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Translational Regulatory Mechanisms in Persistent Forms of Synaptic Plasticity

Review

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Memory and synaptic plasticity exhibit distinct temporal phases, with long-lasting forms distinguished by their dependence on macromolecular synthesis. Prevailing models for the molecular mechanisms underlying long-lasting synaptic plasticity have largely focused on transcriptional regulation. However, a growing body of evidence now supports a crucial role for neuronal activity-dependent mRNA translation, which may occur in dendrites for a subset of neuronal mRNAs. Recent work has begun to define the signaling mechanisms coupling synaptic activation to the protein synthesis machinery. The ERK and mTOR signaling pathways have been shown to regulate the activity of the general translational machinery, while the translation of particular classes of mRNAs is additionally controlled by gene-specific mechanisms. Rapid enhancement of the synthesis of a diverse array of neuronal proteins through such mechanisms provides the components necessary for persistent forms of LTP and LTD. These findings have important implications for the synapse specificity and associativity of protein synthesis-dependent changes in synaptic strength.

Introduction

The hypothesis that memory traces may depend on the production of new proteins was first put forward more than half a century ago (Katz and Halstead, 1950). It has now been 40 years since the initial demonstration that memory formation in mammals requires protein synthesis (Flexner et al., 1963; reviewed in Davis and Squire, 1984), but only in recent years have the molecular and cellular mechanisms by which regulated protein synthesis contributes to long-term memory formation begun to come to light.

Experimental studies in the rodent hippocampus have delineated distinct temporal phases of memory and synaptic plasticity that share a number of corresponding features. Most notably, short-lived forms of synaptic plasticity and memory can be established in the absence of new mRNA and protein synthesis, whereas long-lasting forms require macromolecular synthesis (Kandel, 2001). These and other observations suggest that a close mechanistic connection exists between protein synthesis-independent forms of synaptic plasticity and memory on the one hand and protein synthesis-dependent forms of synaptic plasticity and long-term memory on the other hand. In support of this view, parallel deficits in protein synthesis-dependent synaptic plasticity and memory, with preservation of protein synthesis-independent processes, have been observed as a result of several pharmacologic and genetic manipulations (Abel et al., 1997; Wong et al., 1999; Frey, 2001; Kang et al., 2001; Kelleher et al., 2004). Therefore, we will focus on mechanistic studies of long-term synaptic plasticity, which are likely to be relevant to the mechanisms underlying memory consolidation.

The dual requirement for new mRNA and protein synthesis has suggested that transcriptional regulation represents the principal control point for the consolidation of synaptic plasticity, with translation of newly synthesized mRNAs playing a more permissive or secondary role. Indeed, intensive investigations have delineated a variety of signaling pathways and phosphorylation events linking synaptic activity to changes in the activity of key transcriptional regulatory proteins (West et al., 2002). In this review, we will outline several lines of evidence that have revealed a crucial and specific role for neuronal activity-dependent regulation of mRNA translation in long-lasting synaptic plasticity.

A consideration of the role of new protein synthesis in enduring forms of synaptic plasticity raises a number of interesting issues. The protein products required for the establishment of long-term synaptic plasticity are thought to be utilized by activated synapses to stabilize modifications in synaptic strength. Since input or synapse specificity is one of the hallmarks of synaptic plasticity, there must therefore exist a cellular mechanism to restrict or localize these protein products to activated synapses. Furthermore, since enduring forms of both LTP and LTD depend upon new protein synthesis, it is unclear how these newly synthesized protein products can be utilized by activated synapses in a way that results in an appropriate pattern of synaptic modification. Specifically, an important question is whether long-lasting LTP and LTD are differentiated by the synthesis of limited sets of proteins that distinguish the two processes or whether the components essential for each process are differentially captured from a broader mixture of newly synthesized proteins. We will discuss recent progress on definition of the molecular mechanisms regulating protein synthesis in response to synaptic activity, which offers important insights into these problems.

Distinct Temporal Phases of Synaptic Plasticity

A requirement for newly synthesized proteins in long-lasting LTP was initially described in the rodent hippocampus in vivo (Krug et al., 1984), and subsequent studies in hippocampal slice preparations have distinguished temporal phases of LTP on the basis of their differing

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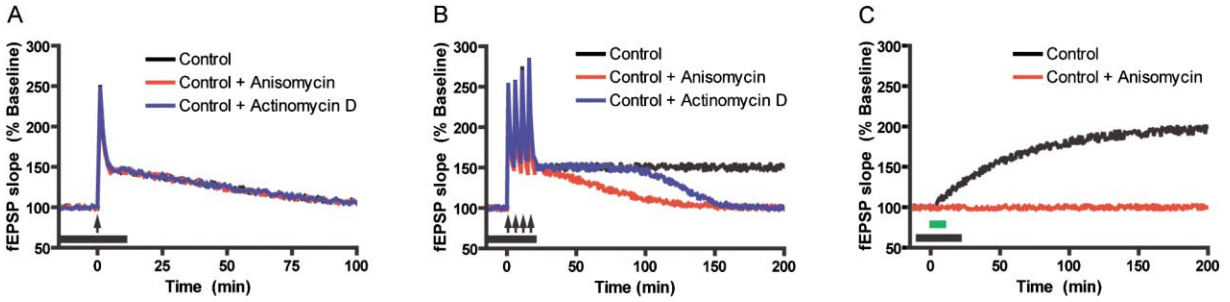

Figure 1. Schematic Representation of the Temporal Phases of LTP at CA1 Synapses

(A) A single train of tetanic stimulation produces a decremental potentiation lasting for about 1 hr. This early phase of LTP (E-LTP) is insensitive to translational inhibition (anisomycin) and transcriptional inhibition (actinomycin-D). The solid bar represents the time period of inhibitor treatment. The arrow indicates the time of delivery of tetanic stimulation.

(B) Four spaced trains of tetanic stimulation induce a persistent potentiation lasting for many hours. This late phase of LTP (L-LTP) is converted to a decremental potentiation that resembles E-LTP when a translational inhibitor is present during the repeated tetanization. When a transcriptional inhibitor is similarly present during tetanization, L-LTP remains unaffected during the initial 60–90 min, after which it decays to baseline. The solid bar represents the time of inhibitor treatment. The arrows indicate the time of delivery of the four tetanic trains.

(C) An incremental and persistent form of LTP can also be induced by treatment (indicated by green bar) with BDNF or cAMP/PKA agonists. This form of potentiation is completely blocked by inhibition of translation during stimulation (indicated by the black bar).

sensitivities to inhibitors of mRNA and protein synthesis (Krug et al., 1984; Frey et al., 1988, 1996; Huang and Kandel, 1994; Nguyen et al., 1994). An "early phase" of LTP (E-LTP) lasting 1–2 hr, which is typically induced by a single train of high-frequency tetanic stimulation, is unaffected by transcriptional and translational inhibition (Figure 1A). In contrast, the "late phase" of LTP (L-LTP) differs from E-LTP in its greater amplitude and longer duration (>3 hr), its recruitment by repeated, spaced tetanizations (typically three to four tetanic trains separated by 5–10 min), and its critical dependence on new mRNA and protein synthesis (Figure 1B). These properties of LTP have been observed in each of the three major hippocampal excitatory synaptic pathways (Huang et al., 1996). Though the distinctions between E-LTP and L-LTP have been best characterized with high-frequency stimulation delivered in the form of tetanization, temporal phases of LTP induced by theta burst stimulation have also been described (Nguyen and Kandel, 1997). Just as with tetanus-induced LTP, the late phase of theta burst-induced LTP requires more prolonged stimulation as well as new mRNA and protein synthesis. In the well-studied case of the Schaeffer collateral pathway (CA3-CA1 synapses), the induction of both E-LTP and L-LTP requires NMDAR activation, but further mechanistic investigations of L-LTP have largely focused upon its unique requirement for macromolecular synthesis.

The reported kinetic effects of protein synthesis inhibition on L-LTP have been somewhat variable, likely owing to differences in methodology, particularly the time of application of protein synthesis inhibitors. When inhibitors are applied during a preincubation period immediately prior to repeated tetanization, thereby allowing sufficient time for drug penetration and protein synthesis blockade, inhibition of the early expression of L-LTP immediately following induction is generally observed (Otani et al., 1989; Huang and Kandel, 1994; Frey and Morris, 1997; Scharf et al., 2002; Kelleher et al., 2004). Moreover, when the effects of single and repeated tetanization were comparatively analyzed at CA1 synapses, treatment with the reversible protein synthesis inhibitor anisomycin prior to repeated tetanization transformed L-LTP into a decremental potentiation resembling E-LTP (Frey and Morris, 1997). As observed in studies of long-term memory formation, effective blockade of L-LTP requires treatment with translational inhibitors around the time of L-LTP induction, whereas treatment after L-LTP has been established produces no effect (Otani et al., 1989; Frey and Morris, 1997). These observations indicate that repeated tetanization recruits a rapid enhancement of protein synthesis that is necessary for the full expression of L-LTP. Indeed, increased rates of protein synthesis can be detected rapidly following L-LTP induction (Kelleher et al., 2004). Similarly, the failure of translational inhibitors to affect already established L-LTP argues against a simple requirement for ongoing protein synthesis to maintain steady-state protein levels. Rather, this temporal window for L-LTP blockade further suggests that the increase in translational rate that accompanies L-LTP induction is transient. The apparent ability of protein synthesis inhibitors to block the enhanced protein synthesis stimulated by L-LTP induction without depleting proteins necessary for basal neuronal and synaptic function presumably reflects the brief duration of inhibitor treatment (typically 30–45 min) relative to the turnover rates of neuronal proteins.

