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Compartmentalized Synthesis and Degradation of Proteins in Neurons

Review

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An important aspect of gene expression in neurons involves the delivery of mRNAs to particular subcellular domains, where translation of the mRNAs is locally controlled. Local synthesis of protein within dendrites plays a key role in activity-dependent synaptic modifications. In growing axons, local synthesis in the growth cone is important for extension and guidance. Recent evidence also documents the existence of mechanisms permitting local protein degradation, providing bidirectional control of protein composition in local domains. Here, we summarize what is known about local synthesis and degradation of protein in dendrites and axons, highlighting key unresolved questions.

A cornerstone of our understanding of the neuron as a cell came from findings in the late 19th century that axons and dendrites grew out from the cell body, and depended on the cell body for their survival. These findings led Ramon y Cajal to conclude that the cell body was the "trophic center of the neuron." Later cell biological studies revealed that the machinery for macromolecular synthesis and posttranslational processing was present in neuronal cell bodies, and that there were selective axonal and dendritic transport mechanisms capable of delivering proteins anywhere within neurons. Together, these discoveries led to a central tenet of neuronal cell biology—that the protein building blocks of axons and dendrites were synthesized exclusively in the soma, and transported to their final destinations.

In the early 1980s, however, there was increasing evidence for an alternative view that certain proteins could be synthesized outside the neuronal cell body using mRNAs that were selectively positioned in particular cytoplasmic microdomains. A story line on dendritic protein synthesis was launched by the discovery that polyribosomes and associated membranous cisterns were selectively localized beneath postsynaptic sites on dendrites (Steward, 1983; Steward and Fass, 1983; Steward and Levy, 1982). The selectivity of the localization suggested that (1) synapse-associated polyribosomes synthesized key molecular constituents of the synapse, (2) local translation was regulated by signaling events at the synapse, and (3) local translation played a key role in synapse plasticity. Studies over the last 21 years have confirmed and extended these hypotheses (Steward and Schuman, 2001).

Running in parallel with the story of local protein synthesis in dendrites was a story line that developed in fits and starts regarding local protein synthesis in axons. There was consistent evidence for axonal protein synthesis in invertebrate systems (reviewed in Alvarez et al., 2000), but whether similar mechanisms existed in vertebrate axons remained controversial. Recent studies, however, have established the existence of protein synthetic machinery and mRNAs in growing vertebrate axons, especially growth cones, and demonstrated that this machinery and the local protein synthesis it allows plays a key role in growth cone function (for a review, see Steward, 2002).

A key advantage conferred by mechanisms that allow local protein synthesis is the ability to regulate protein composition in local domains on a moment-by-moment basis. If this is advantageous, it is obvious to ask whether there are also mechanisms that could mediate local protein degradation. The story here is less developed, but recent evidence indicates that such mechanisms do exist, and are important for neuronal function.

Here, we summarize what is known about the mechanisms for local protein synthesis and degradation, highlighting some of the key unresolved questions. We focus here on cell biological issues, and mention only briefly the role of local synthesis in synaptic plasticity, which has been considered in other recent reviews (Steward and Schuman, 2001).

Machinery for Local Translation in Dendrites

Structural features of organelles provide important clues about their function, and so we begin with a consideration of the features of synapse-associated polyribosome complexes (SPRCs). Electron microscopic analyses revealed that the majority of the polyribosomes in dendrites are selectively positioned beneath postsynaptic sites (Steward and Levy, 1982). At spine synapses, SPRCs are most often localized at the base of the spine in the small mound-like structures from which the neck of the spine emerges. At nonspine synapses (both excitatory and inhibitory), SPRCs are localized beneath the postsynaptic membrane specialization (Steward et al., 1996). In their location beneath the synapse, SPRCs are ideally situated to be influenced by ionic and/or chemical signals from the synapse as well as by events within the dendrite proper. An important implication of this selective localization is that there must be some mechanism that causes ribosomes, mRNA, and other components of the translational machinery to dock selectively in the postsynaptic cytoplasm. The details of the mechanisms underlying this selective localization remain to be established.

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Local Translation: Ubiquitous or Synapse Specific?

What proportion of synapses has underlying SPRCs? Estimates vary depending on the quantitative methods used and the cell type being evaluated. Reconstructions of dendrites in the dentate gyrus of adult rats reveal that about 25% of the spine synapses on mid-proximo-distal dendrites have underlying polyribosomes (Steward and Levy, 1982). The incidence was higher at synapses on proximal dendrites. Studies of ribosomes (not polyribosomes) in serially reconstructed spines revealed that most spines on pyramidal neurons in the cerebral cortex contained ribosomes, whereas the incidence of ribosomes was lower in spines on cerebellar Purkinje cells (Spacek and Hartmann, 1983). Thus, the prevalence of subsynaptic ribosomes varies by neuron type.

The incidence of polyribosomes also varies across development. During periods of maximal synaptogenesis, most synapses have underlying polyribosomes, and many synapses have multiple clusters, implying that local synthesis is especially important during periods of synapse growth (Steward and Falk, 1986).

The fact that polyribosomes are present at some, but not all synapses in mature animals raises the question of whether SPRCs can shuttle from one synapse to another, or whether the presence of the machinery marks synapses that are capable of, and in the process of, local translation. It is of interest in this regard that following synaptic stimulation leading to LTP in the CA1 region of the hippocampus, polyribosomes appear to translocate from the base of the spine into the spine head (Ostroff et al., 2002). These results document that polyribosome localization can be modified by signals generated at the synapse, but leave open whether polyribosomes can move from one synapse to another. Also unknown is whether a given polyribosome moves as a unit, or whether the individual ribosomes dissociate from the polyribosome, move, and then reinitiate on some other mRNA.

