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Victoria M. Ho, *et al.*

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The Cell Biology of Synaptic Plasticity

Victoria M. Ho,[1] Ji-Ann Lee,[2] Kelsey C. Martin[2,3,4]*

Synaptic plasticity is the experience-dependent change in connectivity between neurons that is believed to underlie learning and memory. Here, we discuss the cellular and molecular processes that are altered when a neuron responds to external stimuli, and how these alterations lead to an increase or decrease in synaptic connectivity. Modification of synaptic components and changes in gene expression are necessary for many forms of plasticity. We focus on excitatory neurons in the mammalian hippocampus, one of the best-studied model systems of learning-related plasticity.

The circuitry of the human brain is composed of a trillion (10^{12}) neurons and a quadrillion (10^{15}) synapses, whose connectivity underlies all human perception, emotion, thought, and behavior. Studies in a range of species have revealed that the overall structure of the nervous system is genetically hardwired but that neural circuits undergo extensive sculpting and rewiring in response to a variety of stimuli. This process of experience-dependent changes in synaptic connectivity is called synaptic plasticity.

Studies of synaptic plasticity have begun to detail the molecular mechanisms that underlie these synaptic changes. This research has examined a variety of cell biological processes, including synaptic vesicle release and recycling, neurotransmitter receptor trafficking, cell adhesion, and stimulus-induced changes in gene expression within neurons. Taken together, these studies have provided an initial molecular biological understanding of how nature and nurture combine to determine our identities. As a result, research on synaptic plasticity promises to provide insight into the biological basis of many neuropsychiatric disorders in which experience-dependent brain rewiring goes awry.

Here we focus on long-lasting forms of plasticity that underlie learning and memory. We consider, in turn, each component of the synapse: the presynaptic compartment, the postsynaptic compartment, and the synaptic cleft, and discuss processes that undergo activity-dependent modifications to alter synaptic efficacy. Long-lasting changes in synaptic connectivity require new RNA and/or protein synthesis, and we discuss how gene expression is regulated within neurons. We concentrate on studies of learning-related plasticity at excitatory chemical synapses in the rodent hippocampus because these provide extensive evidence for the cell biological mechanisms of plasticity in the vertebrate brain. Space constraints prevent us from addressing any single mechanism in depth; instead, our aim is to provide a framework for understanding the cell biology of synaptic plasticity.

Hippocampal Synaptic Plasticity

The successful study of the cell biology of synaptic plasticity requires a tractable experimental model system. Ideally, such a model should consist of a defined population of identifiable neurons and be amenable to electrophysiological, genetic, and molecular cell biological manipulations. A well-studied model system for studying plasticity in the adult vertebrate nervous system is the rodent hippocampus (Fig. 1). Critical for memory formation, the anatomy of the hippocampus renders it particularly suitable for electrophysiological investigation. It consists of three sequential synaptic pathways (perforant, mossy fiber, and Schaffer collateral pathways), each with discrete cell body layers and axonal and dendritic projections (Fig. 1). Synaptic plasticity has been studied in all three hippocampal pathways. Distinct stimuli elicit changes in synaptic

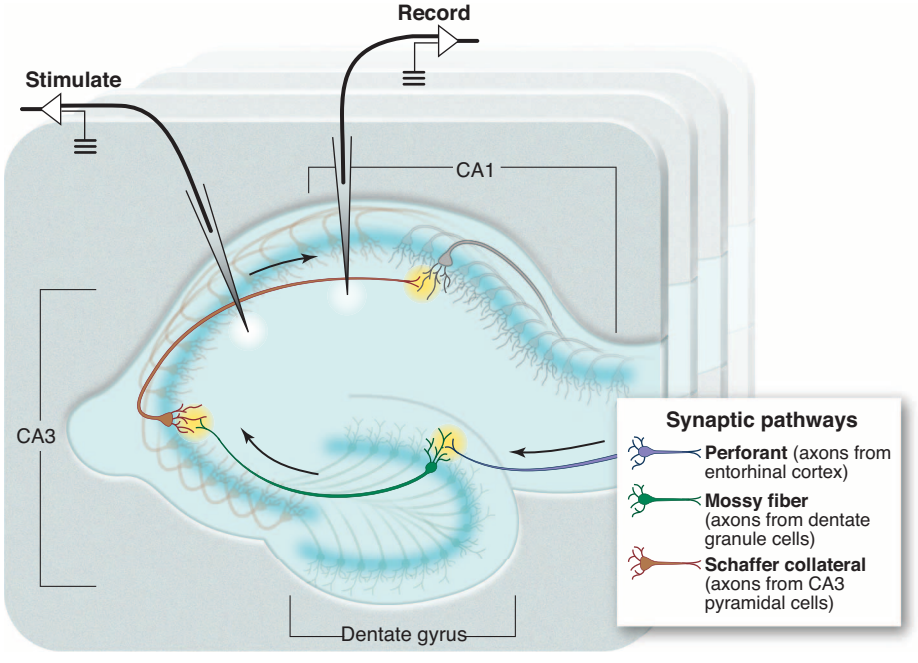

Fig. 1. Hippocampal synaptic plasticity. The rodent hippocampus can be dissected and cut into transverse slices that preserve all three synaptic pathways. In the perforant pathway (purple), axons from the entorhinal cortex project to form synapses (yellow circles) on dendrites of dentate granule cells; in the mossy fiber pathway (green), dentate granule axons synapse on CA3 pyramidal neuron dendrites; and in the Schaffer collateral pathway (brown), CA3 axons synapse on CA1 dendrites. The dentate, CA3, and CA1 cell bodies form discrete somatic layers (dark blue lines), projecting axons and dendrites into defined regions. Electrodes can be used to stimulate axonal afferents and record from postsynaptic follower cells, as illustrated for the Schaffer collateral (CA3-CA1) pathway.