Several observations indicate that the effects of protein synthesis inhibitors on long-lasting synaptic plasticity are likely to be a specific consequence of their translational blockade, rather than any nonspecific inhibitory effects or toxicity. First, their action is specific for long-lasting forms of synaptic plasticity, without interference with more transient forms of synaptic plasticity or other synaptic processes; for example, the widely used inhibitor anisomycin does not affect calcium influx evoked by depolarization or mGluR activation (e.g., Linden, 1996). Second, protein synthesis inhibitors typically interfere with the induction but not the maintenance of long-lasting synaptic plasticity. Third, both L-LTP and L-LTD (see below) are blocked by protein synthesis inhibitors, arguing against the induction of an antagonistic synaptic potentiation or depression. Fourth, long-lasting synaptic

plasticity is specifically inhibited in both vertebrate and invertebrate systems by multiple protein synthesis inhibitors with distinct structures and mechanisms of action, including mRNA 5′ cap analogs, which interfere with recognition of capped mRNAs by the translational machinery (Huber et al., 2000; Beaumont et al., 2001).

Long-lasting protein synthesis-dependent LTP can also be induced by treatment with several agonists, including the neurotrophins BDNF and NT-3, forskolin and the membrane-permeable cAMP analog Sp-cAMPS, and dopamine receptor type D1/D5 agonists (Frey et al., 1993; Huang et al., 1994; Huang and Kandel, 1995; Kang and Schuman, 1995). In contrast to L-LTP that is induced by repeated tetanization, the L-LTP that is elicited by all of these agents develops gradually, requiring 1–2 hr to reach maximal levels, and is entirely abolished by pretreatment with protein synthesis inhibitors (Figure 1C). Thus, these "incremental" forms of L-LTP appear to be wholly dependent on newly induced protein synthesis. Pharmacologic studies have suggested that L-LTP that is induced by repeated tetanization and L-LTP that is induced by elevation of intracellular cAMP levels share a common protein synthesis-dependent mechanism. Treatment with an inhibitory cAMP analog (RpcAMPS) or establishment of cAMP-induced LTP prior to repeated tetanization blocks L-LTP, resulting in a residual decremental potentiation resembling E-LTP (Frey et al., 1993; Huang and Kandel, 1994). Similar inhibition and occlusion of tetanus-induced L-LTP was observed using agonists and antagonists of the dopamine D1/D5 receptors, which appear to contribute to LTP primarily via cAMP-dependent mechanisms (Frey et al., 1991; Huang and Kandel, 1995).

Although E-LTP and L-LTP have been interpreted as sequential "phases" or components of a single process, their differing induction protocols and biochemical features suggest that they may represent distinct processes that function in parallel. In particular, experimental procedures that give rise to L-LTP recruit a protein synthesis-dependent potentiation in the earliest minutes following stimulation. Whereas treatment with protein synthesis inhibitors prior to repeated tetanization yields a decremental potentiation resembling E-LTP, protein synthesis inhibition entirely abrogates the potentiation induced by neurotrophins or cAMP agonists, suggesting that these stimuli can induce L-LTP independent of E-LTP. In contrast, the potentiation induced by repeated tetanization appears to represent a composite of gradually decaying E-LTP and more persistent protein synthesis-dependent mechanisms.

The capacity for bidirectional modifiability of enduring synaptic changes is presumably provided by complementary forms of protein synthesis-dependent LTP and LTD. Consistent with this expectation, long-lasting forms of hippocampal LTD have recently been described. Activation of type I metabotropic glutamate receptors, either through direct stimulation with the agonist DHPG or through paired-pulse low-frequency stimulation (ppLFS), produces L-LTD at CA1 synapses that is completely blocked by pretreatment with anisomycin (Huber et al., 2000, 2001). Low-frequency stimulation (LFS) of the Schaeffer collateral afferents in organotypic hippocampal slices has also been shown to induce stable protein synthesis-dependent L-LTD (Kauderer and Kandel, 2000). The mechanisms of these two forms of L-LTD differ, since the induction of mGluR-dependent L-LTD does not require NMDA receptor activity, whereas the LFS-induced form of L-LTD is NMDA receptor dependent. Nevertheless, the inhibitory effect of anisomycin pretreatment on both forms of L-LTD is evident immediately after completion of the induction period, again arguing for a rapid contribution of newly synthesized proteins to persistent forms of synaptic plasticity. A protein synthesis-dependent late phase of cerebellar LTD has also been described in cultured Purkinje neurons (Linden, 1996).

Differing requirements for macromolecular synthesis have also been described in distinct temporal phases of synaptic strengthening in invertebrate systems. In *Aplysia*, sensitization of the gill-withdrawal reflex is associated with synaptic facilitation in the sensorimotor neural circuit mediating the reflex (Kandel, 2001). A single sensitizing stimulus induces short-term memory that depends on covalent modifications of existing proteins, while multiple, spaced sensitizing stimuli induce long-term memory that requires new mRNA and protein synthesis (Castellucci et al., 1989). Similarly, short-term facilitation (STF) of the sensorimotor synapse can be produced by a single application of serotonin independent of macromolecular synthesis, but long-term facilitation (LTF) elicited by multiple, spaced applications of serotonin is dependent on new mRNA and protein synthesis (Montarolo et al., 1986).

In contrast to the predominantly postsynaptic mechanism of hippocampal LTP expression, LTF of the sensorimotor neuron synapse in *Aplysia* is mediated by a presynaptic enhancement of neurotransmitter release. Unlike the axons and dendrites of mammalian neurons, the processes or "neurites" of *Aplysia* neurons are not polarized and can form both presynaptic and postsynaptic terminals. In fact, the sensory neuron in the neural circuit that mediates the gill-withdrawal reflex is presynaptic to the motor neuron but postsynaptic to the serotonergic interneurons responsible for LTF induction (which is therefore heterosynaptic). Thus, new gene expression is stimulated in the sensory neuron by postsynaptic signaling mechanisms, as in the case of hippocampal L-LTP, but the new gene products then act at presynaptic terminals within the same neuron to enable persistent facilitation of transmitter release.

A Requirement for Translation Independent of Transcription in L-LTP and L-LTD

The requirement for new mRNA synthesis in the establishment of long-term memory and long-lasting forms of synaptic plasticity suggested that induction of gene expression at the transcriptional level may provide the primary regulatory mechanism underlying these processes. According to this view, the observed protein synthesis dependence simply reflected the need for constitutive translation of newly produced mRNAs. In recent years, however, several lines of evidence have revealed important dissociations between transcriptional and translational regulation in the establishment of long-term forms of synaptic plasticity, demonstrating that upregulation of the translational rate makes an important contribution to these processes. Earlier investigations of L-LTP had suggested that pretreatment with transcriptional inhibitors produced a delayed effect on

L-LTP maintenance, whereas pretreatment with translational inhibitors had an immediate effect (Nguyen et al., 1994; Frey et al., 1996; Frey and Morris, 1997; Scharf et al., 2002). More recently, direct comparison of the kinetic effects of transcriptional and translational blockade on L-LTP confirmed this temporal dissociation, defining an early translation-dependent, transcription-independent phase of L-LTP during the initial 60–90 min following induction and a subsequent transcription- and translation-dependent phase (Kelleher et al., 2004). Importantly, the delayed kinetic effect of transcriptional blockade is unlikely to be explained by delayed action of the transcriptional inhibitor, since a 30 min pretreatment with a transcriptional inhibitor terminating 45 min prior to tetanization produced a comparable delayed effect on L-LTP (Frey et al., 1996). The parsimonious explanation for this difference in the inhibition kinetics is that the early effect of translational inhibition reflects the rapid translation of *preexisting* mRNAs, while the delayed effect of transcriptional inhibition reflects the time needed for the synthesis, processing, transport, and translation of *newly synthesized* mRNA. This interpretation is consistent with evidence outlined above for a rapid contribution of new protein synthesis to long-lasting forms of LTP and LTD.

