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Protein synthesis in the dendrite

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In neurons, many proteins that are involved in the transduction of synaptic activity and the expression of neural plasticity are specifically localized at synapses. How these proteins are targeted is not clearly understood. One mechanism is synaptic protein synthesis. According to this idea, messenger RNA (mRNA) translation from the polyribosomes that are observed at the synaptic regions provides a local source of synaptic proteins. Although an increasing number of mRNA species has been detected in the dendrite, information about the synaptic synthesis of specific proteins in a physiological context is still limited. The physiological function of synaptic synthesis of specific proteins in synaptogenesis and neural plasticity expression remains to be shown. Experiments aimed at understanding the mechanisms and functions f synaptic protein synthesis might provide important information about the molecular nature of neural plasticity.

Keywords: neuron; dendrite; synapse; messenger RNA; protein synthesis; synaptic plasticity

1. INTRODUCTION

Many dendritic proteins are specifically localized at synapses. The specific complement of proteins present at a synapse determines its structure and function. Synaptic proteins are a diverse group that includes receptors, as well as structural and signalling molecules (Harris & Kater 1994). In the spine—a postsynaptic structure that protrudes from dendritic shafts—these proteins form a highly ordered structure (e.g. the postsynaptic density) that detects and transduces information. How are dendritic proteins, in particular synaptic proteins, deposited? The classical view assumes that these proteins are first synthesized in the soma and then transported to specific synaptic regions. An alternative view is that some dendritic proteins are synthesized in the dendrite, close to the synaptic site. These two hypotheses are not mutually exclusive; both proposed mechanisms probably have roles in dendritic protein targeting. In this review, we focus on aspects of dendritic protein synthesis. New protein synthesis in dendrites, especially at synaptic sites, might allow neurons to maintain their structural and functional diversity and plasticity.

2. EVIDENCE FOR DENDRITIC PROTEIN SYNTHESIS

The possibility of dendritic protein synthesis was first suggested by the observations of messenger RNAs (mRNAs) and polyribosomes in dendrites (Bodian 1965; Steward & Levy 1982). In hippocampal neurons, several mRNAs are selectively delivered to dendritic processes (Steward 1997). For example, the mRNA for MAP2 (Garner *et al.* 1988), αCaMKII (Burgin *et al.* 1990) and several other proteins has been detected by *in situ* hybridization dendrites of the rat hippocampus (Steward 1997). Results from differential display experiments carried out on isolated, single neurites indicated that other mRNA species in dendrites might be abundant (Miyashiro *et al.* 1994). Using poly(dT) as an *in situ* hybridization probe, poly(A) mRNAs were detected in dendrites, with concentrated labelling at the base of dendritic spines. Sometimes, mRNA labelling was also observed near the synapses that terminate on dendritic shafts (Martone *et al.* 1996).

The presence of polyribosomes beneath postsynaptic sites in the neurons of the central nervous system (CNS) was shown by electron microscopy (EM) studies (Bodian 1965; Palacios-Pru *et al.* 1981; Steward & Levy 1982). Polyribosomes have been observed in more than 75% of visual cortical spines (Spacek 1985). In hippocampal CA1 and dentate gyrus neurons, polyribosomes are selectively located at the base of spines and less frequently in spine heads (Steward & Levy 1982). This distribution pattern resembles that of mRNAs (Martone *et al.* 1996). Further EM studies revealed that about half of the polyribosomes present beneath the base of spines were associated with membranous cisterns, whereas the polyribosomes in the head of spines were rarely associated with cisterns (Steward & Reeves 1988). A spectrum of translational machinery components, including ribosomal proteins, translational initiation and elongation factors, and transfer RNAs (tRNAs) were identified in dendritic compartments (Tiedge & Brosius 1996). Using molecular markers, the presence of endoplasmic reticulum, Golgi apparatus and translocation machinery has also been shown (Gardiol *et al.* 1999; Pierce *et al.* 2000). Together, these observations indicate that neuronal dendrites are equipped with

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machinery for both translation and post-translational modifications.