Definitive Evidence for Local Synthesis

To definitively establish that protein synthesis is indeed occurring in local compartments, be it dendrites or axons, the cell body must be ruled out as a protein synthesis source. Studies using synaptodendrosomes or neurosomes are inadequate in this regard because these are usually contaminated by fragments of cell bodies and glia. A capacity for local incorporation of amino acid precursors has been documented in physically isolated dendrites (Torre and Steward, 1992), and recent studies have shown that physically and optically isolated dendrites are capable of synthesizing GFP-tagged proteins (Aakalu et al., 2001; Job and Eberwine, 2001) and that dendritic synthesis was stimulated by BDNF. Interestingly, there were persistent hotspots for protein synthesis along the length of the dendrite that were located near ribosomes and synapses, lending support to the idea that dendritic sources of protein synthesis may subserve a small synaptic domain.

Presence of Elements of the ER and Golgi in Dendrites

Integral membrane proteins (receptors, for example) and proteins for release are synthesized by rough endoplasmic reticulum (RER), and are usually glycosylated. Thus, an important issue has been whether ribosomes are present on membranes in an RER-like configuration, and whether ER and Golgi enzymes are present that could mediate posttranslational modifications. Reconstructions of dendrites of dentate granule cells and hippocampal pyramidal cells indicate that about 50% of the polyribosomes beneath synapses are associated with tubular cisterns, suggesting that the SPRC/cisternal complex may be a form of RER (Steward and Reeves, 1988). Immunocytochemical studies have revealed the presence of different markers of the RER in dendrites of neurons in culture, including ribophorin I (Torre and Steward, 1996), the signal sequence receptor TRAPP, and the signal recognition particle (SRP), which directs nascent polypeptide chains to the RER (Tiedge and Brosius, 1996). Electron microscopic immunocytochemical studies indicate that the membranous cisterns that are present near spine synapses stain for Sec6lα protein complex, which is part of the machinery for translocation of proteins through the RER during their synthesis (Pierce et al., 2000). Moreover, dendrites that have been separated from their cell body incorporate sugars in a tunicamycin-sensitive fashion (Torre and Steward, 1996), and are capable of translating membrane protein-encoding mRNAs (glutamate receptors) and inserting these into the plasma membrane (Kacharmina et al., 2000). Together, these studies indicate that elements of RER and Golgi apparatus are present in dendrites in a configuration that can support synthesis, posttranslational processing, and membrane insertion of locally synthesized proteins.

In nonneuronal cells, secretory and membrane proteins are synthesized on RER, packaged into post-ER carriers (coated vesicles), and transported to a centrally located Golgi apparatus, where protein processing and sorting occurs. Live-cell imaging using a GFP-tagged temperature-sensitive mutant of vesicular stomatitis virus glycoprotein (VSVG), and ER and Golgi markers, has documented the presence of functional ER, post-ER carriers, Golgi elements, and post-Golgi trafficking in dendrites (Horton and Ehlers, 2003). VSVG-GFP moves from ER elements in dendrites into highly mobile tubero-vesicular structures, and the tubero-vesicular structures then move bidirectionally in dendrites, fusing with stationary structures that stained for Golgi markers (GM130). The accumulation of VSVG-GFP into tubero-vesicular structures occurred at defined immobile foci that were positive for Sec13, a marker of ER exit sites in other cells.

In the context of a consideration of local synthesis, it is noteworthy that VSVG-GFP-containing tubero-vesicular structures were transported bidirectionally for tens of micrometers. This raises a puzzling issue. Why go to the trouble of distributing protein synthetic machinery throughout dendrites if the proteins that are produced are rapidly transported to other locations? Perhaps VSVG-GFP is missing some critical address tag that is possessed by proteins that are locally synthesized that would target the proteins to the nearby synapse. Alternatively, perhaps our ideas about the purpose of local synthesis still need fine-tuning.

There is still uncertainty about the organelle responsible for Golgi-like activity in dendrites. Electron microscopic immunocytochemical studies indicate that certain Golgi marker proteins are present in cisterns in spines (Pierce et al., 2000). On the other hand, the Golgi elements identified by live-cell imaging are localized in

the dendritic shaft, and are not associated with synapses (Horton and Ehlers, 2003). In either case, the spine apparatus, which has a form that has invited speculation that it might be a mini-Golgi apparatus (Steward and Reeves, 1988), has not been implicated in Golgi function. Thus, some 50 years after its discovery, the function of the spine apparatus and the related cisternal apparatus at nonspine synapses remains a mystery.

What mRNAs Are Present in Dendrites and What Proteins Are Synthesized Locally at Synapses?

These related questions have been addressed using different but complementary approaches. Biochemical studies have focused on the protein products; molecular biological studies have focused on the mRNAs.

One approach has relied on subcellular fractionation techniques that allow the isolation of synaptosomes with attached fragments of dendrites that retain their cytoplasmic constituents, including polyribosomes and associated mRNAs. Subcellular fractions, termed synaptodendrosomes (Rao and Steward, 1991) or synaptoneurosomes (Weiler and Greenough, 1993; Weiler et al., 1997), have been used for biochemical studies of proteins that are locally synthesized, and for the isolation of mRNAs.

For biochemical approaches, the strategy was to incubate synaptodendrosomes with [^{35}S]methionine, and use subcellular fractionation techniques to prepare synaptic plasma membranes and fractions enriched in synaptic junctional complexes (the postsynaptic membrane specialization and associated membrane). Protein species were then characterized by polyacrylamide gel electrophoresis combined with fluorography and/or by immunoprecipitation of the labeled peptides. This strategy revealed that a number of proteins become labeled, indicating local synthesis (Leski and Steward, 1996; Rao and Steward, 1991). Some of the proteins have been identified, including the α subunit of calcium/calmodulin-dependent protein kinase II (CAMKII; Sheetz et al., 2000), Fragile X mental retardation protein (FMRP; Weiler et al., 1997), and Arc (Zalfa et al., 2003).