Investigations of mGluR-dependent L-LTD have provided perhaps the most compelling evidence that translational activation of preexisting mRNAs can support rapid and long-lasting modulation of synaptic efficacy. mGluR-dependent L-LTD is insensitive to transcriptional inhibition, and CA1 dendrites from which the cell bodies have been excised continue to support the full expression of LTD (Huber et al., 2000). These findings further imply not only that nuclear mRNA synthesis is unnecessary for this form of L-LTD but also that the dendritic compartment contains all of the necessary mRNA and translational components. A similar sufficiency of isolated CA1 dendrites for the expression of BDNF-induced L-LTP has also been demonstrated (Kang and Schuman, 1996), but the sensitivity of this form of L-LTP to transcriptional inhibition has not been directly investigated. Interestingly, isolation of CA1 dendrites from their somata did not affect the early expression of tetanus-induced L-LTP but abolished its maintenance beginning about 3 hr postinduction; in contrast, protein synthesis inhibition in control slices gave rise to an immediate and progressive impairment (Frey et al., 1989). Thus, dendritic translation of preexisting mRNAs appears to be sufficient for the early expression of tetanus-induced L-LTP, but the contribution of nuclear mRNA synthesis seems necessary for its maintenance.

Dissection of the temporal phases of synaptic facilitation in *Aplysia* has provided additional support for a translational requirement independent of new transcription in synaptic strengthening. Interestingly, an intermediate phase of synaptic facilitation (ITF) with shorter duration than LTF is induced by multiple, spaced serotonin applications and requires new translation but not new transcription (Ghirardi et al., 1995; Mauelshagen et al., 1996). Modulation of synaptic efficacy on intermediate timescales comparable to *Aplysia* ITF has been shown in other invertebrate systems to be similarly dependent on new translation but independent of new transcription (Beaumont et al., 2001). The distinct temporal and mechanistic features of *Aplysia* ITF are paralleled by an intermediate phase of memory (ITM), which also requires new protein but not mRNA synthesis (Sutton et al., 2001).

Dendritic versus Somatic Protein Synthesis in Long-Lasting Synaptic Plasticity

Further support for a specific role for translation in long-term synaptic plasticity has emerged from investigations of protein synthesis in the synaptodendritic compartment (reviewed in Steward and Schuman, 2003). The presence of ribosomal assemblies in neuronal dendrites and the ability of isolated synaptic fractions to support de novo protein synthesis were first reported over 25 years ago (Bodian, 1965; Autilio et al., 1968; Morgan and Austin, 1968). Nevertheless, it had long been presumed that the soma represented the primary site of macromolecular synthesis in the neuron and that synapses depended on this synthesis for their function. This view was challenged in the early 1980s by observations of dendritic polyribosomes preferentially localized near postsynaptic sites, particularly under conditions of synapse formation or replacement, suggesting that local protein synthesis may be important for synaptic growth (Steward and Levy, 1982; Steward and Fass, 1983). Subsequent work documented the presence in dendrites of endoplasmic reticulum and Golgi elements, as well as the capacity for posttranslational modification and membrane insertion of newly synthesized proteins (Steward and Reeves, 1988; Tiedge and Brosius, 1996; Torre and Steward, 1996; Kacharmina et al., 2000; Pierce et al., 2000).

How diverse is the repertoire of proteins synthesized in the dendritic compartment? In order to address this question, the population of mRNAs present in this compartment has been investigated. Early in situ hybridization studies suggested that a relatively small number of neuronal mRNAs are localized within the dendritic compartment, most notably highly abundant mRNAs such as those encoding MAP-2 and αCaMKII (Garner et al., 1988; Burgin et al., 1990), while the vast majority of mRNAs were restricted to the cell body. Later introduction of more sensitive methodologies has greatly expanded this list of mRNAs to include $\sim$400 distinct species in hippocampal dendrites (Miyashiro et al., 1994; Eberwine et al., 2002) and $\sim$260 species in *Aplysia* neurites (Moccia et al., 2003). In general, mRNAs appear to exhibit either somatic localization or distribution throughout both the somatic and dendritic compartments, and exclusively dendritic mRNA localization has not been observed.

Although most dendritic mRNAs appear to display a diffuse distribution within the dendritic compartment, there is some evidence for neuronal activity-induced trafficking and localization of dendritic mRNAs. Much of this evidence derives from the selective translocation of newly synthesized mRNA for the activity-regulated cytoskeletal protein (Arc) to dendritic segments activated by high-frequency tetanic stimulation (Steward et al., 1998). These observations have led to the proposal that the local translation of mRNAs targeted to activated synapses may contribute to synaptic plasticity and growth (Steward and Worley, 2001). However, due to the limited resolution of available methods, it has not yet been shown that the translocation of Arc mRNA is truly restricted to tetanized synapses.

Based on the original observations of ribosomal assemblies in close proximity to individual synapses, spatially restricted translation at activated synapses was initially envisioned as a potential mechanism for synapse-specific delivery of proteins essential for plasticity and growth (Steward and Levy, 1982). However, the conceptual focus has shifted in recent years to protein synthesis occurring more broadly in the dendritic compartment, emphasizing the distinction with protein synthesis occurring in the cell body followed by protein transport into dendrites (Steward and Schuman, 2003). Leaving aside the issue of synapse specificity, dendritic protein synthesis affords a mechanism for rapid changes in protein content in response to synaptic activity, subject to the limitations on protein repertoire that are imposed by the dendritic localization of a subset of mRNAs. The capacity of the dendritic compartment to effect rapid increases in mRNA translation in response to various forms of neuronal activity, in a manner independent of the neuronal soma, has now been amply demonstrated. Early evidence for protein synthesis originating in the dendritic compartment was derived from the observation of rapid incorporation of radiolabeled amino acid in CA1 dendrites following low-frequency stimulation of Schaeffer collateral pathway in hippocampal slices (Feig and Lipton, 1993). A subsequent study in hippocampal slices demonstrated that tetanic stimulation evoked a rapid increase in αCaMKII immunoreactivity in distal CA1 dendrites that was unlikely to be explained by somatic protein synthesis, based on estimated rates of cytoskeleton-dependent anterograde protein transport (Ouyang et al., 1999). Several recent reports have provided more direct visualization of dendritic protein synthesis in response to neuronal stimulation. Increased translation of a synthetic GFP reporter mRNA bearing the α*CaMKII* 5′ and 3′ untranslated regions (UTRs) was observed in dendrites transected from the cell body of cultured hippocampal neurons within 45–60 min of BDNF treatment (Aakalu et al., 2001). Similarly, translation of *GluR2* and *GFP* mRNAs transfected into isolated hippocampal dendrites in culture was rapidly stimulated in response to DHPG treatment, with the increased expression detected as early as 5–10 min following stimulation (Kacharmina et al., 2000; Job and Eberwine, 2001). A novel fluorescent affinity tag methodology has recently been employed to demonstrate enhanced expression of exogenously introduced constructs encoding GluR1 and GluR2 in transected dendrites within 1 hr following DHPG treatment or membrane depolarization (Ju et al., 2004).

The rapid induction of dendritic protein synthesis in response to forms of neuronal activity associated with L-LTP and L-LTD, such as those induced by BDNF and DHPG, is compatible with a role in long-lasting synaptic plasticity. Indeed, the immediate requirement for translation of preexisting mRNAs inferred from studies of L-LTP and L-LTD argues that somatic translation and subsequent "somatofugal" protein transport to dendrites is unlikely to contribute to the protein synthesis-dependent potentiation, at least during the initial postinduction period. Experiments conducted in hippocampal slices in which CA1 dendrites were transected from their cell bodies have demonstrated that protein synthesis in the dendritic compartment is sufficient for BDNF-induced L-LTP and DHPG-induced L-LTD (Kang and Schuman, 1996; Huber et al., 2000). Although protein synthesis in isolated dendrites is not sufficient to maintain normal tetanus-induced L-LTP throughout the entire time course, it does appear to be sufficient for the early expression of a translation-dependent (and presumably transcription-independent) component (Frey et al., 1989). While these studies demonstrate the sufficiency of dendritic translation for the expression of some forms of L-LTP and L-LTD, protein synthesis in the dendritic compartment of intact neurons has not yet been shown to be necessary for these processes. Investigations of branch-specific LTF in a bifurcated sensory neuron-motor neuron coculture system in *Aplysia* perhaps come closest to illustrating such a requirement for extrasomatic translation. Inhibition of protein synthesis at one sensory neuron branch during serotonin application blocked LTF at that branch but had no effect on serotonin-induced LTF at the other branch (Martin et al., 1997).