[1]Interdepartmental Program in Neurosciences, University of California–Los Angeles (UCLA), BSRB 390B, 615 Charles E. Young Drive South, Los Angeles, CA 90095–1737, USA. [2]Department of Biological Chemistry, UCLA, BSRB 310, 615 Charles E. Young Drive South, Los Angeles, CA 90095–1737, USA. [3]Department of Psychiatry and Biobehavioral Sciences, UCLA, BSRB 390B, 615 Charles E. Young Drive South, Los Angeles, CA 90095–1737, USA. [4]Brain Research Institute, UCLA, BSRB 390B, 615 Charles E. Young Drive South, Los Angeles, CA 90095–1737, USA.

*To whom correspondence should be addressed. E-mail: kcmartin@mednet.ucla.edu

efficacy; high-frequency stimuli produce synaptic strengthening called long-term potentiation (LTP), and low-frequency stimulation produces synaptic weakening, called long-term depression (LTD). LTP and LTD can also be produced by spike timing–dependent plasticity, in which the relative timing of pre- and postsynaptic spikes leads to changes in synaptic strength (*1*). Different patterns of stimulation elicit changes in synaptic strength that persist over various time domains, with long-lasting forms, but not short-term forms, requiring new RNA and protein synthesis (*2*).

Hippocampal plasticity is studied in in vivo and in vitro preparations. Implanted electrodes can be used to stimulate and record from hippocampal pathways in living animals. The hippocampus can be dissected out of the brain and cut into 300- to 500-μm-thick transverse slices that can be maintained and recorded from for hours (Fig. 1). Slices can also be kept as organotypic slice cultures for weeks, preserving many aspects of their architecture. Finally, hippocampal neurons can be studied in dissociated cultures, which are particularly amenable to manipulation and dynamic imaging of individual neurons and synapses. The development of genetically modified mice and vectors for acute manipulation of gene expression complete a rich tool-kit for studies of the cell and molecular biology of hippocampal synaptic plasticity.

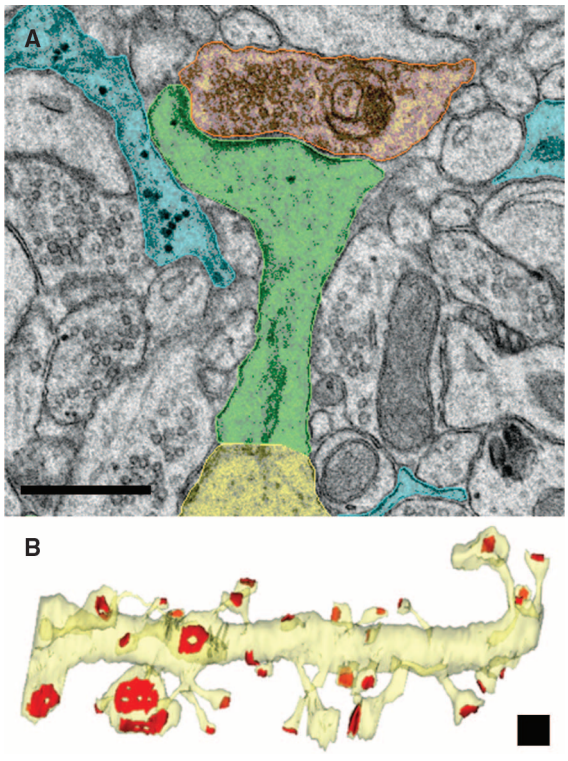

Fig. 2. The ultrastructure of the synapse. Neurons communicate with one another at chemical synapses. (**A**) Electron micrograph from area CA1 in adult rat hippocampus. The CA1 dendritic shaft is colorized in yellow, the spine neck and head in green, the presynaptic terminal in orange, and astroglial processes in blue. Scale bar, 0.5 μm. (**B**) Three-dimensional reconstruction of an 8.5-μm-long dendrite (yellow) with the PSDs labeled in red. Note the variation in spine and PSD size and shape. Scale cube, 0.5 μm^3. Reproduced with permission from Elsevier (*63*).