The other approach is to identify the mRNAs present in synaptodendrosomes or synaptoneurosomes. The problem, however, is that the fractions are often contaminated with fragments of neuronal and glial cell bodies. For example, high levels of the mRNA encoding glial fibrillary acidic protein are present (Chicurel et al., 1993; Rao and Steward, 1991). There have been continuing efforts to refine subcellular fractionation approaches so as to yield purer fractions. In this regard, one recent study reported a fractionation approach that yields synaptosomes in which GFAP mRNA is not detected by RT/PCR (Bagni et al., 2000), suggesting a lack of contamination by glial fragments. Previously identified dendritic mRNAs such as CAMKII, Arc, and an inositol 1,4,5-trisphosphate receptor (InsP3R1) were detected in the fraction, and also the mRNA for FMRP (more on this below). Thus, these fractions may represent a purer population of synaptodendrosomes than have been available previously, which could provide a means to identify novel dendritic mRNAs.

mRNAs in the Dendrites of Neurons In Vivo

Another approach to identifying mRNAs in dendrites has been to use in situ hybridization. In tissue sections, dendritic localization is inferred by the pattern of labeling in brain regions where there are distinct neuropil layers that contain dendrites but few neuronal cell bodies (cortical regions including the hippocampus and the cerebellar cortex). It is important to establish that the mRNA is in fact present in dendrites and not in glial cells, which can be done using nonisotopic in situ hybridization techniques.

Table 1 lists the mRNAs that show substantial dendritic localization in vivo (i.e., labeling that extends for several hundred μm from the cell body). The list includes mRNAs encoding cytoplasmic, cytoskeletal, integral membrane, and membrane-associated proteins that have quite different functions. The different mRNAs are expressed differentially by different neuron types, and exhibit somewhat different localization patterns within dendrites. Some of the mRNAs (the mRNA for calmodulin, for example) are present in dendrites during early development, and absent in mature neurons (Berry and Brown, 1996). The table does not include mRNAs that extend for only a few tens of micrometers into proximal dendrites (for example, mRNAs for the protein kinase C substrates GAP43 and RC3).

It is noteworthy that FMRP mRNA has not been detected in dendrites using in situ hybridization techniques (Hinds et al., 1993; Valentine et al., 2000), despite the fact that FMRP mRNA can be detected (using RT-PCR) in isolated synaptodendrosomes (Zalfa et al., 2003). It is possible that FMRP mRNA is not recognized by the probes that have been used, perhaps because of its close association with mRNA binding proteins (including FMRP itself). Nevertheless, the complete lack of evidence for dendritic labeling for FMRP mRNA remains a paradox.

In situ hybridization data indicate that several other mRNAs are present in the dendrites of young neurons developing in vitro, including mRNAs for BDNF and trkB receptors (Tongiorgi et al., 1997), the mRNA for a fatty-acylated membrane-bound protein called ligatin (Severt et al., 2000), and the mRNA for β-actin (Tiruchinapalli et al., 2003). The mRNAs are usually present in granules, and live-cell imaging has documented the movement of mRNA-containing granules within dendrites (Knowles et al., 1996). The extent of dendritic labeling and movement of mRNA-containing granules into dendrites has been shown to be enhanced by neurotrophin treatment (Knowles and Kosik, 1997) and depolarization by KCl (Tiruchinapalli et al., 2003), indicating that the distribution of mRNA in the dendrites of neurons in culture may be dependent in part on different growth substrates and signaling molecules.

Despite prominent dendritic labeling in cultured neurons, the mRNAs for BDNF and trkB receptors appear to be largely restricted to the region of the cell body in young neurons in vivo (Dugich et al., 1992). The same is true of ligatin (Perlin et al., 1993). These results raise the possibility that neurons developing in culture have a different complement of dendritic mRNAs than their counterparts in vivo.

In situ hybridization evidence suggests that only a small number of mRNAs are localized in dendrites, whereas the vast majority of mRNAs that have been evaluated are localized exclusively in neuronal cell bodies. A different approach, however, has provided evi-

Table 1. mRNAs that Have Been Shown to Be Localized within Dendrites of Mature Neurons In Vivo by In Situ Hybridization

mRNA	Cell Type	Localization in Dendrites	Class of Protein	Protein Function
MAP2[a]	Cortex, hippocampus, dentate gyrus	Proximal 1/3–1/2	Cytoskeletal	Microtubule-associated
CAMII kinase[b] α subunit	Cortex, hippocampus, dentate gyrus	Throughout	Membrane-associated NRC/psd protein	Multifunctional kinase Ca^{2+} signaling
Arc/Arg 3.1[c]	Cortex, hippocampus, dentate gyrus depending on inducing stimulus	Throughout (when induced)	Cytoskeleton-associated, activity-induced NRC/psd protein	Actin binding?
Dendrin[d]	Hippocampus, dentate gyrus, cerebral cortex	Throughout	Putative membrane	Unknown
G protein[e] γ subunit	Cortex, hippocampus, dentate gyrus, striatum	Throughout	Membrane-associated	Metabotropic receptor signaling
Calmodulin[f]	Cortex, hippocampus, Purkinje cells	Proximal-middle	Cytoplasm- and membrane-associated	Ca^{2+} signaling in conjunction with CAMII kinase
NMDAR1[g]	Dentate gyrus	Proximal-middle?	Integral membrane	Receptor
Glycine receptor[h] α subunit	Motoneurons	Proximal	Integral membrane	Receptor
Vasopressin[i]	Hypothalamo-hypophyseal	Proximal-middle	Soluble	Neuropeptide
Neurofilament protein 68[j]	Vestibular neurons	Proximal-middle	Cytoskeletal	Neurofilament
Neuralized[k]	Widespread	Throughout	Cytoplasmic	Transcription repressor
MRG15[l]	Widespread	Proximal 1/3–1/2	Chromodomain-containing	Nucleolar?
Shank[m] AKA SSTRIP	Widespread	Throughout	NRC/psd protein	Links InsP3 to GKAP/psd95
InsP3 receptor[n]	Purkinje cells	Throughout	Integral membrane (endoplasmic reticulum)	
L7[o]	Purkinje cells	Throughout	Cytoplasmic?	Homology to c-sis PDGF oncogene signaling?
PEP19[n]	Purkinje cells	Proximal 1/3	Cytoplasmic	Ca^{2+} binding