Because chemical- or agonist-induced synaptic plasticity is not input specific and involves direct and widespread synaptic activation, the possible spatial restriction of the induced dendritic protein synthesis to activated synapses could not be addressed. The spatial extent of the protein synthesis induced by serotonin in branch-specific LTF is similarly unclear. Though most studies of dendritic protein synthesis have not examined its spatial extent, available evidence suggests that induced translation products can exhibit either diffuse or punctate patterns of localization, and in some cases the apparent size of puncta approached synaptic dimensions (Aakalu et al., 2001; Job and Eberwine, 2001). It remains to be seen whether such localization patterns contribute to synapse-specific processes.

Synapse Specificity in Protein Synthesis-Dependent Plasticity

Synaptic Tagging and Capture

The input specificity of synaptic plasticity necessitates a mechanism for selective delivery or localization of the protein components required for L-LTP and L-LTD to activated synapses. In principle, three basic solutions to the problem can be envisioned: (1) essential plasticity proteins are synthesized and retained locally at activated synapses; (2) mRNAs for essential plasticity proteins are localized to activated synapses, and their protein products are retained locally; or (3) neither mRNAs nor protein products are strictly localized to activated synapses, but essential plasticity proteins are somehow "captured" only by activated synapses.

The first possible solution is embodied by the "local protein synthesis hypothesis," which proposes that activated synapses synthesize locally, and use locally, the protein components that are required for L-LTP or L-LTD (Figure 2). According to this mechanism, protein synthesis is stimulated locally only at activated synapses, and localization of essential mRNAs at activated synapses is therefore not required. The second possible solution is exemplified by the "mRNA targeting hypothesis," i.e., newly synthesized mRNAs that encode essential plasticity proteins are targeted to activated synapses. The specific targeting of essential mRNA(s) in this model would obviate the need for a strictly localized pattern of protein synthesis at activated synapses. The input specificity of transcription-independent forms or phases of L-LTP

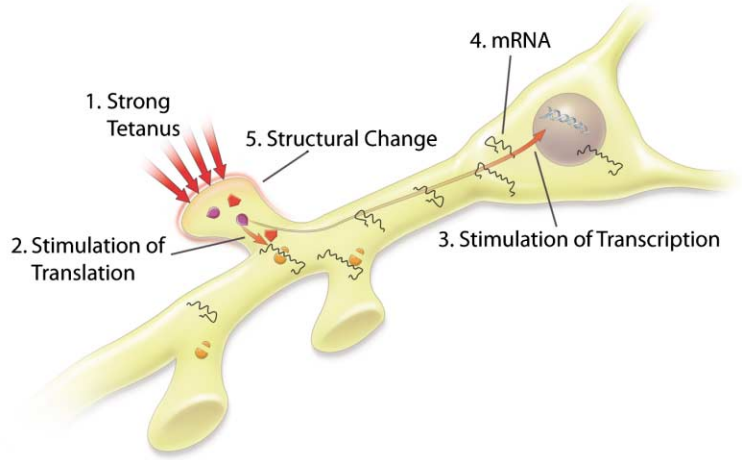

Figure 2. Model for Input Specificity Conferred by Local Protein Synthesis

When a synapse is stimulated strongly (1), local translation (2) and transcription (3) are activated. Transcription causes production of mRNAs necessary for the expression of plasticity. These mRNAs are diffusely distributed throughout the neuron, possibly in the form of mRNPs or RNA granules (4). The induction of translation (2) at the stimulated synapse in a spatially restricted manner results in the production (and retention) of proteins and synaptic modifications (5) only at the site of stimulation.

and L-LTD, however, would be difficult to explain by the mRNA targeting hypothesis, since it would not be possible to target preexisting mRNAs prior to synaptic activation. Synapse specificity in both of these candidate mechanisms relies on a highly spatially localized event (translation or mRNA targeting) and equally localized confinement or retention of the resulting protein products. Importantly, both mechanisms also require new protein synthesis at the time of L-LTP or L-LTD induction.

The third "synaptic tag" hypothesis does not demand these highly localized events or protein products. Instead, synaptic specificity is conferred by a synaptic "tag," whose molecular nature is as yet unknown but is conceptualized as comprising a relatively immobile synaptic component that has been modified by synaptic activity. This tag functions to sequester or "capture" proteins newly synthesized as a result of L-LTP induction (Figure 3). Evidence supporting this hypothesis was first obtained by Frey and Morris (1997), who reported a novel long-term heterosynaptic facilitation of L-LTP when examining two independent synaptic inputs in the Schaeffer collateral pathway. Repeated tetanization of the first input resulted in the establishment of homosynaptic protein synthesis-dependent L-LTP, which was inhibited by pretreatment with a protein synthesis inhibitor (either anisomycin or emetine). One hour later, repeated tetanization was delivered to the second input in the presence of a protein synthesis inhibitor, and normal L-LTP was paradoxically observed, suggesting that the proteins synthesized in response to L-LTP induction in the first input also enabled the establishment of L-LTP in the second input (Figure 3A). This observation is incompatible with both the local protein synthesis and the mRNA targeting hypotheses, which would have predicted that only the first input would have been potentiated. Consistent with this interpretation, delivery of a single tetanus to the second input, which would normally produce protein synthesis-independent E-LTP, instead resulted in L-LTP, provided that L-LTP had been established in the first input within 1–2 hr (either before or after) of tetanization of the second input (Figure 3B). Thus, provision of the newly synthesized proteins produced in response to L-LTP induction appears sufficient for the conversion of E-LTP to L-LTP. The limited time window for coincidence of the synaptic tag and the new protein synthesis presumably reflects the temporal decay of the tag.

These observations suggest that induction of either E-LTP or L-LTP results in the creation of a protein synthesis-independent synaptic tag, which is able to provide synapse specificity by capturing the necessary protein components. These observations have been confirmed and extended by Kandel and colleagues, who additionally found that formation of the synaptic tag requires the activity of both NMDARs and PKA and that synaptic tagging and capture can also be observed with L-LTD (Kauderer and Kandel, 2000; Barco et al., 2002). Recent work from the Morris and Frey groups has also shown that the new protein synthesis that enables synaptic capture requires activation of NMDA and D1/D5 receptors (O'Carroll and Morris, 2004; Sajikumar and Frey, 2004). The availability of the proteins synthesized as a result of L-LTP induction at one input for capture by a second independent input further argues against synapse-specific localization of protein synthesis or mRNA targeting as the operative mechanisms. Rather, the induced translation products appear to be available to additional synapses within the same dendritic compartment, but the spatial and temporal limits on the availability of such protein products for capture have yet to be defined.

Recent work from Frey and colleagues has also provided evidence for synaptic tagging and capture in L-LTD (Sajikumar and Frey, 2004). Specifically, L-LTD induction at one input enables the capture of L-LTD at an independent input receiving either L-LTD-inducing stimulation in the presence of protein synthesis inhibition or stimulation that would normally induce only E-LTD. Interestingly, in a process that the authors term "cross-tagging," if an L-LTP stimulus is followed by an E-LTD stimulus, the E-LTD is converted to L-LTD, and conversely, if an L-LTD stimulus is followed by an E-LTP stimulus, the E-LTP is converted to L-LTP (Figure 3C and 3D) (Sajikumar and Frey, 2004). These observations surprisingly show that L-LTP and L-LTD can exhibit long-term heterosynaptic associativity. Thus, L-LTP and L-LTD appear to induce the synthesis of a set of proteins capable of supporting both processes, and it is the nature of the tag that differentiates between expression of

