-dependent LTP and is required for the expression but not the induction of LTP (*29*). The activation of PI3K is also important in the amygdala for synaptic plasticity and memory consolidation (*30*). Because PI3K is required for NMDAR-stimulated delivery of AMPARs to the neuronal surface (*31*), PI3K may act in LTP to enhance the synaptic delivery of AMPARs (see below).

Rac, Rap. These small GTPases and their regulators [the RapGAP spine-associated RapGap (*32*); the RacGEF kalirin (*33*)] have been identified in the PSD and NMDAR complex (Fig. 1), suggesting that these small GTPases are also involved in postsynaptic signaling. Rac, Rap, and kalirin are implicated in the regulation of dendritic spine morphology, presumably via actions on the actin cytoskeleton (*32*–*34*). In contrast to Ras, which is required for LTP, Rap activation is required for hippocampal LTD (*26*). It is unclear how Rap activity is regulated by NMDARs. Calcium influx could stimulate the production of cAMP by Calcium-CaM-dependent adenylate cyclases, leading to activation of cAMP-responsive RapGEFs (*35*) (Fig. 2).

Nonreceptor tyrosine kinases. Nonreceptor tyrosine kinases of the Src family stimulate NMDAR activity (*36*, *37*), probably by direct phosphorylation. Src family kinases can be coimmunoprecipitated with NMDARs, and inhibitors of these kinases prevent the induction of hippocampal LTP (*36*, *37*). CAKβ/PYK2 (a tyrosine kinase of the focal adhesion kinase family) appears to mediate the activation of Src (*37*). CAKβ/PYK2 becomes phosphorylation-activated after LTP-inducing stimulation and is necessary for hippocampal CA1 LTP. How CAKβ/PYK2 is stimulated by synaptic activity is uncertain, but the kinase is activated by both calcium and PKC. Thus, CAKβ/PYK2 could integrate signals from NMDARs and other postsynaptic sources, including metabotropic glutamate receptors (mGluRs), which couple to the PLC-PKC pathway (Fig. 2). By activating Src and hence NMDAR function, CAKβ/PYK2 could enhance calcium influx in a feed-forward manner during strong synaptic stimulation, thereby promoting synaptic potentiation (*36*).

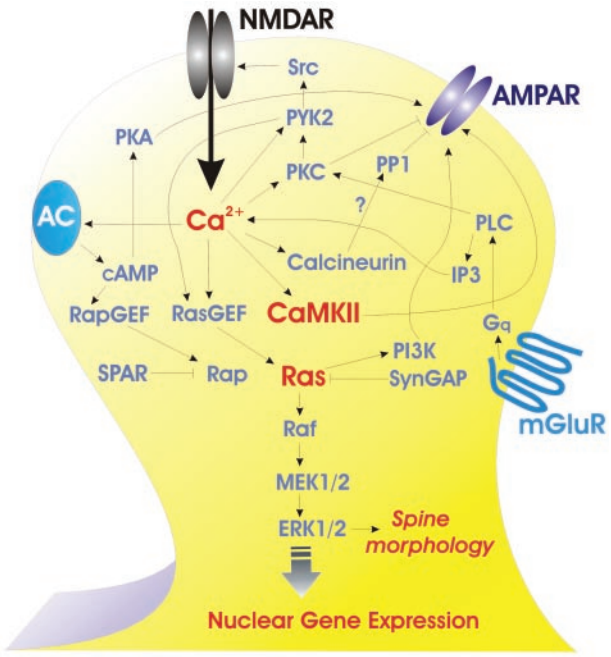

Fig. 2. Postsynaptic glutamate receptor signaling pathways. The diagram focuses on the signaling pathways activated by calcium influx through NMDARs, and how these mechanisms interact with mGluRs, AMPARs, and adenylate cyclase (AC). Gq, G protein q.

Phosphatases and synaptic depression. Hippocampal NMDAR-dependent LTD requires the activation of the protein phosphatases calcineurin (a calcium-CaM-regulated phosphatase, also termed PP2B) and PP1 (*38*), both of which are abundant in the PSD. Protein phosphatases bind to various anchoring proteins that target them to specific subcellular sites and substrates (*39*). Recent studies indicate that the proper targeting of PP1 to synapses is important for LTD. LTD is associated with a redistribution of PP1 to synapses, and peptides that interfere with the binding of PP1 to its postsynaptic anchoring proteins block NMDAR-dependent LTD (*40*). How PP1 causes synaptic depression is not clear, but a final outcome may be the internalization of AMPARs, which is correlated with dephosphorylation of the GluR1 subunit on Ser^{845}, a protein kinase A (PKA) site (*41*).