Not shown are mRNAs that are localized only in the most proximal segments.
[a] Garner et al., 1988.
[b] Burgin et al., 1990.
[c] Link et al., 1995; Lyford et al., 1995.
[d] Herb et al., 1997.
[e] Watson et al., 1994.
[f] Berry and Brown, 1996.
[g] Benson, 1997.
[h] Racca et al., 1997.
[i] Prakash et al., 1997.
[j] Paradies and Steward, 1997.
[k] Timmusk et al., 2002.
[l] Matsuoka et al., 2002.
[m] Zitzer et al., 1999.
[n] Furuichi et al., 1993.
[o] Bian et al., 1996.

dence for a large and heterogeneous complement of dendritic (or at least neuritic) mRNAs in neurons in culture. Miyashiro et al. (1994) used patch pipettes to aspirate the cytoplasmic contents of individual neurites of hippocampal neurons in culture and then used RNA amplification techniques and mRNA expression profiling. A large number of different mRNAs were detected, some of which have not been detected by in situ hybridization analyses. It is possible that the amplification techniques are capable of detecting mRNAs that are not accessible to probes in situ (the paradox of Fragile X comes to mind). Alternatively, the amplification techniques are extremely sensitive, and may detect mRNAs that are present at levels that are below the threshold for detection by in situ hybridization. If the latter interpretation holds, the issue arises of what level of mRNA is necessary to generate biologically significant amounts of protein.

Local Synthesis of Components of Multimolecular Structures

It is interesting that several dendritic mRNAs encode proteins that are components of a highly organized multimolecular structure specialized for postsynaptic signal transduction termed the NMDA receptor complex (NRC; Husi et al., 2000), including CAMKII, shank, InsP3 receptor, and Arc. The existence of the complex was inferred by identifying the proteins that coprecipitate with the NMDA receptor (Husi et al., 2000). A related study that used mass spectroscopy to identify protein constituents of the "core postsynaptic density" revealed a similar set of proteins (Walikonis et al., 2000), suggesting that the core psd may be a scaffold made up largely of the NRC. Thus, the postsynaptic density/NRC appears to be a highly organized multimolecular structure specialized for postsynaptic signal transduction (Sheng and Lee, 2000).

It is likely that proper signaling would require a precise stoichiometric relationship between the different molecules making up the NRC, raising the question of how such a complex is assembled. One possibility is that the complex is assembled away from the synapse, and inserted into the psd. Alternatively, local synthesis at synapses provides a mechanism that could allow the different molecular constituents of the NRC to be replaced by direct substitution into existing complexes at the postsynaptic density. In this regard, it is of interest that one of the components of the NRC (Arc) is expressed as an IEG, and disappears within hours after induction, whereas the other component proteins have much longer half-lives (Ehlers, 2003).

If constituents of the NRC are replaced individually, then ribosomes and other components of the translational machinery would have to be closely associated, perhaps embedded within the postsynaptic density as they synthesize molecules of the NRC. Direct electron microscopic visualization of ribosomes is problematic because of the electron-dense nature of the postsynaptic density, but studies using subcellular fractionation and EM immunocytochemical techniques have provided evidence for the localization of several components of the translational machinery and ribosomal protein in postsynaptic densities, which would be consistent with the presence of ribosomes in association with the density (Asaki et al., 2003).

It is also noteworthy that strong synaptic activation, which is associated with an increase in the synthesis of molecules that are assembled into the NRC (Arc and CAMKII), causes a translocation of ribosomes from the spine base out into the spine head, where they would be closer to the postsynaptic density itself (Ostroff et al., 2002). Moreover, a more recent study reveals that a similar stimulation paradigm caused a 3-fold increase in the levels of CAMKII mRNA in isolated synaptodendrosomes without any change in overall CAMKII mRNA levels, suggesting translocation of preexisting CAMKII mRNA from the shaft of the dendrite out into the spine head (Havik et al., 2003). The translocation of polyribosomes and mRNAs encoding components of the NRC into the spine cytoplasm would mean newly synthesized proteins would emerge from the translational apparatus very close to the psd.

Dendritic Transport and Synaptic Targeting of mRNA

We consider here only in passing two important subjects that have been considered in previous reviews—how mRNAs are delivered into dendrites and how they are targeted to synaptic sites (Steward and Worley, 2001; Tiedge et al., 1999).