Presynaptic Mechanisms of Plasticity

Communication at chemical synapses involves the release of neurotransmitter from the presynaptic terminal, diffusion across the cleft, and binding to postsynaptic receptors (Figs. 2 and 3). Chemical neurotransmission is rapid (occurring in milliseconds) and highly regulated. The presynaptic terminal contains synaptic vesicles filled with neurotransmitter and a dense matrix of cytoskeleton and scaffolding proteins at the site of release, the active zone. Varying the probability of neurotransmitter release provides one mechanism for altering synaptic strength during neuronal plasticity.

Synaptic vesicle release can be subdivided into distinct steps, including vesicle mobilization, docking, priming, fusion, and recycling. Although each of these steps may be regulated by activity, we will highlight three: vesicle mobilization, docking, and priming.

Synapsins and synaptic vesicle mobilization. The population of synaptic vesicles within a presynaptic terminal exist in three states: the readily releasable pool docked at the active zone; the recycling pool, which can be released with moderate stimulation; and the reserve pool, which is only released in response to strong stimuli. A family of phosphoproteins called synapsins tether synaptic vesicles to the actin cytoskeleton and to one another. Neuronal stimulation activates kinases that phosphorylate synapsins to modulate synaptic vesicle tethering and thereby alter the number of synaptic vesicles available for release (*3*). Synapsin knockout mice have reduced reserve pools of synaptic vesicles and demonstrate deficits in learning and memory as well as various forms of plasticity (*4*), indicating that activity-dependent modulation of synaptic vesicle mobilization is critical to neuronal and behavioral plasticity.

RIM proteins and synaptic vesicle docking and priming. For synaptic vesicles to become fusion competent, they must undergo docking and priming, in which vesicle and plasma membrane soluble NSF-attachment protein receptor (SNARE) proteins are brought into close contact to allow rapid fusion following calcium influx. The Rab3-interacting molecule (RIM) family of proteins is critical for this process (*5*). As large, multidomain proteins, RIMs act as scaffolding proteins to cluster calcium channels in the active zone (*6*) and interact with Munc-13 (*7*), a priming factor required for efficient SNARE complex formation and membrane fusion. RIM is a substrate for phosphorylation by protein kinase A (PKA) and is required for mossy fiber LTP (*8*).

Postsynaptic Mechanisms of Plasticity

Most principle neurons in the brain are studded with membrane protuberances called dendritic spines, which are the postsynaptic compartments. Spines are heterogeneous in shape (Fig. 2), but consist of a bulbous head and a thinner neck that connects the spine to the dendritic shaft; the size of the spine head and the volume of the spine correlate with synaptic strength (*9*, *10*), with large spine heads containing more neurotransmitter receptors, reflecting greater synaptic strength. Spines serve as compartmentalized signaling units, and the number and shape of spines change during synaptic plasticity (*11*). At the ultrastructural level, the postsynaptic compartment is characterized by an electron-dense postsynaptic density (PSD), which consists of neurotransmitter receptors and an extensive network of scaffolding proteins.

Activation of postsynaptic kinases in the spine: CaMKII and PKMζ. LTP and LTD induction are both dependent on postsynaptic increases in intracellular calcium, with LTP requiring large increases in calcium concentrations and LTD being dependent on smaller calcium increases. The increase in calcium activates multiple downstream signaling enzymes, including the kinases calcium/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC).

LTP induction in the CA1 region of the hippocampus requires CaMKII activity (*12*, *13*), and transgenic mice lacking the α isoform have defective LTP and spatial learning (*14*, *15*). CaMKII undergoes autophosphorylation in response to increases in Ca^{2+}-bound calmodulin, which renders the kinase autonomously active. Neuronal activity also translocates CaMKII to the PSD, where it can phosphorylate many PSD proteins, including glutamate receptors. The autophosphorylation of CaMKII is essential for LTP induction and, perhaps, its maintenance (*16*) [but see (*17*)].

The brain-restricted atypical PKC isoform, protein kinase M zeta (PKMζ), is constitutively active and thus phosphorylates targets in the absence of extracellular stimulation. PKMζ mRNA is targeted to dendrites where activity-dependent signaling cascades regulate its local translation during LTP and LTD (*18*). PKMζ is sufficient and necessary for LTP maintenance and for the maintenance of long-term memories, and PKMζ activation may perpetuate synaptic plasticity and memory (*18*, *19*).

Activity-dependent modulation of postsynaptic glutamate receptors. The main excitatory neurotransmitter in the brain is glutamate, which

activates several postsynaptic receptors. Two types of ionotropic glutamate receptors—α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl D-aspartate (NMDA)—have central roles in hippocampal synaptic plasticity. Both are ligand-gated ion channels and have unique properties that subserve different phases of synaptic plasticity. NMDA-type glutamate receptors (NMDARs) are calcium permeable and, when activated, allow an influx of calcium needed for the induction of LTP. However, NMDARs do not conduct current at resting potentials because their channel pores are blocked by magnesium cations. Consequently, NMDARs have been called "coincidence detectors" because, to conduct current, they require both presynaptic transmitter release as well as postsynaptic depolarization to relieve the magnesium block. AMPA-type glutamate receptors (AMPARs) are important for the expression and maintenance of LTP. Unlike NMDARs, AMPARs can be activated by ligand binding at resting potentials to allow current flow. Increased conductance through AMPARs is responsible for the increase in synaptic strength during NMDAR-dependent LTP at CA1 synapses.