Notably, PKA binds to some of the same anchoring proteins that interact with protein phosphatases (e.g., yotiao, which binds to PP1, and AKAP79/150, which binds calcineurin). Yotiao binds directly to NMDARs (*42*), whereas AKAP79/150 associates indirectly with both AMPA and NMDARs (*43*). Thus, signaling complexes containing both kinases and their counterpart phosphatases are specifically targeted to glutamate receptors, thereby facilitating their bidirectional regulation during synaptic plasticity (*42*, *44*).

LTP Versus LTD

A central puzzle in synaptic plasticity is how the activation of and calcium influx through NMDARs can give rise to opposite results (LTP or LTD). One simple idea is that high levels of postsynaptic calcium lead to stimulation of CaMKII and LTP, whereas moderate levels result in activation of phosphatases and LTD. However, the temporal pattern of calcium increase is also likely to be important, because changing the relative timing of pre- and postsynaptic activation by just tens of milliseconds can reverse the direction of synaptic modification (*45*). In addition, the mode of postsynaptic calcium elevation affects the polarity of synaptic change; for instance, a blockade of intracellular calcium released through IP_3 receptors can convert LTD to LTP (*46*). Thus, most likely, it is the precise spatiotemporal pattern of postsynaptic calcium that determines which signaling pathways are activated and whether synaptic strengthening or weakening ensues.

Some insight into the spatiotemporal specificity of postsynaptic signaling has come from

the realization that NMDARs exist outside as well as within synapses and that extrasynaptic and synaptic receptors have different properties. The selective stimulation of extrasynaptic NMDARs promotes synaptic depression, cell death, and dephosphorylation of CREB, whereas the stimulation of synaptic NMDARs promotes synaptic potentiation, neuronal survival, and phosphorylation-activation of CREB (*47*, *48*). Whether conventional hippocampal LTP and LTD obtained with synaptic stimulation involves differential activation of synaptic and extrasynaptic NMDARs is unknown.

Why are synaptic and extrasynaptic NMDARs different? One possibility is that the subunit (such as NR2A and NR2B) composition of NMDARs differs according to location (*49*). Synaptic and extrasynaptic receptors may be associated with different signaling proteins, perhaps because of distinctive protein interactions of NR2A and NR2B subunits. A switch in NMDAR signaling properties and in NMDAR-associated proteins has been observed during forebrain development (*50*, *51*).

Recent studies have revealed an unexpected mobility of NMDARs between synaptic and extrasynaptic sites (*52*, *53*). PKC may be involved in the dispersal of NMDARs from synaptic sites (*54*) and in the regulated surface delivery of NMDARs (*55*). Thus, the subcellular distribution of NMDARs appears to be tightly controlled in neurons and highly important for regulation of synaptic plasticity.

AMPAR Regulation

AMPARs contain tetrameric combinations of subunits GluR1 to GluR4, each interacting with a specific set of intracellular proteins (*5*, *7*). In the hippocampus, AMPARs are composed mainly of GluR1-GluR2 and GluR2-GluR3 heteromers (*56*). Because AMPARs mediate most of the EPSC in glutamatergic synapses, a simple way to modify synaptic strength is to change the activity or number of AMPARs in the postsynaptic membrane (Fig. 3).

The activity of AMPARs is regulated by direct phosphorylation. For instance, CaMKII phosphorylation of GluR1 increases single-channel conductance of AMPARs (*57*). Changes occur in the phosphorylation of GluR1 at the CaMKII site (Ser^{831}, also a PKC site) and at the PKA site (Ser^{845}) during hippocampal LTP and LTD (*58*–*60*), consistent with an important role for these modifications in synaptic plasticity.

Synaptic delivery of AMPARs. In addition to phosphorylation, AMPARs show dynamic changes in their subcellular distribution. The physical delivery of AMPARs to the postsynaptic membrane could be a major mechanism underlying NMDAR-dependent LTP (*61*, *62*).