The fact that some mRNAs are abundant in dendrites and undetectable in the axons of the same neurons indicates that there is a selective sorting mechanism. In principle, mRNA could either be excluded from the axon by some mechanism, or there could be dendrite-specific transport mechanisms. Granules containing mRNA are actively transported in dendrites (Knowles et al., 1996), although it remains unclear what features distinguish the transport machinery in dendrites from the machinery in mature axons. In terms of selection for transport, the available evidence suggests that *cis*-acting elements (usually in the 3′UTR) act as "zip codes" for delivery into dendrites (Tiedge et al., 1999). This has been demonstrated convincingly for CAMKII in an experiment in which a mutant mouse was produced in which most of the 3′UTR of α-CAMKII mRNA was replaced by the 3′UTR of bovine growth hormone (Miller et al., 2002). In situ hybridization analyses revealed that CAMKII mRNA lacking the 3′UTR remained in the cell body, and biochemical analyses revealed that CAMKII protein levels in the postsynaptic density were reduced. Physiological and behavioral studies revealed subtle but significant deficits in late-phase LTP and memory in the mutant animals, suggesting that local synthesis of CAMKII protein is important for these processes. It cannot be excluded, however, that local synthesis is required to maintain high levels of α-CAMKII protein in the psd, which in turn is important for the signal transduction events that are critical for late-phase LTP. In this case, local synthesis itself would not play a direct role in bringing about the late-phase modifications, but instead would create a signal transduction-competent synapse (Steward, 2002).

Regulation of mRNA Translation at Synapses

The selective localization of ribosomes at synapses invites the speculation that signals generated by synaptic activity may regulate translation of mRNAs in dendrites. There is a bit of a puzzle, however. Although synapses on proximal dendrites may have multiple clusters of polyribosomes, most synapses on middle and distal dendrites have only one to two polyribosome clusters (Steward, 1983). If each cluster is associated with an individual mRNA, this means that one to two mRNAs are being translated at any given time. Given that there are a number of different mRNAs present in dendrites (even with the more conservative list of mRNAs that are evident by in situ hybridization), there must be competition for initiation and translation. How this is orchestrated is just now being investigated, and it appears that the story will be complex.

Studies of the local translation of CAMKII mRNA have suggested that local synthesis is regulated via NMDA receptor activation. In two studies, it was shown that high-frequency stimulation designed to induce LTP (which activates NMDA receptors) led to a rapid increase

in immunostaining for CAMKII within dendrites. One study involved hippocampal neurons in slices (Ouyang et al., 1999); the other involved dentate granule neurons in vivo (Steward and Halpain, 1999). The increases in immunostaining were blocked by NMDA receptor antagonists (Steward and Halpain, 1999), implying a role for NMDA receptors in translational activation. One puzzle was that increases in immunostaining were blocked by protein synthesis inhibitors applied to hippocampal slices (Ouyang et al., 1999), but not when inhibitors were delivered in vivo (Steward and Halpain, 1999).

Subsequent studies of CAMKII synthesis in isolated synaptodendrosomes provided clarification on the issue of sensitivity to protein synthesis inhibitors, and also on the mechanisms of translational activation (Sheetz et al., 2000). CAMKII synthesis in synaptodendrosomes (as measured by incorporation of [^{35}S]methionine into CAMKII protein) was enhanced by NMDA receptor activation. Interestingly, there was also increased phosphorylation of the initiation factor eIF2, which would be expected to decrease the rate of polypeptide elongation. This apparent paradox can be explained by the fact that decreases in elongation rate favor the translation of weakly initiated mRNAs, and CAMKII is one of the mRNAs for which initiation is inefficient. Consequently, decreases in elongation following eIF2 phosphorylation could lead to increases in CAMKII synthesis. In support of this idea, Sheetz et al. showed that low to moderate concentrations of cycloheximide (which inhibit elongation) increased incorporation into CAMKII at the same time that overall levels of protein synthesis were diminished. These results may explain why protein synthesis inhibitors failed to block the increases in immunostaining after synaptic activation in vivo (Steward and Halpain, 1999).

Other recent studies have revealed another mechanism for regulating the translation of CAMKII mRNA at synapses involving a cytoplasmic polyadenylation element (CPE) that increases poly(A) tail length, promoting translation initiation (Wu et al., 1998). CPEs regulate the translation of maternal mRNAs in oocytes by repressing translation until fertilization. At fertilization, CPEs are activated, triggering cytoplasmic polyadenylation of mRNAs with the appropriate CPE element. CAMKII mRNA contains a consensus sequence for the binding of CPE in its 3′ untranslated region (UTR). Wu et al. (1998) demonstrated that NMDA receptor activation triggered polyadenylation of the mRNA for CAMII kinase, which in turn was associated with an increased synthesis of CAMKII protein. Interestingly, this mechanism could be triggered by behavioral experience (the first light exposure for animals that had been raised in the dark). Subsequent immunocytochemical studies revealed that a number of factors known to control polyadenylation-induced translation in oocytes were present in dendrites, and regulated by NMDA receptor-dependent signal transduction pathways (Huang et al., 2002). Moreover, glutamate stimulation of neurons in culture triggered translation of reporter constructs that contained intact CPEs (Wells et al., 2001). Together, these results document the presence of a mechanism capable of regulating local translation of CAMKII mRNA at synapses in response to signals generated by synaptic activation.

Whereas the evidence to date indicates that translation of CAMKII is regulated via NMDA receptor activation, translation of other mRNAs may be regulated in other ways. Local synthesis of FMRP appears to be regulated by mGluR activation; for example, treatment of synaptoneurosomes with agonists for metabotropic glutamate receptors causes a rapid increase in the amount of FMRP detectable by Western blot (Weiler et al., 1997). The translation of other dendritic mRNAs appears to be insensitive to neurotransmitter activation. For example, using another measure of translation (association of mRNAs with polysomes), Bagni et al. (2000) confirmed that glutamate application or depolarization recruited CAMKII mRNA to polyribosomes, but did not recruit mRNAs for InsP3R1 or Arc.