Several approaches have been used to show the competence of dendritic compartments for protein synthesis. In one early study, radiolabelled leucine was injected into the left lateral ventricle and the distribution of silver grains in the hippocampus was examined by light and electron microscopy. Silver grains were observed in dendrites, mainly in proximal compartments, although some grains also appeared in more distal compartments (Kiss 1977). An obvious limitation of this approach is that a potential somatic source of the silver grains in dendrites cannot be ruled out. To overcome this limitation, later experiments were carried out on dendrites that were dissociated from their cell bodies in culture. Torre & Steward (1992) examined the incorporation of ^{3}H-leucine in dissociated dendrites after pulse labelling for 30 min and found that MAP2-positive dendritic processes were heavily labelled, as shown by autoradiography. Consistent with these observations, Crino & Eberwine (1996) showed that transfection of isolated neurites with an *in vitro* transcribed mRNA led to synthesis of the corresponding polypeptides. More recently, Aakalu *et al.* (2001) showed that a dentritically targeted mRNA for green fluorescent protein (GFP) could be translated following treatment with a growth factor. Moreover, time-lapse measurements of GFP fluorescence indicated that there were apparent dendritic hotspots for translation that were persistent over time. These data indicate that dendrites are competent to translate mRNAs into proteins. To assess directly the competence of the synaptic subcellular compartments in protein translation, Wedege *et al.* (1977) showed that synaptosomes—a preparation that contains resealed pre- and postsynaptic entities—are able to synthesize proteins. Subsequently, by analysing the proteins that were synthesized in synaptosomes, Rao & Steward (1991) suggested that protein constituents of postsynaptic membrane specializations were locally synthesized at synaptic sites. Other experiments showed K^{+}-induced depolarization or that the addition of glutamate was able to stimulate protein synthesis in synaptoneurosomes (Weiler & Greenough 1991, 1993). One concern about the results from experiments with synaptosome preparations (or synaptoneurosome preparations used in other experiments discussed in this review) is the purity of the preparations, as previous studies have indicated glial contaminations in such preparations (Henn *et al.* 1976).

There are a limited number of studies that identify the specific proteins synthesized at synaptic sites. One such protein is fragile X mental retardation protein (FMRP). FMRP mRNA is present in synaptoneurosomes and is rapidly associated with polyribosomes after metabotropic glutamate receptor (mGluR1) stimulation. Furthermore, stimulation of mGluRs leads to increased expression of FMRP (Weiler *et al.* 1997). αCaMKII kinase is another protein that is potentially synthesized at the synaptic sites. Induction of long-term potentiation (LTP) in hippocampal slices leads to a rapid increase of αCaMKII in the dendritic region. The rapid increase in dendritic αCaMKII argues against a somatic transport mechanism, favouring a dendritic location for synthesis (Ouyang *et al.* 1997, 1999).

3. REGULATION OF DENDRITIC PROTEIN SYNTHESIS

In eukaryotic cells, several mechanisms have been described to regulate translation. These include the control of mRNA stability, post-transcriptional modification of mRNAs, translation initiation and elongation. Recent experiments have indicated that such mechanisms are also probably involved in the modulation of protein synthesis in dendrites.