Given the importance of AMPARs in determining synaptic strength, much effort has focused on delineating the mechanisms that regulate their function. Regulated phosphorylation can change AMPAR function by changing the open probabilities and conductances of the receptors. However, changes in channel properties are unlikely to account for the drastic changes in AMPAR function seen with LTP (*20*). Instead, changes in AMPAR function during synaptic plasticity are mostly due to phosphorylation-induced changes in its abundance at the synapse.

AMPARs traffic constitutively to and from the plasma membrane via recycling endosomes (*21*) (Fig. 3). Delivery of AMPARs to synapses is believed to occur first by exocytosis at extrasynaptic sites followed by lateral diffusion within the plasma membrane to PSDs, where the mobility of the receptors is greatly reduced. During removal of synaptic AMPARs, receptors diffuse away from the PSD and then undergo clathrin-mediated, dynamin-dependent endocytosis. After endocytosis, small GTP-binding proteins of the Rab family and effector proteins direct AMPARs either to early (sorting) endosomes or back to the plasma membrane (*22*).

AMPAR trafficking occurs constitutively under basal conditions and is modulated by activity through changes in actin and myosin dynamics (*23*), as well as AMPAR interactions with scaffolding proteins and accessory subunits. One of these accessory subunits, Stargazin, mediates the interaction between AMPARs and the PSD protein PSD-95, and this interaction is important for synaptic localization of AMPARs (*24*). Activity alters the phosphorylation of Stargazin, with phosphorylated Stargazin decreasing the