Another recent study reports, however, that Arc mRNA translation in synaptosomes is strikingly upregulated by exogenous recombinant reelin acting through integrin receptors (Dong et al., 2003). This induction was blocked by echistatin (which blocks integrin receptors) and by rapamycin, implying that translation is regulated through the rapamycin-sensitive kinase mammalian target of rapamycin (mTOR, also known as FRAP kinase and RAFT-1). Previous studies had shown that several putative components of this translational signaling pathway, including mTOR, 4E-BP1, 4E-BP-2, and eIF-4E, are present in dendrites (Tang et al., 1998), and that rapamycin blocks several forms of protein synthesis-dependent synaptic plasticity (Steward and Schuman, 2001; Tang et al., 1998). It remains to be seen how integrin receptor-mediated signals and signals generated by neurotransmitters are integrated by the translational machinery at synapses.

Adding still further to the complexity is the evidence that several dendritically localized mRNAs have internal ribosome entry sites (IRESs), including CAMKII, Arc, dendrin, MAP2, and RC3, and that these mRNAs can be translated in a cap-dependent or cap-independent fashion (Pinkstaff et al., 2001). Interestingly, studies of dicistronic constructs with two different reporters revealed that IRES-mediated translation was relatively more efficient in dendrites (Pinkstaff et al., 2001), and studies using bicistronic constructs in *Aplysia* neurons have revealed that egg-laying hormone, which triggers a bout of intense activity, causes a switch from cap-dependent to cap-independent translation (Dyer et al., 2003). It remains to be seen whether IRES-mediated translation is regulated by synaptic activation in mammalian neurons or by other signals impinging on dendrites.

The Fragile X/BC1 Connection

Still another mechanism for controlling translation at synapses involves Fragile X mental retardation protein (FMRP) and a pol-3 RNA transcript called BC1. Fragile X mental retardation syndrome is caused by a mutation in the gene encoding FMRP (usually an expanded trinucleotide repeat that is hypermethylated, inhibiting gene transcription). FMRP is an RNA binding protein, and EM immunocytochemical studies revealed that FMR protein is concentrated around SPRCs (Feng et al., 1997). On this basis, Feng et al. proposed that FMRP might be involved in targeting mRNAs to dendrites or regulating their translation. Studies of FMRP knockout mice revealed that there were no gross abnormalities in the dendritic localization of representative dendritic mRNAs (MAP2, CAMKII, and Arc), but this study did

not exclude the possibility of subtle deficits in mRNA targeting (Steward et al., 1998).

Subsequent studies sought to define the mRNAs bound by FMRP. One identified a "G quartet" domain that appears to be one motif that mediates binding of mRNA to FMRP, but none of the principle "dendritic" mRNAs (Table 1) have this domain (Darnell et al., 2001). Another study identified a different set of mRNAs that interact with FMRP (Miyashiro et al., 2003), some of which are localized in proximal dendrites, but again none of the mRNAs that are abundant in dendrites turned up.

A new twist to the story has come from a very recent study that indicates that FMRP acts as a repressor of translation of several of the principle dendritic mRNAs including CAMKII and Arc, as well as β-actin and FXR2 (a protein related to FMRP) (Zalfa et al., 2003). This study also showed that FMRP interacts with the regulated mRNAs via a noncoding pol-3 transcript called BC1, which has previously been shown to be localized in dendrites (Tiedge et al., 1991). Interestingly, BC1 contains sequences that are predicted to base pair with sequences in MAP1B, CAMKII, and Arc mRNAs. These results suggest that BC1 may link particular mRNAs to FMRP, leading to repression of translation. This ties in nicely with other work implicating BC1 as a regulator of translation initiation of dendritic mRNAs (Wang et al., 2002). These findings have led to the interesting idea that the loss of FMRP in Fragile X mental retardation syndrome could lead to a dysregulation of mRNA translation at the synapse, disrupting synaptic function (Zalfa et al., 2003).

As is evident, the story regarding translational regulation is rapidly evolving, and the final answer is likely to be complex. Translation of certain mRNAs appears to be regulated by signals generated by particular neurotransmitter receptors as well as other signals, and the translation of different mRNAs appears to be controlled in different ways. It remains to be seen whether different control mechanisms exist at different types of synapses.

Protein Synthesis in Axons

The idea for local protein synthesis in axons has been controversial until recently (for a review, see Giuditta et al., 2002). It is now well accepted, however, that mRNAs and translational machinery are present in the neurites of invertebrates, which have the characteristics of both axons and dendrites (van Minnen and Syed, 2001). There is abundant evidence that local synthesis in invertebrate neurites is critical for several different forms of activity-dependent synaptic plasticity (Martin et al., 1997; Sutton et al., 2001). It is not yet clear whether the critical protein synthesis-dependent events are on the presynaptic or postsynaptic side, or whether different mechanisms operate for different types of synaptic modification.

In adult vertebrate axons, polyribosomes are present beneath synapses on axon initial segments (Steward and Ribak, 1986), but are generally not detectable in distal axons. mRNAs encoding the neuropeptide neurotransmitters oxytocin, vasopressin, and prodynorphin are present in axon terminals of the hypothalamo-hypophyseal tract (Mohr et al., 1991), and mRNAs for the olfactory marker protein and various odorant receptors are present in the axon terminals of olfactory neurons that terminate in the olfactory bulb (Ressler et al., 1994; Vassar et al., 1994). The significance of the localization of mRNAs in these axons is not clear, because ribosomes have not been detected in these axon terminals.

Protein Synthesis in Growing Axons and Growth Cones

Local protein synthesis appears to be especially important in growing axons, especially within growth cones. There is evidence, for example, that local synthesis of β-actin is important for cytoskeletal remodeling at the leading edge of the growth cone and within filopodia (Bassell et al., 1998). β-actin mRNA is localized in growth cones, and neurotrophin treatments that stimulate axon growth in culture also enhance the localization of β-actin mRNA in growth cones (Zhang et al., 1999). Localization of β-actin mRNA appears to be mediated by a *cis*-acting sequence (termed the zip code) that is recognized by a protein called the zip code binding protein (ZBP). Disruption of the interaction between ZBP and β-actin mRNA disrupted mRNA localization, reduced β-actin protein levels within growth cones, and impaired growth cone motility (Zhang et al., 2001).