(a) *Regulation of dendritic RNA targeting*

The selective appearance of specific mRNAs rather than all RNA species in dendrites indicates that dendritic RNA distribution might involve an active transport mechanism rather than simple diffusion. The targeting of specific mRNAs into dendrites often requires *cis*-elements in mRNAs. For example, the 3′ untranslated region (UTR) of *αCaMKII* mRNA is required for the dendritic localization of this mRNA (Mayford *et al.* 1996). Importantly, in dendrites, the expression level of the β-galactosidase (*lacZ*) reporter gene that was fused to *αCaMKII* 3′ UTR was considerably higher than the same reporter that lacks the *αCaMKII* 3′ UTR. This observation is consistent with the idea that at least some of the LacZ reporter protein that is present in dendrites is synthesized in them, rather than synthesized in the soma and then transported. 3′ UTRs are also required for the dendritic targeting of *MAP2* mRNAs (Blichenberg *et al.* 1999). However, the *cis*-acting dendritic localization signals of *BC1* mRNA resides in the 5′ region (Muslimov *et al.* 1997). Recently, two *cis*-elements have been identified in *αCaMKII* 3′ UTR. One of them is a 30-nucleotide sequence that is essential for dendritic targeting of the green fluorescent protein reporter mRNA (Mori *et al.* 2000). Interestingly, a homologous sequence was found in the 3′ UTR of neurogranin mRNA, which is also present in dendrites (Landry *et al.* 1994). Furthermore, in this *cis*-element, there is a 10-nucleotide sequence that shows very strong homology to the RNA transport signal in the myelin basic protein (*MBP*) 3′ UTR that is required for transport of the *MBP* mRNA into oligodendrocytic processes (Mori *et al.* 2000). The conservation of *cis*-elements in different neuronal and oligodendrocytic mRNAs indicates the existence of common mechanisms for RNA transport. However, sequences that are homologous to this particular *cis*-element are not found in many other dendritic mRNAs including *MAP2* and *Arc* (activity-regulated cytoskeleton) (Mori *et al.* 2000). The second *cis*-element identified in *αCaMKII* 3′ UTR has a negative role in regulating dendritic RNA targeting (Mori *et al.* 2000). However, its inhibitory effect on the dendritic targeting of mRNAs can be overridden by neuronal depolarization (Mori *et al.* 2000). These findings indicate that dendritic targeting of *αCaMKII* mRNA might be coordinated by activity-sensitive functional interactions between these two *cis*-elements.

RNAs are transported into dendrites in RNA–protein complexes, which probably contain components of translational machinery and *trans*-acting factors that are responsible for RNA transportation (Knowles *et al.* 1996). Protein synthesis is not required for dendritic RNA transport, but might have a role in restricting particular mRNAs to the soma (Kleiman *et al.* 1993). Identification of the minimal *cis*-elements sufficient for dendritic RNA

targeting should facilitate the characterization of the *trans*-acting factors that are involved in dendritic RNA transport. Several proteins that are involved in RNA targeting and asymmetrical distribution have been described in *Drosophila melanogaster* and *Xenopus laevis*. One such molecule is Staufen, a protein that is required for the asymmetrical distribution of specific mRNAs in *Drosophila* oocytes and neuroblasts. Recently, several mammalian *Staufen*-related genes have been identified (Kiebler *et al.* 1999; Marion *et al.* 1999; Tang *et al.* 2001; Wickham *et al.* 1999). At least two of them are present in hippocampal neurons. Immunostaining experiments indicate somatodendritic distribution patterns of the Staufen protein in hippocampal neurons, with higher signals in the proximal dendrites compared with the distal dendrites. The Staufen distribution pattern overlaps with that of dendritic RNA in cultured neurons (Tang *et al.* 2002). In addition, Staufen protein particles that contain RNA move along microtubules in the dendrites (Kohrmann *et al.* 1999; Tang *et al.* 2002). *Staufen* binds many mRNAs, including the mRNA for *MAP2* (Monshausen *et al.* 2001). Overexpression of wild-type *Staufen* in cultured hippocampal neurons enhances dendritic RNA transport, whereas the expression of a truncated form of *Staufen* inhibits this process. These findings suggest that the *Staufen* protein is necessary and sufficient for dendritic RNA targeting (Tang *et al.* 2002).

Neuronal activity might affect dendritic RNA targeting as indicated by various studies. For example, K^+ depolarization of hippocampal neurons has been shown to enhance the targeting of brain derived neurotrophic factor (*BDNF* and *TrkB*) mRNAs into dendrites (Tongiorgi *et al.* 1997). In addition, in the dentate gyrus, electrical stimulation of a selected population of afferents led to increased dendritic targeting of *Arc* mRNA (Steward *et al.* 1998), an immediate-early gene induced by neuronal activity (Lyford *et al.* 1995; Link *et al.* 1995). The application of the neurotrophic factor NT3 can also promote the movement of RNA particles into dendrites (Knowles & Kosik 1997). The mechanisms by which neuronal activity or neurotrophic factors can initiate or enhance dendritic RNA transportation remain unknown. As indicated from studies on other eukaryotic cells, the availability of dendritic RNA for translation might also be affected by RNA stability and RNA release from RNA–protein particles (Theodorakis & Cleveland 1996). There is, however, little information available about these areas of regulation in neurons. Neuronal activity might affect dendritic RNA targeting.