The synaptic delivery of GluR1-containing AMPARs is induced by the activation of NMDARs and CaMKII, resulting in synaptic potentiation (*63*). In contrast, the synaptic incorporation of GluR2 is activity-independent and occurs by exchange with existing synaptic AMPARs, with no net change in synaptic strength (*64*) (Fig. 3). The delivery of GluR1 from intracellular compartments to the neuronal surface is inducible by NMDAR activation, whereas the exocytosis of GluR2 is constitutively rapid (*31*). The distinctive trafficking behaviors of GluR1 and GluR2 are determined by their COOH-terminal cytoplasmic tails, with GluR1 being dominant over GluR2 in heteromeric GluR1-GluR2 receptors (*31*, *63*, *64*). Thus, the synaptic level of AMPARs is determined, at least in part, by regulated exocytosis from intracellular compartments. Consistent with an important role for GluR1 in NMDAR-dependent delivery of AMPARs to synapses, hippocampal CA1 LTP is lost in adult mutant mice lacking GluR1 (*65*). The precise molecular mechanisms by which NMDA-receptor activation stimulates exocytosis of AMPARs are unclear, but they involve CaMKII and PI3K (*31*).

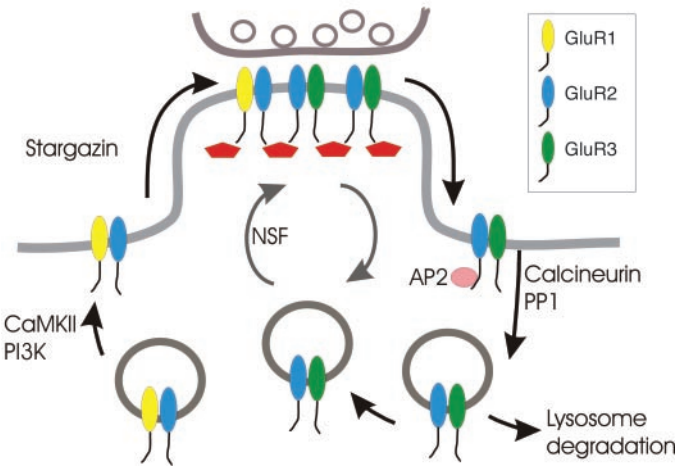

Fig. 3. Model of AMPAR trafficking. AMPARs cycle between the postsynaptic membrane and intracellular compartments. Via a NSF-dependent mechanism, intracellular GluR2-GluR3 receptors exchange constantly with synaptic receptors. GluR1-GluR2 heteromeric receptors are "retained" in intracellular compartments but are delivered to the dendrite surface upon activation of NMDARs CaMKII and PI3K. Exocytosis of GluR1-GluR2 receptors occurs at extrasynaptic sites and is followed by lateral translocation into synapses (both these steps requiring Stargazin). The number of postsynaptic AMPARs also depends on the availability of specific anchoring proteins (red pentagons) that bind to AMPAR subunits. The activation of calcineurin and PP1 leads to the recruitment of the AP2 clathrin adaptor complex to AMPARs, resulting in endocytosis. Internalized AMPARs can be recycled to the surface or sorted to lysosomes for degradation. Circles in presynaptic terminal represent synaptic vesicles.

Where does the exocytosis of AMPARs occur on the dendritic surface? Indirect evidence indicates that GluR1-containing receptors first surface at extrasynaptic sites, followed by lateral translocation into synapses (*31*). However, newly secreted GluR2 was found at synaptic sites even at the earliest time point examined, consistent with direct exocytosis at synapses in exchange for existing synaptic receptors (*31*).

Studies of the mouse mutant Stargazer also imply a two-step mechanism for AMPAR targeting to synapses. Stargazin, the protein defective in the Stargazer mouse, is required for synaptic expression of AMPARs, but mutations in the COOH-terminal tail of Stargazin dissociate surface delivery from synaptic accumulation of AMPARs (*66*). Stargazin can bind to AMPARs, but precisely how it functions in the surface delivery and synaptic incorporation of AMPARs is unclear.

In live, cultured hippocampal neurons, AMPARs show rapid lateral mobility in the plasma membrane, interspersed with periods of immobilization in the vicinity of synapses and in response to elevated intracellular calcium (*67*). Thus, mounting evidence suggests that surface translocation from nonsynaptic to synaptic sites may be an additional regulated step in synaptic targeting of AMPARs.