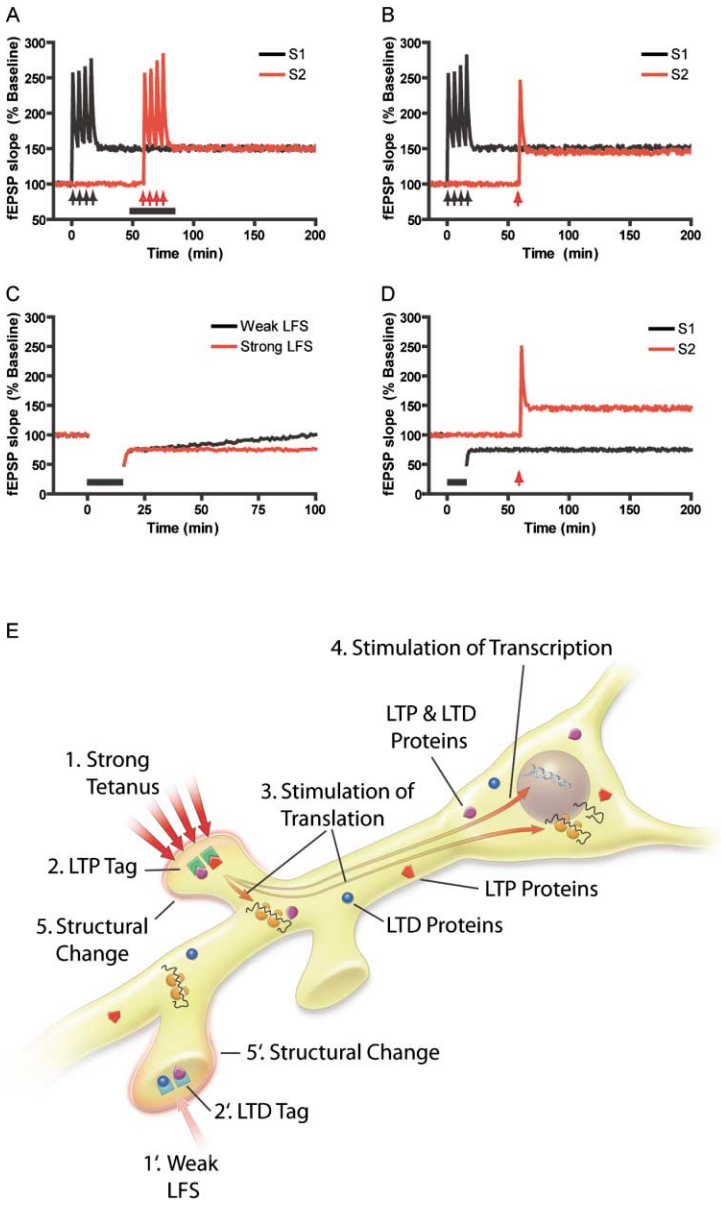

Figure 3. Evidence and Model for Synaptic Tagging and Capture

(A) When input 2 is stimulated with four tetanic trains in the presence of anisomycin, L-LTP is obtained if input 1 is stimulated in the absence of anisomycin in close temporal proximity to the tetanization at input 2. The bar indicates anisomycin treatment.

(B) If a single tetanic train is applied at one input in close temporal proximity to four tetanic trains applied at another input, E-LTP is converted to L-LTP.

(C) Weak low-frequency stimulation (W-LFS) induces transient E-LTD, whereas more persistent L-LTD is produced by strong low-frequency stimulation (S-LFS). L-LTD is dependent on protein synthesis and is converted to E-LTD if protein synthesis inhibitors are present at the time of the stimulation (not shown). The bar represents delivery of LFS.

(D) If a single tetanic train is delivered to input 2 in close temporal proximity to S-LFS delivered to input 1, E-LTP is converted to L-LTP. Conversely, E-LTD can be converted to L-LTD if the W-LFS stimulus is delivered in close proximity to four spaced tetani (not shown). The bar represents delivery of LFS.

(E) Model for conversion of E-LTD to L-LTD via synaptic capture. When four tetanic trains are delivered to a synapse (1), an LTP tag (2) is formed at the synapse (this tag may also be induced by a single tetanic train), and translation and transcription (3 and 4) are induced. The newly synthesized proteins can support expression of both L-LTP and L-LTD and are thus presumed to include products necessary for L-LTP, L-LTD, or both L-LTP and L-LTD ("LTP," "LTD," and "LTP and LTD proteins," respectively). These newly synthesized proteins are also available to other synapses, but only the synapse bearing the LTP tag captures the proteins necessary for L-LTP, causing that synapse to express L-LTP and the accompanying structural changes (5). If a second synapse then receives W-LFS (1′) in close temporal proximity to stimulation of the first synapse, transcription and translation are not induced, but an LTD tag is created (2′). This synapse then captures the proteins necessary for L-LTD (which were synthesized in response to L-LTP induction at the first synapse), resulting in structural changes (5′) and expression of L-LTD at the second synapse. A similar process also occurs when E-LTP is converted to L-LTP. Since the tag has a half-life of approximately 1–2 hr, the weak stimulation can occur either before or after the strong stimulation.

L-LTP and L-LTD at a particular input. Recent evidence suggests that L-LTP and L-LTD induction may upregulate translation via similar mechanisms (see below), providing a molecular basis for this associativity.

LTF in *Aplysia* also appears to be associated with the creation of a synaptic tag or "mark" that allows the capture of essential protein components (Martin et al., 1997). However, the situation in *Aplysia* is more complex with respect to the dependence of the synaptic tag on protein synthesis. Specifically, LTF captured in the presence of protein synthesis inhibition is expressed normally at 24 hr postinduction but is impaired at 72 hr postinduction (Casadio et al., 1999). Thus, synaptic capture of LTF appears to involve two distinct tags, a protein synthesis-independent tag required for capture at 24 hr (an "induction" tag) and a protein synthesis-dependent tag required for capture at 72 hr (a "maintenance" tag). In addition, capture at 72 hr depends upon branch-specific protein synthesis induced by a single serotonin application. These findings imply that a single serotonin application, which normally produces protein synthesis-independent STF, nevertheless induces branch-specific protein synthesis required for the formation of a maintenance tag, which in turn captures protein components necessary for stabilization of captured LTF between 24 and 72 hr following induction.

The Nature of the Tag

If the tagging hypothesis is correct, what is the nature of the tag? A number of different processes may contribute to the tag, and thus, the tag need not be a single

molecule; however, the experimental evidence dictates that the tag must satisfy a number of criteria: (1) the tag is induced in a protein synthesis-independent manner, (2) the tag possesses a finite lifetime of 1–2 hr, (3) the tag is induced both by E-LTP/E-LTD and by L-LTP/L-LTD, (4) the tag is induced in an input-specific and physically immobile manner, (5) the tag interacts with the proteins required for L-LTP/L-LTD to facilitate capture, and (6) distinct tags are created as a consequence of LTP and LTD induction.

A number of possible postsynaptic modifications have been enumerated as candidates for the synaptic tag (Frey and Morris, 1998; Martin and Kosik, 2002). The most straightforward of these possibilities is one or more specific phosphorylation or dephosphorylation events associated with the induction of LTP or LTD. For example, it is known that LTP requires the activation of various kinases, such as αCaMKII, while LTD requires the activation of various phosphatases, such as calcineurin (CaN). Another possibility could be a change in cytoskeletal dynamics. There is evidence that cytoskeletal changes occur during LTP and LTD; these changes, along with changes in the molecular motors that interact with the cytoskeleton, could form the mechanism behind the tag and capture process. Alterations in membrane receptor number, molecular architecture of the synapse, localized protein degradation, or conformational changes in particular molecules, among other possibilities, could also form the basis for the tag. The interaction of the tag with the protein(s) to be captured need not be complex; in the limit, the tag need only facilitate the capture of a single component whose function is rate limiting for the expression of L-LTP or L-LTD.

If the expression mechanism of L-LTP necessarily involves both synaptic tagging and capture, then the observations that agonists such as BDNF, Sp-cAMPS, and DHPG are sufficient to induce L-LTP may provide some clues to the nature of the tag. Although these forms of L-LTP are not input specific by nature and may bypass the requirement for formation of a synaptic tag, the mechanistic similarities between cAMP-induced and tetanus-induced L-LTP and between DHPG-induced and ppLFS-induced L-LTD (see above) suggest that similar synaptic modifications, such as the formation of a synaptic tag, may be involved. Consistent with this notion, synaptic tagging in L-LTP has been reported to require cAMP-dependent signaling (Barco et al., 2002). Some insight into the identity of the proteins that are required for synaptic capture is provided by the observation that constitutive activation of CRE-dependent gene expression results in the conversion of E-LTP to L-LTP (Barco et al., 2002), suggesting that the capture of one or more protein products of CRE-bearing genes is rate limiting for the expression of L-LTP.

Recent studies of the *Aplysia* homolog of the cytoplasmic polyadenylation element binding protein (ApCPEB) have led to a novel proposal for the nature of the synaptic tag that mediates capture of LTF. The CPEBs are a family of RNA binding proteins that regulate the polyadenylation and translation of a class of mRNAs bearing a specific recognition sequence in their 3′ UTRs (see below). Interestingly, ApCPEB was found to be capable of adopting a prion-like state in a yeast assay system, and this prion-like state was proposed to be the active species in mRNA binding and translational stimulation (Si et al., 2003b). In *Aplysia* neurons, branch-specific inhibition of ApCPEB expression impaired the maintenance of LTF at 72 hr (Si et al., 2003a). Based on these findings, the prion-like properties of ApCPEB (which have not yet been demonstrated in neurons) were hypothesized to enable the formation of a self-perpetuating and synapse-specific mark that mediates maintenance or stabilization of LTF through persistent translational activity at stimulated synapses. It is unclear, however, whether a persistent upregulation of translation would be necessary for LTF stabilization. Available evidence suggests that initial establishment of LTF or L-LTP requires a transient burst of enhanced protein synthesis that is essential for long-term synaptic modifications, whereas subsequent maintenance of these changes appears to be largely insensitive to translational inhibition. Once essential protein components have been captured by activated synapses, the protein composition of the modified synapses could in principle be maintained by basal or steady-state mechanisms for protein turnover. Recent work in *Aplysia* has shown that maintenance of LTF at 72 hr was blocked by application of protein synthesis inhibitor at 6 hr but not at 12 hr following induction, indicating that continuous upregulation of translation is not required for maintenance (Giustetto et al., 2003). Given that the prion state is extremely stable, the reversibility of plastic changes will have to be explained with an additional unknown mechanism.