In contrast to the situation in adult organisms, where polyribosomes are not detected in axons except in initial segments, polyribosomes are abundant in growth cones of at least some growing axons in vitro (Bassell et al., 1998) and in vivo (Tennyson, 1970). It is interesting that the significance of the latter observation is only now becoming apparent.

Recent evidence also implicates local protein synthesis within growth cones in growth cone turning in response to guidance cues. Growth cones extend and retract filopodia and ruffled membranes, and the net direction of extension is determined by where extension/retraction occurs. Turning is caused by extension on one side and collapse on the other, and is triggered by local attractive and repulsive cues in the environment. Studies of chemotropic responses of growth cones of *Xenopus* retinal ganglion cells have demonstrated that growth cone collapse and turning away in response to sema3A are blocked by protein synthesis inhibitors, even in growth cones that had been separated from their cell bodies (Campbell and Holt, 2001). Protein synthesis inhibitors also blocked the attractive turning response normally seen when retinal growth cones from young embryos (stage 24) were exposed to a gradient of netrin-1 as well as the repulsive turning induced by netrin-1 when neurons were grown on laminin. Together, these results indicated that local protein synthesis within the growth cone is essential for both repulsive and attractive guidance mechanisms, regardless of the exact extracellular stimulus that induces the collapse or turning response. Moreover, exposure to sema3A or netrin-1 triggered a burst of protein synthesis within growth cones, as evidenced by a rapid phosphorylation of the elongation factor eIF-4E, and increased incorporation of labeled amino acids.

One paradox is that protein synthesis is important for both collapse (a deconstruction of the growth cone) and turning toward netrin-1 (a positive response). A role for local protein synthesis in growth toward an attractant is consistent with the idea that local synthesis is important for extension of the growth cone. What role new protein synthesis plays in growth cone collapse remains

to be established, but the opposite responses to netrin-1 indicate that particular extracellular signals can be "translated" in different ways.

A somewhat different role for local protein synthesis was suggested by studies that revealed adaptation of *Xenopus* spinal growth cones to the chemoattraction produced by gradients of netrin-1 and BDNF (Ming et al., 2002). The main finding was that attractive turning of the growth cone toward a netrin-1 or BDNF source was attenuated by the presence of background concentrations of either chemoattractant in the bath. This "desensitization" disappeared within 30 min after removing netrin-1 from the bath, and resensitization was blocked by protein synthesis inhibitors.

A still different role for local protein synthesis in axonal growth cones is suggested by the work of Brittis et al. (2002), who document the existence of a mechanism that could allow the local synthesis of membrane receptors for axon guidance molecules. Spinal commissural axons grow initially toward the midline floor plate in response to attractive guidance cues. After crossing the midline, the axons lose their responsiveness to midline attractants and gain responsiveness to a new set of guidance cues so that the axons grow longitudinally toward the brain (Stein and Tessier-Lavigne, 2001). One possible explanation for the change in responsiveness is a local synthesis of receptors for guidance molecules just after growing commissural axons reach the midline. Evidence for such a mechanism came from studies of the expression of an EphA2 receptor-GFP reporter construct in commissural axons. EphA2 is one of several proteins that are expressed selectively on the distal segments of axons of commissural neurons after they have crossed the midline. Commissural neurons transfected with constructs made up of the 3′UTR of EphA2 and a fluorescent reporter protein (GFP) exhibited protein expression in cell bodies, but not in proximal segments of the axons prior to midline crossing. In contrast, the protein was expressed at high levels in the distal segments of the axons that had extended beyond the midline.

These results reveal a mechanism that could allow the local synthesis of receptors for guidance cues. One can imagine a scenario in which one set of receptors is expressed until growing axons reach intermediate point A; signals from point A then trigger the translation of mRNAs for a different set of receptors that are critical to guiding the axon to the next intermediate station. It should be emphasized, however, that the study involved an exogenously transfected reporter that had the 3′UTR from EphA receptor mRNA, and did not directly demonstrate the presence of EphA receptor mRNA in growing axons. It also remains to be established whether there is functional ER and Golgi in axonal growth cones. A key proof of principle would be to show that disrupting local synthesis would disrupt axon guidance.

Interesting new evidence indicates that axonal transport of mRNA may be reinitiated during axonal regeneration. Regenerating axons of adult dorsal root ganglion cells and spinal motoneurons contain ribosomal proteins, translation initiation factors, and rRNA (Zheng et al., 2001). Axons of dorsal root ganglion cells that have been induced to regenerate by a conditioning lesion also contain mRNAs for actin and neurofilament protein, and blocking protein synthesis within these axons causes growth cone retraction. Together, these findings suggest that local protein synthesis is a critical factor in successful axon regeneration.

Protein Degradation

For the reasons mentioned earlier, the idea of local protein synthesis has great appeal as a mechanism to regulate synaptic function and plasticity. It follows that any cellular process that regulates protein availability is of potential importance for synaptic function. Stepping on the heels of the flurry of local protein synthesis studies come several recent studies implicating the ubiquitin-proteasome pathway in the control of synaptic development and plasticity. The degradation of proteins via the ubiquitin-proteasome system requires three basic steps: the recognition of the target protein via specific signals, the tagging of the target protein with a ubiquitin chain, and the delivery of the target protein to the 26S proteasome, a protein complex that degrades the ubiquitinated proteins. The ubiquitination of target proteins is a highly regulated process; the basic biology of the ubiquitin-proteasome pathway is described by Ciechanover and Brundin (2003; this issue of *Neuron*).