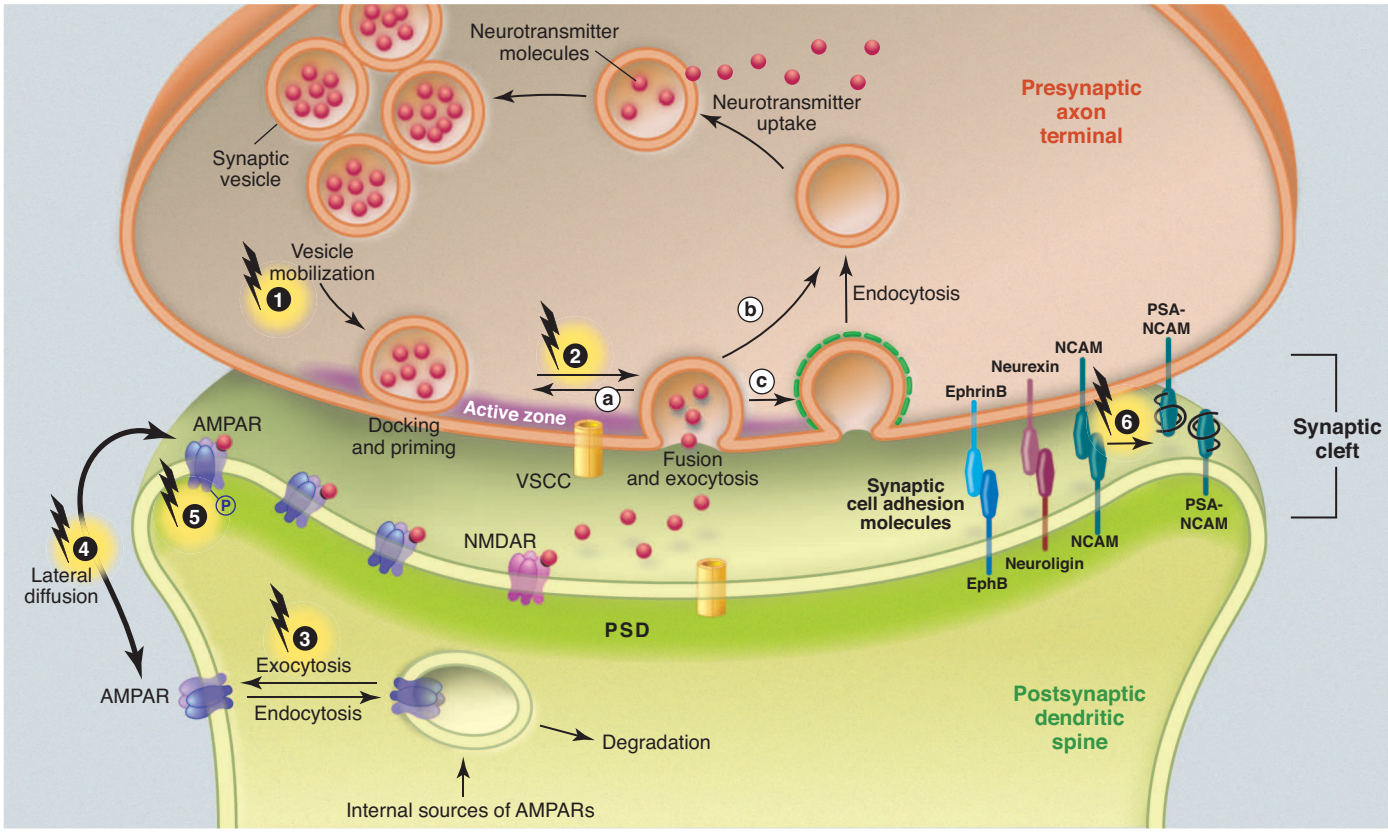

Fig. 3. Activity-dependent modulation of pre-, post-, and trans-synaptic components. Presynaptic: Neurotransmitter vesicle cycling. Neurotransmitter release starts with the filling of synaptic vesicles, which then dock and undergo priming at the active zone. Arrival of an action potential induces calcium influx through voltage-sensitive calcium channels (VSCCs), which triggers membrane fusion and exocytosis. The synaptic vesicles are then recycled via local reuse (a; "kiss and stay"), fast recycling (b; "kiss and run"), or clathrin-mediated endocytosis (c). Neurotransmitter release can be regulated during plasticity as exemplified by the regulation of synapsin phosphorylation (1) and the regulation of RIM protein phosphorylation (2). Postsynaptic: AMPA receptor trafficking. Locally and somatically synthesized AMPARs enter a pool of endosomes that undergo constitutive and regulated membrane trafficking. During potentiation, greater receptor insertion (3) increases the concentration of AMPARs at the synapse, where they are anchored by interactions at the PSD. During synaptic depression, AMPARs are endocytosed (3). The preferential location of endocytosis and exocytosis is probably extrasynaptic. Within the plasma membrane, trafficking of AMPARs between the synapse and the point of insertion or removal occurs by lateral diffusion. Extrasynaptic movement of AMPARs increases with neuronal activity (4). Receptor trafficking is modulated by phosphorylation of AMPAR subunits (5), which influences interactions with scaffolding proteins. Trans-synaptic: Synaptic cell adhesion molecules. PSA-NCAM is increased following neuronal activity (6). Lightning bolts indicate activity-dependent processes.

mobility of AMPARs and enhancing AMPAR function. Blocking Stargazin phosphorylation or dephosphorylation blocks LTP and LTD, respectively (*25*).

AMPARs exist as tetramers made up of different combinations of the four subunits, GluA1 through 4. The cytoplasmic tails of each subunit contain multiple phosphorylation sites that regulate the trafficking of AMPARs. For example, PKA phosphorylation of S845 in the long cytoplasmic tail of GluA1 increases GluA1 surface expression due to both enhanced insertion and attenuated internalization (*26*). Conversely, LTD of dissociated cultures and brain slices results in dephosphorylation of S845 and is correlated with an increased rate of AMPAR endocytosis (*27*). Knock-in mice with phosphorylation-deficient mutations at both S831A and S845A display a loss of NMDA-induced AMPAR internalization, deficits in LTP and LTD, and have impaired spatial memory (*28*).

Although studies of posttranslational modifications at individual sites have established a role for regulating GluA1 trafficking and channel properties, they do not fully account for the changes in GluA1 function observed with synaptic plasticity (*29*). Activity-modified residues continue to be discovered, including, for example, the highly conserved T840 phosphorylation site, the phosphorylation of which correlates remarkably well with synaptic strength (*30*). It is likely that complex patterns of phosphorylation and of other post-translational modifications (e.g., palmitoylation or ubiquitination) combine to regulate AMPAR localization.

Trans-Synaptic Signaling; the Synaptic Cleft

The synaptic cleft is a ~20-nm junction between the pre- and postsynaptic compartments, consisting of a space through which neurotransmitters diffuse to bind postsynaptic receptors, as well as a network of cell adhesion molecules (CAMs) that keeps the synapse together. These adhesive interactions are so strong that it is impossible to separate intact pre- from postsynaptic compartments biochemically.

Role of CAMs in synaptic plasticity. The CAMs that localize to the synaptic cleft include members of the cadherin, integrin, and immunoglobulin-containing CAMs, as well as neurexins and neuroligins. Much research has focused on trying to understand whether and how CAMs mediate synapse specificity during neural circuit formation. Here we focus on the regulation of synaptic CAMs during experience-dependent synaptic plasticity, limiting our discussion to just two of many examples.

One such example involves the addition of large sialic acid homopolymers to the neural cell adhesion molecule (NCAM) to form polysialylated NCAM (PSA-NCAM), which decreases homophilic adhesion to allow new synaptic remodeling and growth. The ratio of PSA-NCAM