From Synapse to Ribosome: Translational Regulatory Mechanisms in L-LTP and L-LTD

Until recently, little was known about the mechanisms that may couple synaptic activity to changes in translational efficiency, but several routes from the synapse to the translational machinery have now come to light. These mechanisms provide some insight into several important questions regarding the role of protein synthesis in long-term synaptic plasticity. For example, how do the protein synthesis requirements that are associated with long-lasting forms of both LTP and LTD allow for synaptic modifications of opposing strengths? Are long-lasting synaptic modifications enabled by the synthesis of a limited number of essential proteins, which may differ for LTP and LTD? What are the molecular mechanisms that govern translation in the contexts of LTP and LTD?

Broadly speaking, translational regulatory mechanisms fall into two categories: gene-specific mechanisms, which apply to the translation of a particular subset of mRNAs bearing a specific *cis*-acting sequence, and general mechanisms, which apply to translation of many or all mRNAs (Figure 4). Both gene-specific and general mechanisms are employed in mitotic cells for inducible translational responses to extracellular stimuli, and recent work indicates that neurons employ both classes of mechanisms in the context of long-term synaptic plasticity.

General Translational Mechanisms

Eukaryotic translation is primarily regulated at the level of initiation, and studies in mitotic cells have defined the key events that are involved in this process (Raught et al., 2000). Translation initiation factors orchestrate the rate-limiting step of ribosomal recruitment to the mRNA 5′ cap, which is present on all nuclear-tran-

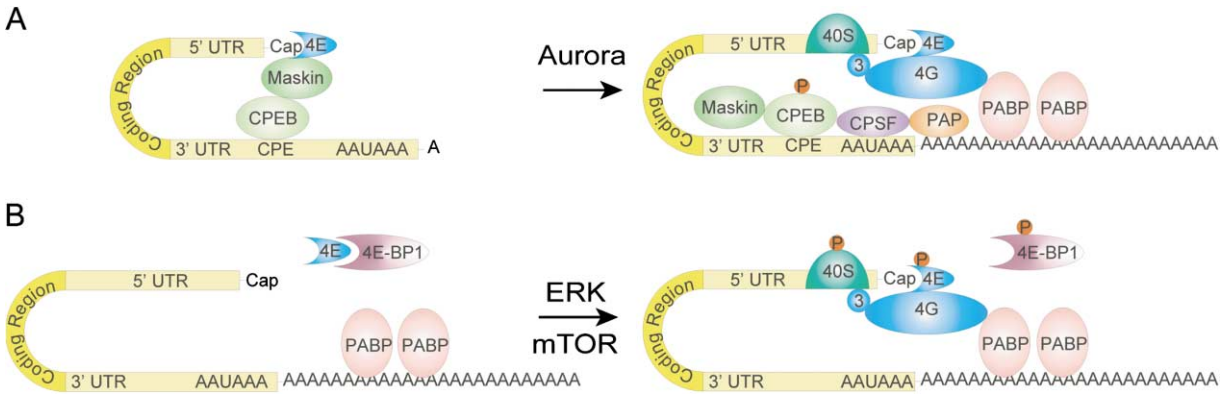

Figure 4. Molecular Mechanisms of Gene-Specific and General Translational Regulation

(A) Gene-specific control is governed by *cis*-acting sequences in specific mRNAs, here exemplified by the cytoplasmic polyadenylation element (CPE). CPEs in the distal 3′ UTRs of particular mRNAs (e.g., α*CaMKII*) are recognized by a specific binding protein, CPEB. CPEB phosphorylation by Aurora kinase in response to synaptic activity is proposed to result in polyadenylation, displacement of Maskin, and poly(A) binding protein (PABP)-mediated recruitment of eIF4G.

(B) General translational control is exerted primarily at the level of translation initiation. Recognition of the mRNA 5′ cap by eIF4E and subsequent recruitment of the 40S ribosomal subunit by eIF4G are key steps in this process. Phosphorylation of eIF4E and its inhibitor, 4E-BP1, regulates the activity and availability of eIF4E for cap recognition and interaction with eIF4G. Phosphorylation of eIF4E, 4E-BP1, and S6 in response to synaptic activity and L-LTP is mediated by the ERK and mTOR signaling pathways. See text for details and references.

scribed mRNAs. The mRNA cap is specifically recognized by the cap binding factor eIF4E, which then recruits the large ribosomal subunit through an interaction with eIF4G. The translational activity of eIF4E is regulated both through specific phosphorylation by the ERK-dependent kinases Mnk1/2 and through its association with inhibitory eIF4E binding proteins 4E-BP1/2 (Gingras et al., 1999). The 4E-BPs sequester eIF4E, preventing its phosphorylation and its association with eIF4G. The ability of the 4E-BPs themselves to interact with eIF4E is regulated by multiple ERK- and mTOR-dependent phosphorylation events (Gingras et al., 2001; Herbert et al., 2002). 4E-BP hyperphosphorylation causes eIF4E release, enabling cap recognition and ribosomal recruitment. General ("cap-dependent") regulation of mRNA translation is thus accomplished through modulation of the activity of the translation initiation factors eIF4E and 4E-BP1/2. Synthesis of the translation machinery itself is regulated by a *cis*-acting repressor sequence, termed a 5′ oligopyrimidine tract (5′TOP), which occurs adjacent to the 5′ cap in the mRNAs encoding ribosomal subunits and a number of translation factors (Meyuhas, 2000). 5′TOP-dependent translational repression thus represents a gene-specific control mechanism, but it is considered here due to its potential to increase general translational efficiency and capacity. Derepression of 5′TOP-dependent translation is highly associated with mTOR-dependent phosphorylation of ribosomal protein S6, but the precise mechanism mediating derepression is unclear.

Recent evidence has shown that neuronal activity-dependent modulation of translation initiation factor activity by the ERK MAPK signaling pathway plays an important role in the establishment of L-LTP. In hippocampal neurons, multiple forms of neuronal activity, including BDNF treatment, excitatory synaptic activity, and membrane depolarization, stimulate translational efficiency in association with increased phosphorylation of eIF4E, 4E-BP1/2, and rpS6 in an ERK-dependent manner (Kelleher et al., 2004). Importantly, the establishment of L-LTP has been shown to stimulate protein synthesis as well as phosphorylation of eIF4E, 4E-BP1/2, and rpS6, and all of these increases were inhibited in the hippocampus of transgenic mice expressing a dominant-negative ERK kinase, which exhibit selective defects in the translational component of L-LTP and memory consolidation (Kelleher et al., 2004). The relevance of these findings with L-LTP to long-term memory formation was supported by the demonstration of similar ERK-dependent changes in hippocampal eIF4E, 4E-BP1/2, and rpS6 phosphorylation occurring in response to contextual fear conditioning. In light of the established roles of eIF4E and 4E-BP1/2 phosphorylation in regulation of cap-dependent translation in mitotic cells, these findings suggest that L-LTP induction and long-term memory formation recruit a general enhancement of mRNA translation. Consistent with this view, electrophoretic analysis of metabolically pulse-labeled translation products following synaptic activation and L-LTP induction revealed a general increase in protein synthesis across the range of resolved molecular weights (Kelleher et al., 2004).

In addition, the observed increases in rpS6 phosphorylation suggest that L-LTP and long-term formation also stimulate 5′TOP-dependent translation, possibly allowing for increased synthesis of ribosomal components and translation factors. The combined effects of dual cap- and 5′TOP-dependent mechanisms may therefore allow for concerted increases in both translational efficiency and translational capacity during the establishment of long-lasting forms of synaptic plasticity and memory. Increases in ribosome number (Wenzel et al., 1993) or translocation of ribosomes within dendrites without a change in total number (Ostroff et al., 2002) have been reported in response to LTP induction, but the interpretation of these findings is complicated by the fact that the forms of LTP studied were not shown to be protein synthesis dependent.

The involvement of MAPK signaling in regulation of cap-dependent translation in response to neuronal activity is further supported by recent work showing that NMDA receptor activation leads to ERK-dependent phosphorylation of eIF4E in hippocampal area CA1 (Banco et al., 2004). Similarly, BDNF treatment of cultured neurons has been shown to produce ERK-dependent increases in protein synthesis and phosphorylation of eIF4E and 4E-BP1 (Takei et al., 2001). ERK-dependent mechanisms are likely to regulate general translational activity in the synaptodendritic compartment, as ERK-dependent stimulation of protein synthesis and phosphorylation of eIF4E and rpS6 has been observed in synaptoneurosome preparations (Banco et al., 2004; Kelleher et al., 2004), and eIF4E and 4E-BP1/2 have previously been shown to exhibit postsynaptic localization (Tang et al., 2002). Interestingly, *Aplysia* ITF and crayfish LTF, both of which require translation but not transcription, also require MAPK activation (Beaumont et al., 2001; Sharma et al., 2003), suggesting that similar mechanisms may transduce synaptic signals to the translational machinery in invertebrates and vertebrates.