(b) *Polyadenylation of mRNA*

Post-transcriptional modification has important roles in the translational regulation of eukaryotic mRNAs. These include the creation of a 5′ cap structure and polyadenylation. It is known that the presence of a poly(A) tail can increase mRNA stability. Recently, an additional role for the poly(A) tail in translation regulation was shown. Increases in the length of the poly(A) tail were found to coincide with translational activation of several mRNAs (e.g. *tPA* mRNA), and shortening of the poly(A) tail coincided with translational suppression of these mRNAs (Stebbins-Boaz & Richter 1997). Activation of translation by the poly(A) tail might involve an interaction of poly(A) binding protein(s) with the translation initiation complex assembled at the 5′ end of the mRNA (Jacobson 1996). In *Xenopus* oocytes, polyadenylation-activated translation requires the cytoplasmic polyadenylation element (CPE) *cis*-element and the CPE-binding protein (CPEB) *trans*-factor (De Moor & Richter 1997). Recently, CPEB was found at synaptic sites and in the postsynaptic density (Wu *et al.* 1998). Interestingly, *αCaMKII* mRNA, which is present in dendrites, contains two CPE-like elements. These CPE elements can bind CPEB *in vitro*, and can mediate polyadenylation-induced translation when injected into *Xenopus* oocytes (Wu *et al.* 1998). In the intact brain, visual experience can not only induce *αCaMKII* mRNA polyadenylation, but also activate its translation, indicating a possible physiological role for the αCaMKII CPEs (Wu *et al.* 1998). It would be interesting to examine whether the visual-activity-induced *αCaMKII* mRNA translation is a direct consequence of its polyadenylation and whether polyadenylation-related mechanisms are also involved in the translation control of other mRNAs present in dendrites.

(c) *Translation initiation*

Translation initiation is a rate-limiting step in protein synthesis for most mRNAs; initiation is possibly subject to tight control as indicated by many studies carried out in mammalian cells (Mathews *et al.* 1996). The first step of translation initiation is to assemble the initiation complex on the cap structure at the 5′ end of the mRNA. The translation initiation factor eIF-4E is the cap binding protein on which other proteins bind directly or indirectly to form the initiation complex (Sonenberg & Gingras 1998). Phosphorylation, as well as interaction with other proteins, regulates the function of eIF-4E. When associated with 4E-binding proteins (4E-BPs), eIF-4E cannot interact with eIF-4G, and thus the formation of the initiation complex is inhibited. The phosphorylation of 4E-BPs is stimulated by a rapamycin-sensitive kinase mTOR (mammalian target of rapamycin; FRAP; RAFT1), resulting in their dissociation from eIF-4E and the subsquent activation of translation (Beretta *et al.* 1996; Von Manteuffel *et al.* 1996; Gingras *et al.* 1998).

Recent studies have shed light on the potential role of the rapamycin-sensitive translational signalling pathway in dendritic protein synthesis. Immunostaining experiments showed that several components of this pathway, such as eIF-4E, 4E-BP1, 4E-BP2, mTOR and p70S6K, are present in hippocampal dendrites, with eIF-4E, 4E-BP1 and 4E-BP2 enriched at the postsynaptic sites (Tang *et al.* 2002). The presence of these translational regulatory factors in the postsynaptic regions indicates that the dendrites might be capable of initiating new protein synthesis and argues against the possibility that dendritic protein synthesis simply represents the continuation of translation that is initiated in soma and undergoes transport to dendrites. Consistent with a role of this pathway in protein synthesis during LTP expression, rapamycin application leads to specific defects in the expression of the late phase of LTP induced by tetanic stimulation, as well as deficits in BDNF-induced synaptic potentiation (Tang *et al.* 2001*b*). Recent work in *Aplysia* has also shown that rapamycin can partially block protein synthesis in isolated sensory cell neurites (Casadio *et al.* 1999).