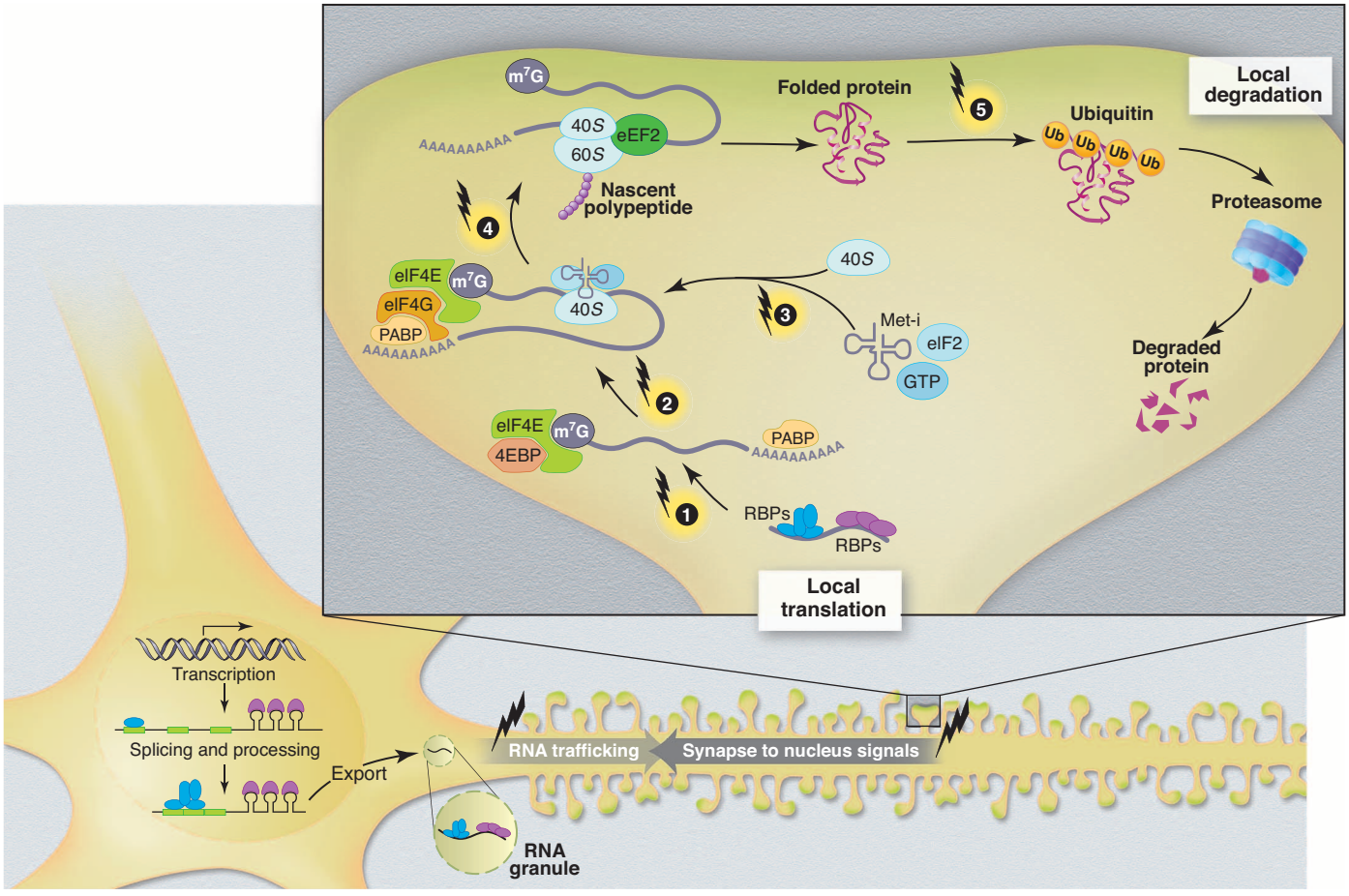

Fig. 4. Local regulation of the synaptic proteome. Synaptic plasticity modifies gene expression at many levels. Strong stimulation of synapses triggers signals that are sent to the nucleus to modify RNA synthesis. Synaptic activity also modifies protein synthesis, and has been found to act at several key steps during translation: (1) Relief of repression, e.g., RISC-mediated repression; (2) modification of translational initiation to allow 4E-4G interaction and recruitment of 40*S*; (3) formation of the preinitiation complex; and (4) dephosphorylation of eEF2 to allow for catalysis of ribosome translocation during translational elongation. To counterbalance local protein synthesis, local protein degradation also occurs at synapses (5). Together, these regulated steps in protein addition and removal allow for rapid, spatially restricted control of the synaptic proteome. Lightning bolts indicate activity-dependent processes. RBP, RNA binding proteins such as exon junction complexes, RISC machinery, Staufen, CPEB, etc. (Note: Although local translation in dendrites is a well-accepted phenomenon, it has not been demonstrated to occur in spines.)

to NCAM increases following hippocampal learning tasks, and inactivation of the enzyme that adds the polysialic moieties blocks hippocampal learning and plasticity (*31*). The increase in PSA-NCAM is thought to promote synaptic remodeling during persistent forms of plasticity.

Another family of CAMs that play a role in hippocampal plasticity includes the synaptically localized receptor tyrosine kinase ephrins and ephrin receptors (Eph receptors). Initially studied in the context of neural development, ephrins and Eph receptors have also been found to be essential for hippocampal LTP and LTD in the adult brain (*32*). Specific ephrins and Eph receptors regulate the localization and function of NMDA receptors, and can thereby modulate synaptic strength in response to activity. Experiments using inhibitory ephrin and Eph receptor peptides have revealed that both molecules are required, in a kinase-independent manner, for mossy fiber hippocampal LTP (*33*).