If synaptic AMPARs are captured from an extrasynaptic pool, the number of AMPAR-tethering proteins ("slots") at the postsynaptic membrane could be an important determinant of AMPAR density. One molecular candidate for the AMPAR "slot" is PSD-95, which can bind to the COOH-terminus of Stargazin and hence indirectly to AMPARs (*66*). The synaptic targeting of PSD-95 requires the modification of PSD-95 by a fatty acid (palmitate), a dynamic process that is influenced by synaptic activity (*68*). Regulated palmitoylation of PSD-95 may contribute to the control of synaptic strength by affecting the synaptic content of AMPARs (*68*, *69*).

AMPAR internalization. The internalization of AMPARs from the neuronal surface is emerging as a major mechanism for synaptic depression in the hippocampus (CA3-CA1 synapse) and the cerebellum (parallel fiber–Purkinje cell synapse) (*70*–*73*). AMPAR endocytosis occurs via a dynamin-dependent, clathrin-mediated pathway and is enhanced by factors that can induce synaptic depression, such as NMDAR stimulation (*41*, *72*, *74*, *75*).

Similar to LTD, NMDAR-triggered AMPAR internalization requires calcium influx and protein phosphatase activity and is correlated with dephosphorylation of GluR1 (*41*, *75*). Conversely, AMPAR endocytosis and LTD are associated with increased phosphorylation of the COOH-terminus of GluR2, an event that switches the binding preference of GluR2 from one PDZ protein (GRIP) to another (PICK-1) (*76*–*79*) [however, see (*80*)]. The binding of the clathrin adaptor complex AP2 to GluR2 also correlates with endocytosis of AMPARs and is required for NMDAR-dependent internalization of GluR2 and hippocampal LTD (*72*, *81*). It is unclear how AP2 is recruited to AMPARs after NMDAR activation, or whether AMPARs are internalized directly from the postsynaptic membrane or after lateral movement to extrasynaptic regions of the dendritic surface. In some circumstances, the degradation of AMPARs after internalization may provide an additional mechanism for controlling the level of synaptic AMPARs (*41*, *82*).

Constitutive cycling of AMPARs. After endocytosis, most of the internalized AMPARs are recycled quickly to the surface and to synapses (*31*, *41*, *74*). The basal rate of AMPAR cycling is quite rapid even in mature cultures of hippocampal and cortical neurons. About 10 to 20% of surface AMPARs are internalized from the surface in 10 min, depending in part on endogenous synaptic activity in culture (*41*, *74*). Whether such a high rate of cycling occurs in the intact brain is unknown, but there is evidence for rapid AMPAR redistribution to and from cortical synapses during synaptic plasticity in vivo (*83*). The hexameric adenosine triphosphatase (ATPase) N-ethylmaleimide-sensitive factor is likely involved in the constitutive cycling of AMPARs or synaptic stabilization of AMPARs, because disruption of the interaction between NSF and GluR2 in neurons leads to rundown of EPSCs within minutes (*84*–*87*).

Conclusions

A bewildering array of postsynaptic signaling pathways can be activated by synaptic activity and the opening of NMDARs, and this is reflected in the complexity of protein interactions in the PSD. A key challenge of the future is to understand in quantitative terms how these various postsynaptic mechanisms are coupled to synaptic stimulation and how they cross-talk with each other. In particular, dissecting the fine spatiotemporal parameters of postsynaptic signaling will be crucial to understanding how different patterns of synaptic activity lead to distinct responses. As befits the versatility of postsynaptic signal transduction, it is increasingly clear that synaptic plasticity is protean in nature, and multiple forms can occur at different synapses or within the same synapse.

For NMDAR-dependent LTP and LTD, some progress has been made into the molecular mechanisms of induction and expression, but how the two phases are connected remains mysterious. On the postsynaptic side, how do the various NMDAR-activated pathways (CaMKII, Ras, calcineurin, PP1, etc.) control the trafficking of AMPARs to and from the postsynaptic membrane? And what is the "retrograde message" that must be transmitted from the postsynaptic side to the axon terminal to effect the presynaptic changes of plasticity?

Even though the precise mechanisms of postsynaptic signaling and plasticity are still murky, a picture is emerging of a postsynaptic membrane whose molecular organization is amazingly dynamic and heterogeneous. These properties maximize the readiness of synapses for rapid change but are finely balanced by multiple controls to ensure stability of synaptic transmission.

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