The function of eIF-4E might be regulated by other molecules in addition to 4E-BPs, as indicated by the recent identification of a protein called Maskin from *Xenopus* oocytes (Stebbins-Boaz *et al.* 1999). Maskin is a CPEB-associated protein and also binds to eIF-4E. The interaction of eIF-4E, Maskin and CPEB is thought to bring the 5′ end of the associated mRNA close to the 3′ end, which results in a circular conformation of the mRNA. The association of Maskin with eIF-4E blocks the interaction between eIF-4E and eIF-4G, and this inhibits translation initiation. During oocyte maturation, Maskin is partially dissociated from eIF-4E and probably derepresses translation initiation from previously masked mRNAs (Stebbins-Boaz *et al.* 1999). In contrast to 4E-BPs, Maskin is probably involved in the selective translational regulation of mRNAs that contain CPE elements. It is unknown whether Maskin-mediated mechanisms are involved in the regulation of dendritic protein synthesis.

(d) *Translation elongation*

Translation elongation at synapses might be tightly modulated by synaptic activity. Recent studies showed that the eukaryotic translation elongation factor 2 (eEF2) is phosphorylated in response to *N*-methyl-D-aspartate (NMDA) receptor activation and visual activity in the intact tadpole tecta; phosphorylation of eEF2 occurs at the subsynaptic regions (Scheetz *et al.* 1998). In eukaryotic cells, phosphorylation of eEF2 is associated with reduced protein synthesis, probably by halting ribosomal translocation (Narin & Palfrey 1996). Indeed, in synaptoneurosome preparations, activation of NMDA receptors leads to rapid phosphorylation of eEF2, which is coincident with a decrease in total protein synthesis (Scheetz *et al.* 2000). However, soon after the rapid eEF2 phosphorylation, the phospho-eEF2 levels start to decrease and return to baseline 15 min after the NMDA receptor activation; consistent with the above story, the dynamics of the decrease in phospho-eEF2 is paralleled by an increase in total protein, as well as αCaMKII synthesis (Scheetz *et al.* 2000).

(e) *Synaptic activity and dendritic protein synthesis*

Several studies have shown that synaptic activity can regulate protein synthesis in hippocampal neurons. For example, the induction of LTP at Schaffer collateral-CA1 synapses induced the synthesis of secretory proteins (Duffy *et al.* 1981; Lauri *et al.* 1996). In addition, low-frequency (10 Hz) stimulation of hippocampal slices treated with the cholinergic agonist carbachol can induce new protein synthesis in dendrites (Feig & Lipton 1993). In synaptosome preparations, glutamate treatment leads to polyribosome formation and translation activation (Weiler & Greenough 1993; Bagni *et al.* 2000). Recently, Ouyang *et al.* (1999) reported that LTP induction led to the increase of αCaMKII protein in the dendrites of stimulated hippocampal CA1 pyramidal neurons. This increase in αCaMKII levels occurred within 5 min of LTP induction. The rapid increase of *α*CaMKII protein in activated dendrites is consistent with the hypothesis that synaptic activity triggers local protein synthesis from the dendritically localized *αCaMKII* mRNA. Steward & Halpain (1999) reported that prolonged (1–2 h) high-frequency stimulation of the perforant path projections to the dentate gyrus also resulted in increased *αCaMKII* protein in the activated dendritic domain and increased MAP2 protein in the regions that flank the activated dendritic domain. However, inhibition of protein synthesis diminished only the increase in MAP2, not αCaMKII protein in the dendritic domains (Steward & Halpain 1999), indicating a protein-synthesis-independent mechanism for the observed increase in αCaMKII protein in the activated dendrites. Signals that are mediated by NMDA mGluRs are probably involved in triggering regulated local protein synthesis after synaptic activation (Raymond *et al.* 2000; Scheetz *et al.* 2000). Protein synthesis regulated by synaptic activity has been reported in the sensory neuron processes of *Aplysia*, and local application of protein synthesis inhibitors at a particular synapse can block long-term facilitation (LTF) at this synapse, but not at the other synapses on the same cells (Martin *et al.* 1997).