Evidence for an important contribution of mTOR signaling to long-lasting synaptic plasticity has emerged from the findings that tetanus- and BDNF-induced CA1 L-LTP, as well as branch-specific LTF in *Aplysia*, are sensitive to the mTOR inhibitor rapamycin (Casadio et al., 1999; Tang et al., 2002). Rapamycin also inhibits neuronal activity-dependent protein synthesis and specific phosphorylation of eIF4E, 4E-BPs, and rpS6 in cultured hippocampal neurons, suggesting the ability of mTOR to regulate both cap-dependent and 5′TOP-dependent translation in response to synaptic activity (Kelleher et al., 2004). BDNF treatment of cultured neurons has also been shown to elicit mTOR-dependent increases in global protein synthesis, polysomal association of a large group of mRNAs, and the phosphorylation of eIF4E, 4E-BP1, and S6 kinase (Takei et al., 2001; Schratt et al., 2004). Consistent with the demonstrated involvement of cAMP-dependent signaling in protein synthesis-dependent LTP, forskolin treatment induces eIF4E phosphorylation, and NMDA-induced eIF4E phosphorylation requires PKA activity (Banco et al., 2004).

Recent work on mGluR-dependent L-LTD has suggested that similar translational mechanisms may be at play in both long-lasting LTP and LTD. mGluR-dependent L-LTD has recently been shown to be dependent on both ERK and mTOR signaling (Gallagher et al., 2004; Hou and Klann, 2004). Moreover, mGluR activation is associated with enhanced ERK-dependent phosphorylation of eIF4E and 4E-BP1/2 (E. Klann, personal communication). Therefore, it seems likely that mGluR-dependent L-LTD and L-LTP recruit enhanced synthesis of essential protein components at least in part through similar mechanisms, specifically a general stimulation of cap-dependent translation.

Translation may also be globally regulated at the level of elongation (Proud, 2000). Though polypeptide chain elongation does not generally represent a rate-limiting step in translation, alterations in elongation rate have been observed under some conditions, including changes in the levels of nutrients, cAMP, or cytoplasmic calcium. Inducible phosphorylation of eEF2, a GTP/GDP binding factor that mediates ribosomal translocation, is associated with inhibition of elongation rates. In addition, the levels of eEF2 and another elongation factor, eEF1A, appear to be regulated by 5′TOP elements in the cognate mRNAs. Interestingly, brief NMDAR activation at developing synapses induces a biphasic temporal modulation of the global translation rate, with a rapid depression of the translation rate occurring in association with eEF2 phosphorylation, followed by a more prolonged increase in translation rate (Scheetz et al., 2000). The synthesis rate of αCaMKII was enhanced despite the transient decline in the global translation rate, leading to the proposal that neuronal activity-dependent regulation of elongation may promote the translation of a subset of dendritic mRNAs.

Gene-Specific Translation Mechanisms

Sequences in the UTRs of mRNAs have been found to function as *cis*-acting control elements for post-transcriptional regulation of gene expression in a variety of systems (Macdonald, 2001). In neurons, the 3′ UTR of the *αCaMKII* transcript has been proposed to regulate its translation in response to neuronal activity, and recent evidence suggests that cytoplasmic polyadenylation may mediate this effect (Mayford et al., 1996; Wu et al., 1998; Wells et al., 2000). Inducible cytoplasmic polyadenylation of maternal mRNAs in oocytes is governed by U-rich sequences, termed cytoplasmic polyadenylation elements (CPEs), located in the distal 3′ UTR near the conserved hexamer sequence (AAUAAA) required for polyadenylation (Mendez and Richter, 2001). Polyadenylation is associated with enhanced rates of mRNA translation, possibly by promoting the formation of a circular translation initiation complex that facilitates reinitiation. CPEs are recognized by a sequence-specific RNA binding protein, CPEB, whose activity is regulated in oocytes by progesterone-induced phosphorylation. In the unphosphorylated state, CPEB recruits a translational repressor termed Maskin, which blocks access of eIF4G and the large ribosomal subunit to the eIF4E-mRNA cap complex. CPEB phosphorylation causes Maskin dissociation and recruitment of a multiprotein complex that catalyzes polyadenylation; these two events jointly stimulate mRNA translation. Thus, CPEB functions as both a translational repressor and activator, such that CPE-containing mRNAs may experience large relative increases in translational efficiency as a result of CPEB phosphorylation.

The distal *αCaMKII* 3′ UTR contains a pair of consensus CPE sequences, which have been implicated in neuronal activity-dependent polyadenylation (Wu et al., 1998; Richter and Lorenz, 2002). NMDA receptor activation induces CPEB phosphorylation by Aurora kinase and polyadenylation of endogenous *αCaMKII* mRNA in synaptosomes, as well as enhanced expression of a GFP reporter construct bearing the *αCaMKII* 3′ UTR in cultured neurons (Wells et al., 2001; Huang et al., 2002). Other studies have demonstrated increases in endogenous *αCaMKII* translation in the synaptodendritic compartment, but the mechanism(s) responsible for these increases has not been established (Ouyang et al., 1999; Bagni et al., 2000).

A second gene-specific mechanism for regulation of neuronal translation involves the fragile X mental retardation protein (FMRP), whose function is altered in fragile X syndrome (Antar and Bassell, 2003; Jin and Warren, 2003). FMRP, an RNA binding protein, is abundant in

brain and associates with polysomes in the cell body and dendrites of neurons, suggesting a role in translational regulation. Two distinct mechanisms for mRNA recognition have been proposed for FMRP, the first involving direct mRNA binding mediated by G quartet structures in target mRNAs (Darnell et al., 2001), and the second involving indirect binding to target mRNAs mediated by the noncoding dendritic RNA *BC1* (Zalfa et al., 2003). The *BC1*-dependent association of FMRP with several dendritic mRNAs, including α*CaMKII* and *Arc*, appears to result in their translational repression. Recent work suggests a broader role for FMRP in translational repression mediated by microRNAs through the RNA interference machinery (Carthew, 2002; Jin et al., 2004). Microarray-based analysis of FMRP-associated mRNAs led to the identification of 432 distinct mRNAs, at least half of which exhibited abnormal polysome profiles in fragile X cells (Brown et al., 2001). Interestingly, mGluR-dependent L-LTD is enhanced in FMRP null mice, suggesting that the mRNAs regulated by FMRP may encode proteins that facilitate this form of L-LTD (Huber et al., 2002; Bear et al., 2004).

Implications of Translational Regulatory Mechanisms in Long-Lasting Synaptic Plasticity

The Diversity of Protein Synthesis Accompanying L-LTP and L-LTD

As described above, recent work suggests that long-lasting forms of LTP and LTD elicit a global upregulation of protein synthesis through stimulation of the general translational machinery. These findings imply unexpectedly that L-LTP and L-LTD are accompanied by increased synthesis of a relatively large number of neuronal proteins. Stimulation of cap-dependent translation has the potential to enhance the translation of all nuclear-encoded mRNAs, but the degree of stimulation for individual mRNAs will vary, depending on factors such as mRNA abundance, localization, secondary structure, and sequence features surrounding the translation start site (Kozak, 1999; Dever, 2002). The pattern of proteins ultimately synthesized in response to synaptic activation will additionally depend upon the superimposed effects of gene-specific regulatory mechanisms. In general, *cis*-acting mRNA elements function as translational repressors, and cellular stimulation elicits a derepression of these effects. In the cases of CPEB and FMRP, neuronal activity appears to result in increased translation of target mRNAs (Richter and Lorenz, 2002; Bear et al., 2004), which may number in the several hundreds (Brown et al., 2001; Okazaki et al., 2002). Furthermore, individual mRNAs may be subject to coordinate regulation by multiple gene-specific mechanisms, resulting in complex translational responses to synaptic activity. This may be the case with the α*CaMKII* mRNA, which appears subject to both CPEB- and FMRP-dependent regulation (Wu et al., 1998; Zalfa et al., 2003). In response to NMDAR activation, rates of αCaMKII synthesis exhibit a complex pattern of temporal modulation that does not parallel the induced changes in the global translational rate (Scheetz et al., 2000), possibly reflecting the interplay of multiple translational mechanisms. Thus, it is likely that synthesis of a diverse group of neuronal proteins is enhanced during L-LTP and L-LTD, with the patterns and magnitudes of the increases being determined for individual proteins by the combined effects of general and gene-specific translational regulation, as well as the constraints imposed by patterns of mRNA localization and mRNA abundance.