Trans-synaptic signaling by retrograde messengers. Another means of trans-synaptic signaling involves diffusible, membrane-soluble messengers. The CB1 and CB2 cannabinoid receptors were initially identified as receptors for cannabinoid, the active ingredient of THC/marijuana. This led to the identification of endogenous CB1 and CB2 ligands, called endocannabinoids. Endocannabinoids have emerged as important modulators of plasticity, initially at inhibitory synapses, and more recently at excitatory synapses (*34*). Depolarization and activation of a variety of receptors have been shown to activate release of endocannabinoids from the postsynaptic compartment and binding to presynaptic CB receptors, resulting in a suppression of neurotransmitter release (and thus regulating presynaptic plasticity). This form of plasticity is called endocannabinoid-LTD, or eCB-LTD. Endocannabinoid signaling is required for extinction but not acquisition of spatial memories (*35*).

The Tripartite Synapse: Glia and Synaptic Plasticity

Once thought of as the "support cells" of the nervous systems, glial cells are now considered essential partners in synapse formation, synaptic transmission, and plasticity (*36*). Astrocytes surround the synapse (Fig. 2), forming a "tripartite synapse," composed of neuronal pre- and postsynaptic compartments as well as surrounding astrocytes. Synaptically localized glia release neuroactive molecules that influence neuronal communication. For example, release of D-serine (a coactivator of the NMDA receptor) from glia is required for LTP of hippocampal Schaffer collateral synapses (*37*) [although see also (*38*)]. Ephrin and Eph receptor signaling between neurons and glia regulates the uptake of glutamate through glial glutamate transporters and thereby affects neurotransmission and synaptic plasticity (*39*). The release of lactate from astrocytes and uptake by neurons has also been reported to be required for long-term hippocampal memory and plasticity (*40*).

Regulating Gene Expression Within Neurons During Plasticity

Signaling from synapse to nucleus to regulate transcription. Long-lasting forms of synaptic plasticity, such as those underlying long-term memory, require new RNA synthesis (*2*). This indicates that synaptic signals must be relayed to the nucleus to regulate transcription. Synapse-to-nucleus signaling poses a unique set of challenges in neurons, where the distance between the synapse and nucleus can be appreciable. Neurons are specialized for rapid communication between compartments via electrochemical signaling, with depolarization at the synaptic terminal leading to depolarization at the soma in less than a millisecond. Calcium influx can occur through voltage- and ligand-gated ion channels. Cytosolic calcium can also be released from intracellular pools following activation of G_q-coupled receptors such as metabotropic GluRs (mGluRs). Each route of calcium influx induces different programs of gene induction (*41*).

Soluble signals can also be transported from the synapse to the nucleus by slower, microtubule- and motor protein–dependent pathways (*42*). This class of signals includes kinases and transcriptional regulators that function to alter transcription. These slower pathways of signaling to the nucleus may sustain changes in gene expression for time periods extending beyond the initial stimulus.

To obtain a global view of how transcription is altered during activity-dependent plasticity, expression profiling has been used to identify changes in transcription following depolarization of cultured mouse neurons. Such studies have identified several hundred activity-regulated genes (*41*). Genome-wide analyses of transcription factor binding sites of the activated genes have revealed that the transcription factors CREB, MEF2, and Npas4 control the activity-dependent transcription of a large number of downstream activity-regulated genes (*41*). These downstream transcription factors regulate the expression of overlapping but distinct subsets of activity-regulated genes, suggesting that the precise temporal, spatial, and stimulus-specific cellular response is achieved by the combinatorial control by different transcription factors.

Local protein synthesis. Despite requiring new transcription, LTP and LTD can occur in a spatially restricted manner, raising the question of how gene expression in neurons can be limited to subsets of synapses and not generalized to the entire cell. One way of locally changing the proteome in neurons is through regulated translation of localized mRNAs (Fig. 4).

The existence of local translation in dendrites of mature neurons was first suggested by electron micrographic identification of polyribosomes in hippocampal dendrites (*43*). Studies in hippocampal slices in which dendrites had been severed from cell bodies found that such dendrites retain the ability to express long-lasting LTP and LTD, indicating that local translation can mediate long-term modification of synaptic strength (*44*, *45*).

Studies of mRNA localization have led to the identification of cis-acting RNA elements that bind to RNA-binding proteins to undergo export from the soma into the dendrite (*46*). Although several dendritic localization elements have been identified, there is to date no consensus on their sequence or structure. Among the best-studied RNA binding proteins involved in dendritic mRNA localization are Staufen, Zipcode binding protein 1 (ZBP1), and hnRNPA2 (*46*). These proteins bind cis-acting elements and assemble transcripts into larger RNA transport granules, which travel in a kinesin-dependent manner along microtubules to their final destination. Whether localized RNAs undergo directed targeting, anchoring, or stabilization at specific sites remains an open question.