Synaptic activity might regulate dendritic protein synthesis at several levels, including mRNA targeting, translation initiation and elongation. Recent studies showed that the dendritic distribution of *Arc* mRNA is strictly controlled by synaptic activity. Steward *et al.* (1998) examined the distribution of *Arc* mRNA and protein after synaptic stimulation of the dentate gyrus of the hippocampus, and found that strong stimulation induced Arc transcription and led to dendritic transport of the *Arc* mRNA. Furthermore, after repetitive stimulation of a subset of synapses, they found that newly transcribed *Arc* mRNA was specifically enriched in the activated dendrites, but not in the non-activated dendrites, of the same neuron. Arc protein in dendrites also accumulated after stimulation (Steward *et al.* 1998). The coincidence of *Arc* mRNA targeting and Arc protein appearance in activated dendrites indicates the dendritic synthesis of Arc protein by local translational machinery. It remains to be examined if synaptic activity also has a stimulatory role in the initiation of translation of *Arc* mRNA after its arrival at activated dendritic domains.

Synaptic activity might also regulate dendritic protein synthesis by modulating the activity of specific signalling molecules that reside at the synaptic regions and modulate translation. Angenstein *et al.* (1998) identified a group of kinases associated with polyribosomes prepared from cerebral cortical synaptoneurosomes, including p90rsk-1, p90rsk-2, gsk-3β, ERK-2 and p70S6K. Interestingly, stimulation of metabotropic glutamate receptors in hippocampal slices induced a rapid translocation of p90rsk to polyribosomes (Angenstein *et al.* 1998). It was proposed that polyribosome-associated p90rsk might be phosphorylated and activated by its upstream regulator ERK-2 (Zhao *et al.* 1996); activated p90rsk might then phosphorylate its substrate gsk-3β and inhibit its kinase activity (Sutherland *et al.* 1993). One of the gsk-3β substrates is the translation initiation factor eIF-2B, the phosphorylation of which can decrease translation (Welsh *et al.* 1997). Therefore, according to this scenario, activation of p90rsk after its translocation to polyribosomes would lead to an increase in translation. Although attractive, the operation of this signalling pathway in synaptic protein synthesis remains to be shown. Other studies have indicated a possible inhibitory role of synaptic activity on translation. In adult frog tecta, activation of NMDA receptors

and visual activity led to activation of calcium/calmodulin-dependent EF2 kinase, resulting in the phosphorylation of eEF2 at subsynaptic sites (Scheetz *et al.* 1998). It is known that phosphorylation of eEF2 arrests translation elongation. These findings raise the possibility that synaptic activity might both stimulate and inhibit translation at the same synaptic site. One possibility is that synaptic activity halts the ongoing basal level translation and re-directs protein synthesis to a new set of mRNAs that encode proteins involved in activity-induced structural changes at synapses. In support of this hypothesis, Scheetz *et al.* (2000) have reported that, in synaptoneurosome preparations, the increase of phosphorylation of eEF2 immediately after NMDA receptor activation is coincident with the decrease of total protein synthesis and the increase of αCaMKII synthesis.

Synaptic activity might evoke and orchestrate a set of biochemical pathways that lead to protein synthesis at synaptic sites. However, we do not know whether these processes can selectively activate translation of a specific mRNA at a synaptic site. It is possible, for example, that translation of different mRNAs might be differentially controlled at the same synaptic site. For instance, synaptic activity might initiate and direct the targeting of one specific mRNA to the activated synapse and therefore make it available for subsequent translation; activity might also result in polyadenylation and thus activate the translation of another mRNA that resides in the activated synaptic site.

There are many important questions that remain to be answered about synaptic-activity-regulated local protein synthesis. For example, it is not known whether the products of dendritic translation are destined exclusively for the parent synapse, although hot spots for translation have been identified (Aakalu *et al.* 2001). In addition, whether synaptic activity at one synapse can activate protein synthesis at neighbouring synapses is not known. There is, at present, little information available as to how synaptic activity influences the different pathways that lead to mRNA targeting, modification and translation initiation. Unravelling these signalling cascades will significantly contribute to our understanding of the molecular basis of synaptic plasticity.