Further evidence for a diverse pattern of translational induction in response to neuronal activity comes from a recent study of dynamic changes in polysome profiles in cultured neurons (Schratt et al., 2004). Microarray-based analysis revealed redistribution of $\sim$5% of the expressed mRNAs to the translationally active polysome pool upon BDNF treatment, including mRNAs encoding proteins implicated in synaptic function, such as αCaMKII, NMDAR subunits, and Homer. These findings establish a lower limit on the population of mRNAs capable of undergoing a neuronal activity-induced transition from a translationally inactive to translationally active state. Similar analysis in other systems has suggested that as many as 10%–15% of expressed mRNAs may exhibit such a transition in response to cellular stimulation (Pradet-Balade et al., 2001). Moreover, the population of polysomal mRNAs already actively engaged in translation generally undergoes an increase in translational rate in response to stimulation, as indicated by increased ribosome loading within the polysome fraction. These findings are consistent with a number of recent studies documenting a global enhancement of protein synthesis and activation of translation initiation mechanisms in response to several types of neuronal stimulation, including BDNF treatment, NMDAR activation, and L-LTP induction (Takei et al., 2001; Banco et al., 2004; Kelleher et al., 2004; Schratt et al., 2004). The relative contributions of general and gene-specific mechanisms to such broad patterns of translational recruitment have yet to be delineated.

While activation of general translation initiation mechanisms appears to occur uniformly in response to multiple forms of neuronal activity and L-LTP induction (Kelleher et al., 2004), gene-specific mechanisms may respond differentially to such stimuli. For example, NMDA receptor activation evokes specific phosphorylation of CPEB, but mGluR stimulation is ineffective (Huang et al., 2002). Conversely, mGluR activation may exert a preferential effect relative to NMDAR activation in relief of FMRP-mediated translational repression (Bear et al., 2004). Thus, the concerted effects of general and gene-specific regulatory mechanisms are likely to yield upregulation of the synthesis of a diverse set of proteins, but differential responses of translational regulatory mechanisms to induction of L-LTP and L-LTD may result in differential synthesis of a minority of proteins. Nevertheless, the ability of proteins that are induced by either L-LTP or L-LTD to support the opposing process argues for the synthesis of a common set of proteins sufficient for both L-LTP and L-LTD (see below).

Translational Upregulation and the Associativity of L-LTP and L-LTD

Although the notion that long-lasting synaptic plasticity is based on the enhanced synthesis of a relatively large and diverse group of neuronal proteins may at first glance seem counterintuitive, it does in fact make sense, because the synaptic growth and remodeling that are associated with long-lasting synaptic plasticity would require synthesis of the entire protein composition of the dendritic spine and postsynaptic terminal, as well as the presynaptic terminal. In addition, persistent synaptic

modifications may depend upon increased production of the machinery involved in a variety of neuronal functions, such as protein synthesis and posttranslational modifications, protein trafficking and transport, cytoskeletal processes, protein degradation and turnover, energy metabolism, and ionic homeostasis. Gene-specific translational mechanisms may not be sufficient to provide the necessary protein diversity, and therefore, concomitant activation of general translational mechanisms may be required.

With respect to their protein synthesis requirements, the distinction between L-LTP and L-LTD must lie either in the synthesis of a limited number of critical proteins that differ between the two processes or in the creation of distinct synaptic tags that allow recruitment of distinct subsets of proteins from (nearly) identical mixtures. In other words, either the set of newly synthesized proteins that enable capture must be different or the synaptic tags must be different. In addition, if either process induces the synthesis of a subset of proteins sufficient to enable the other process, then they will exhibit temporal associativity. In the broadest case, if L-LTP and L-LTD induce the synthesis of mutually overlapping sets of neuronal proteins, then the two processes should exhibit reciprocal synaptic capture. As described above, experimental evidence for reciprocal capture and heterosynaptic associativity of hippocampal L-LTP and L-LTD has recently been obtained (Sajikumar and Frey, 2004). Since this initial demonstration of "cross-tagging" involved NMDAR-dependent forms of L-LTD and L-LTP, an important question is whether cross-tagging applies generally to L-LTP and L-LTD, independent of the underlying induction mechanisms. For example, it remains to be seen whether mGluR-dependent (ppLFS-induced) L-LTD exhibits such reciprocal associativity with L-LTP. Nevertheless, the phenomenon of cross-tagging between L-LTP and L-LTD implies that it is the tags and not the essential proteins synthesized that differ between the two processes.

Such long-term heterosynaptic effects may have computational value for the neuron by enhancing the persistence of temporally coincident synaptic modifications of both kinds (i.e., both LTP and LTD). New protein synthesis in response to L-LTP or L-LTD may thus create a "plasticity window" signaling that the neuron is participating in a learning event, with the result that changes in synaptic strength occurring during this window are augmented in their strength and duration. The process of synaptic capture may therefore contribute to efficient long-term memory representation by promoting the stabilization of bidirectional modifications of synaptic strength within a local network.

Future Directions

The observations and interpretations summarized above have provided compelling evidence for a crucial contribution of translational control to long-term synaptic plasticity and have begun to illustrate the mechanisms that couple synaptic activity to changes in translational efficiency. In addition, these findings have afforded some insight into the synapse specificity and associativity of protein synthesis-dependent synaptic processes, but several important issues remain.

Perhaps the most significant outstanding questions are the nature of the synaptic tag(s) and the identities of the minimal set of proteins required for synaptic capture and the expression of L-LTP and L-LTD. Notwithstanding the arguments presented herein, it also remains to be demonstrated that LTP and LTD are associated with distinct synaptic tags and, by inference, that the essential set of proteins captured by these tags are also distinct. How do the proteins that are captured enable persistent strengthening or weakening of synaptic strength or the accompanying structural changes of synapse growth or synapse loss? Answers to these questions will depend upon identification of the protein components that participate in these processes, which will likely provide clues to the molecular mechanisms involved. As a starting point, it will be important to define the pool of newly synthesized proteins available to activated synapses during the establishment of L-LTP and L-LTD; this goal will be facilitated by fuller definition of the mRNAs that are localized to the dendritic compartment under various conditions, the spectrum of mRNAs that are subject to neuronal activity-dependent translational control by specific regulatory proteins, such as CPEB and FMRP, and the population of mRNAs whose translation is stimulated by specific forms of neuronal activity. In addition, available evidence suggests that proteins derived from newly synthesized mRNAs may contribute primarily to the maintenance phase of L-LTP. Thus, it will also be necessary to characterize this population of mRNAs and the manner in which their protein products facilitate maintenance of some, but not all, forms of long-term plasticity.

Another intriguing question is the potential role(s) of neuronal activity-dependent translation in processes other than long-lasting synaptic plasticity. Translational changes have in some cases been observed in response to stimulation procedures that would not be expected to induce protein synthesis-dependent forms of synaptic plasticity. Might there be a dose-dependent gradation of translational upregulation in response to synaptic activation that only reaches a sufficient threshold in long-term synaptic plasticity? Or, alternatively, might such translation contribute to homeostatic functions or basal protein turnover? Interestingly, recent data have demonstrated that chronic activity blockade enhances dendritic protein synthesis (Ju et al., 2004; Sutton et al., 2004). In addition, prolonged membrane depolarization causes a mild global repression of protein synthesis but a selective redistribution of somatodendritic mRNAs encoding αCaMKII, trkB, and NMDAR1 from translationally inactive RNA granules to polysomes, possibly also reflecting translational responses to sustained changes in activity level (Krichevsky and Kosik, 2001). These observations suggest that neuronal activity-dependent modulation of dendritic translation may also play a role in homeostatic processes or synaptic scaling (Turrigiano and Nelson, 2004).

An important unresolved problem is the spatial extent of the distribution or availability of newly synthesized proteins, which will in turn determine the spatial limits on synaptic capture and the associativity of protein synthesis-dependent processes. Improved imaging technologies should make it possible to define these spatial limits through direct time-lapse visualization of newly synthesized proteins at high resolution. For reasons outlined above, it is unlikely that the availability of newly

synthesized proteins will be restricted to individual synapses, but some degree of spatial restriction within the dendritic compartment may be both practical and advantageous. Recent evidence suggests that dendritic branches, and not synapses, are the minimal computational unit of the neuron (Liu, 2004; Polsky et al., 2004). New protein synthesis accompanying the induction of L-LTP or L-LTD, followed by protein diffusion or transport, might produce an increase in essential proteins throughout the local dendritic branch, with synaptic tags being used to capture these proteins into specific synapses within the branch. This distribution of essential proteins, whose supply may be limiting, would dictate associative and competitive interactions among nearby synapses, allowing the dendritic branch to serve as the computational unit for protein synthesis-dependent processes.

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