In terms of translational regulation, studies have revealed activity-dependent regulation of translation initiation and elongation. A mechanism of translational regulation known to occur at synapses involves the cytoplasmic polyadenylation element binding protein (CPEB). CPEB binding to 3′ untranslated regions (3′UTRs) represses translation. However, CPEB undergoes phosphorylation in an activity-dependent manner to recruit other proteins that increase the polyadenylate [poly(A)] tails of mRNAs. Subsequently, poly(A) binding protein (PABP) is recruited to the elongated poly(A) tail, which in turn recruits eukaryotic translation initiation factor 4γ (eIF4G) to interact with eukaryotic translation initiation factor 4E (eIF4E) to promote translation initiation (*47*). CPEB localizes to synapses, where it regulates translation of dendritically localized CamKIIα mRNA (*48*, *49*).

Another activity-dependent means of regulating translation initiation involves phosphorylation of eIF4E-binding proteins (4E-BPs). Hypophosphorylated 4E-BPs bind eIF4E and prevent translation initiation; phosphorylated 4E-BP dissociates from eIF4E and relieves translational inhibition. In neurons, activity increases 4E-BP phosphorylation and stimulates translation (*50*). Studies in 4E-BP2 knockout mice found that E-LTP stimulation protocols could induce L-LTP in brain slices. Recently, two additional 4E-BPs have been identified in neurons: neuroguidin and the cytoplasmic FMRP interacting protein (CYFIP). Whereas 4E-BP1 and 2 are believed to affect general translation, these new 4E-BPs may preferentially affect subgroups of transcripts within dendrites (*51*, *52*).

Activity can also regulate translational elongation during synaptic plasticity. For example, the elongation factor eukaryotic translation elongation factor 2 (eEF2) undergoes activity-dependent changes in phosphorylation. Phosphorylation of eEF2 decreases the rate of translation. Whereas action potentials decrease eEF2 phosphorylation

(thereby increasing translation), spontaneous release of neurotransmitter increases eEF2 phosphorylation and decreases translation (*53*). These effects occur locally at synapses, indicating that one function of spontaneous release may be to suppress local translation and thereby stabilize synapses.

Translation may also be regulated through the microRNA (miRNA) pathway, where each miRNA can potentially regulate hundreds of transcripts and hence coordinate the expression of many genes. Many miRNAs are relatively more abundant in, or restricted to, the brain. While miRNAs can regulate cell-wide levels of translation, their posttranscriptional mode of action makes them especially well suited to regulating distally localized transcripts. Specific miRNAs have been found in dendrites and synapses, and components of the RNA-induced silencing complex (RISC) machinery itself have been found to be altered by activity (*54*).

Consistent with the importance of regulating synaptic AMPAR concentrations during plasticity, the mRNAs encoding GluA1 and GluA2 have both been detected in hippocampal dendrites and found to undergo activity-dependent changes in localization and translation (*55*, *56*). Further linking local translation with synaptic AMPAR abundance, local eEF2-dependent translation of Arc mRNA has been shown to trigger endocytosis of AMPARs during mGluR-mediated hippocampal LTD (*57*, *58*).

Local protein degradation. The local proteome is regulated not only by local translation but also by protein degradation through the ubiquitin proteasome system (Fig. 4). Both protein synthesis and degradation are required for the maintenance of late-phase LTP, suggesting that protein degradation is needed to counterbalance protein synthesis during plasticity (*59*). Like local translation, protein degradation can be regulated within dendrites. Ubiquitin and proteasomal subunits have been found in dendrites and at synapses, and stimulation of hippocampal neurons triggers proteasome-dependent changes in the composition of PSD proteins (*60*). Activity-dependent degradation involves redistribution of proteasomes from dendritic shafts to spines (*61*). Notably, the ubiquitin proteasome pathway alters AMPAR trafficking and degradation at synapses during plasticity (*62*).

Perspectives

As the above examples illustrate, cell biological approaches have provided a detailed understanding of many aspects of activity-dependent plasticity. By focusing on molecular processes occurring within individual neurons and subcellular compartments, we now understand specific processes that are modulated by experience to change synaptic strength. These involve alterations in neurotransmitter release, trans-synaptic signaling, postsynaptic receptor dynamics, and gene expression within neurons. Distinct plasticity mechanisms are used at different types of synapses. For instance, LTP at mossy fiber synapses occurs primarily through presynaptic changes, whereas LTP at Schaffer collateral synapses occurs mostly through postsynaptic mechanisms. The end result of many of the processes we have described is to regulate the concentration of glutamate receptors, indicating that this is a major postsynaptic determinant of synaptic strength during plasticity.

Together, each of these cell biological mechanisms provides potential therapeutic targets for diseases in which brain plasticity is dysfunctional. However, they fall short of elucidating how complex circuits are altered by experience to store information and alter behavior. This will require the development of tools for investigating both the dynamic nano-architecture of the synapse and the neural circuit as a whole. A particular challenge is to study plasticity in neural circuits in living animals, and to develop methods to examine, and computational frameworks to understand, how all components of a circuit are regulated to alter circuit function dynamically. The development of methodologies for super-resolution time-lapse imaging of synapses, neurons, and circuits in live animals promises to move the field forward toward a more nuanced and complete understanding of the experience-dependent plastic changes in the brain that mediate learning and memory.

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