4. LOCAL PROTEIN SYNTHESIS DURING SYNAPTIC PLASTICITY AND SYNAPTOGENESIS

It is known that the maintenance of long-lasting synaptic changes, such as LTP, requires new protein synthesis (Stanton & Sarvey 1984; Montarolo *et al.* 1986; Frey *et al.* 1988). According to the classical view, activated synapses send signals to the cell body that result in the translation of certain proteins that are then targeted to the activated synapse(s). Indeed, recent studies have indicated that synaptic activity might generate a tag that serves as an address for protein targeting (Frey & Morris 1997, 1998). Conversely, several lines of evidence indicate that the expression of long-term plasticity can make use of local protein synthesis that is independent of the soma. Kang & Schuman (1996) addressed this possibility using hippocampal slices with microlesions introduced to separate dendrites from their cell bodies in the CA1 region. Similar to intact slices, BDNF induced long-lasting synaptic potentiation in the CA3–CA1 pathway after the microlesion. Importantly, protein synthesis inhibitors blocked this BDNF-induced potentiation in the lesioned slice. These results indicate that there might be protein synthesis events that occur in the transected dendrites that are essential for the expression of BDNF-induced plasticity. Recently, Raymond *et al.* (2000) reported that activation of metabotropic glutamate receptors contributes to LTP expression by triggering new protein synthesis. Interestingly, the newly synthesized proteins promote LTP stability in an input-specific manner. The homosynaptic restriction of the function of newly synthesized proteins is consistent with the idea that at least some of the new proteins that are essential for LTP expression are synthesized at the activated synapses (Raymond *et al.* 2000). Huber *et al.* (2000) recently reported that LTD induced by mGluR stimulation, in CA1 stratum radiatum severed from cell body layers, could be blocked by translational inhibitors, indicating a requirement for local protein synthesis for the expression of this form of LTD.

In the sensory–motor culture system of *Aplysia*, application of serotonin—a neurotransmitter that is released by interneurons during sensitization of the gill-withdrawal reflex—induces LTF that can last for days. The expression of LTF is protein synthesis dependent (Montarolo *et al.* 1986). Martin *et al.* (1997) reported that local application of protein synthesis inhibitors to a specific synapse could block the expression of serotonin-induced LTF in a synapse-specific manner. Results of experiments using microinjection of a membrane-impermeant protein synthesis inhibitor indicated that synaptic protein synthesis might have occurred in the processes of sensory neurons (presynaptic) rather than that of motor neurons (postsynaptic) (Martin *et al.* 1997). Sherff & Carew (1999) described a form of LTF, induced by coordinated serotonin stimulations on cell bodies and remote synapses. Expression of this particular form of LTF requires new protein synthesis at activated synapses immediately after stimulation and delayed protein synthesis in the soma. In interpreting the results of the above experiments, it is important to keep in mind that translation inhibitors probably shut down all protein synthesis, including basal level translation. Thus, the blocking effect of these inhibitors on the expression of long-term plasticity might be due to not only preventing the protein synthesis induced by synaptic stimulations, but also blocking the basal translation of proteins that could be essential for the structural maintenance of dendrites and synapses. In the neuromuscular junctions (NMJs) of *Drosophila* larvae, protein synthesis at the subsynaptic region of muscle cells has been suggested to have a role in the expression of long-lasting plasticity in the junction. Sigrist *et al.* (2000) provided evidence that polyribosomes and translational machinery are present in the subsynaptic reticulum of the muscles of *Drosophila* larvae, and that genetic manipulation of the formation of subsynaptic translational aggregates leads to changes of not only the level of synaptic proteins, but also the morphology and efficacy of the junction.

At present, we know little about the identities of the proteins that are synthesized at synaptic sites and that contribute to long-term plasticity. Advances in this area might require the development of new approaches that can follow and show newly synthesized proteins. However,

mRNAs that are detected in dendrites using *in situ* hybridization techniques have provided some basic information about the proteins that are synthesized at the synaptic regions. For example, *αCaMKII* mRNA is present in dendrites and its translation seems to be rapidly activated in dendritic domains, as well as in the soma after LTP induction (Ouyang *et al.* 1997, 1999). αCaMKII is known to have a vital role during the expression of long-term plasticity and long-term memory (Silva *et al.* 1992*a*,*b*). Despite these strongly suggestive correlations, the requirement of local synthesis of αCaMKII for LTP expression remains to be shown. Synthesis of some other plasticity-related proteins is also known to respond to LTP induction. For example, increases in protein kinase Mζ—the constitutively active catalytic subunit of protein kinase C (PKCζ)—have been detected as soon as 10 min after tetanic stimulation. Translation inhibitors blocked this stimulation-induced synthesis (Osten *et al.* 1996). In addition, it was reported that LTP induction stimulated the synthesis of glutamate receptor subunits GluR1 and GluR2/3, in a cAMP-signalling-mediated, transcription-dependent manner (Nayak *et al.* 1998). It would be interesting to see if synthesis of these plasticity-related proteins after LTP induction involves local translation mechanisms. Several other mRNAs that encode plasticity-related proteins, such as NMDA receptor subunits, BDNF and TrkB, are detected in dendrites (Benson 1997; Gazzaley *et al.* 1997; Tongiorgi *et al.* 1997). It remains to be examined if synaptic stimulation can result in local translation from these dendritic mRNAs.

Expression of long-term plasticity might involve new synapse formation (Trommald *et al.* 1996; Engert & Bonhoeffer 1999; Toni *et al.* 1999). Studies have indicated that protein synthesis is required for synaptogenesis and maintenance of synapses that are already formed (Burry 1985). Although it is not known if local synthesis of synaptic components is involved in the synaptogenesis of central synapses, results of studies on neuromuscular junctions (NMJs) showed that, during NMJ differentiation, some synaptic components, such as acetylcholine receptors, are synthesized in the muscle cell beneath the postsynaptic membrane (Hall & Sanes 1993). By analogy, local protein synthesis might also have an active role during central synaptogenesis. Consistent with this idea, there are significant accumulations of polyribosomes in developing synapses, and the number of polyribosomes under synapses decreases with synapse maturation (Steward & Falk 1986). Furthermore, denervation lesion-induced synaptogenesis was also accompanied by a marked increase of polyribosomes under spines (Steward 1983). What are the specific proteins that are synthesized in the synaptic region and involved in synapse formation and/or differentiation? One of these proteins might be fragile mental retardation protein (FMRP). As discussed in the previous sections, FMRP is probably synthesized at synaptic sites. Genetic disruption of FMRP function in mice leads to immature morphology of dendritic spines, which are often longer and thinner than that of wild-type mice. Thus, it has been proposed that synaptic synthesis of FMRP might be involved in activity-induced synaptogenesis (Comery *et al.* 1997; Weiler & Greenough 1999).

5. CONCLUSIONS

Increasing evidence indicates that protein synthesis occurs in the dendrites of neurons, particularly at the postsynaptic sites. Local synthesis of specific proteins might provide a mechanism for rapid and region-specific structural changes after synaptic activation. To fully understand the physiological role of synaptic protein synthesis, several questions have to be addressed by future experiments. For example, what are the specific proteins that are synthesized at synapses in a physiological context? Although several proteins have been indicated to be synthesized at synaptic regions, it is likely that more dendritically synthesized protein will be characterized, given the increasing number of dendritic mRNAs reported. In addition, how is synaptic protein synthesis regulated during synaptogenesis and synaptic plasticity? As discussed in previous sections, synaptic protein synthesis might be regulated at several levels, including mRNA availability and post-transcriptional modification, as well as translation initiation and elongation. However, little information is available about the signalling cascades that lead to the activation/repression of these processes, and how these processes are modulated by synaptic activity. Last, what is the physiological consequence of specific proteins that are synthesized at synaptic regions during plasticity and synaptogenesis? Although synaptic activation has been reported to lead the synaptic synthesis of several proteins, such as αCaMKII and Arc, there is no direct evidence that shows the functional requirement of these locally synthesized proteins for the expression of plasticity. In summary, protein synthesis at synapses is becoming a focal point of interest in studies of synaptic plasticity, future experiments that address the above problems should provide significant insights into the molecular basis of long-term plasticity of the central synapses during learning and memory.

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