Degradation Machinery

The machinery required to carry out ubiquitin-dependent proteolysis includes the ubiquitin-conjugating enzymes (E1, E2, and E3), ubiquitin, and the 26S proteasome, which is formed by the coassembly of a 20S proteasome (the catalytic component) and 19S cap (the regulatory component). The initial targeting of the substrates to the proteasome is probably accomplished through the recognition of the polyubiquitin chain by the non-ATPase subunits of the 19S cap. The machinery responsible for ubiquitin-dependent degradation has been detected at mature and developing synapses. Ubiquitinated proteins have been detected in synaptic fractions from adult rat brains (Chapman et al., 1994). In addition, immunostaining experiments with antibodies against ubiquitin, and the α or β subunit of the proteasome, show the presence of these proteins in hippocampal dendrites near synapses (G.N. Patrick et al., 2003, Soc. Neurosci., abstract), in retinal growth cones (Campbell and Holt, 2001), and in *Drosophila* presynaptic terminals (Speese et al., 2003).

Degradation in Axon Guidance and Pruning

As discussed above, it has been shown that local protein synthesis is required for some axon guidance decisions. Recent studies indicate that protein degradation is important for both axon guidance and pruning. For example, proteasome inhibitors block the chemotropic responses to netrin-1 and growth cone collapse in response to LPA (Campbell and Holt, 2001). In addition, netrin-1 and LPA can induce the rapid accumulation of ubiquitin-protein conjugates in growth cones. Once they reach the target area, many axons undergo pruning to adjust their synaptic contacts. In *Drosophila*, the pruning of the γ neuron axonal projections requires protein degradation; mutations of either a ubiquitin-activating enzyme or proteasome subunits prevent normal pruning (Watts et al., 2003). Identifying the axonal targets for degradation is the obvious next step.

Proteasome Regulation of Synaptic Form, Function, and Plasticity

Ubiquitin-dependent processes are also clearly important in synapse formation and regulation. For example, the *fat facets* (*faf*) gene in *Drosophila*, which codes for a deubiquitinating enzyme (DUB), is involved in synaptic development in *Drosophila* (DiAntonio et al., 2001). In a screen for genes whose overexpression leads to synaptic growth abnormalities, DiAntonio et al. identified *faf* as a candidate regulator of synapses. Targeted overexpression of *faf* in *Drosophila* results in an increase in synaptic size, synaptic area, and the number of synaptic branches. Interestingly, both the miniature and evoked excitatory junctional potentials (EJP) are markedly decreased despite the increased size of the synapse. There is also a decrease in the frequency of miniature EJP; taken together, these data indicate a defect in neurotransmitter release and suggest that the target for ubiquitination resides in the presynaptic terminal.

A recent study has suggested a potential presynaptic substrate. In *Drosophila*, Broadie and colleagues identified the synaptic vesicle priming protein DUNC-13 as a substrate for ubiquitin-mediated degradation (Speese et al., 2003). In addition, they localize both a ubiquitin-conjugating enzyme and the proteasome to presynaptic terminals; using a fluorescent reporter, they observed rapid local degradation in the nerve ending. The pharmacological blockade of proteasome activity led to an increase in DUNC-13 levels as well as an increase in synaptic strength at the nerve-muscle synapse.

One of the earliest demonstrations of the connection between the ubiquitin proteasome and synaptic plasticity came from studies of synaptic facilitation at sensory-motor synapses in *Aplysia*. The ubiquitin-proteasome pathway is responsible for the decrease in the level of regulatory subunits of the cAMP-dependent protein kinase, permitting the enhanced catalytic activity that is in part responsible for facilitation (Hegde et al., 1993). In addition, injection of proteasome inhibitors into sensory neurons can prevent synaptic facilitation (Chain et al., 1999). A different story emerges, however, from a more recent study (Zhao et al., 2003). In this study, bath application or injection of proteasome inhibitors increased basal synaptic strength and enhanced, rather than prevented, the synaptic facilitation elicited by serotonin treatment (Zhao et al., 2003). These latter data together with the Speese et al. study (Speese et al., 2003) suggest a different function for the proteasome, one in which proteasome activity functions to inhibit plasticity, rather than facilitate it.

Studies of E6-AP, the gene responsible for the human disease Angelman's syndrome, have supported the idea that proteasomal protein degradation is important for both synaptic and behavioral plasticity. E6-AP is a ubiquitin ligase (E3) that is required, together with the papillomavirus E6 oncoprotein, for the ubiquitination and degradation of the tumor suppressor p53 (Scheffner et al., 1993). Mutations in the E6-AP gene (*Ube3a*) cause Angelman's syndrome, a human hereditary disease that results in mental retardation and seizures (Kishino et al., 1997). Hippocampal slices prepared from mice that possess a maternal *Ube3a* null mutation exhibit normal basal synaptic transmission but attenuated short-term LTP (Jiang et al., 1998). The mutant animals also exhibit impaired conditioned contextual, but not cue, fear conditioning (Jiang et al., 1998). As most E3 ligases have a limited number of protein targets, it will be very interesting to identify the (presumably) synaptic targets of E6-AP.

What are the potential synaptic protein substrates for the proteasome? A recent study (Ehlers, 2003) has examined this by looking at regulation of synaptosomal protein levels following blockade or enhanced activity for 24–48 hr. In this study, families of synaptic proteins were identified that showed either consistent increases or decreases in protein levels following activity manipulations. For some of the proteins, the activity-dependent changes in protein levels were sensitive to long-term treatment with proteasome inhibitors, suggesting a role for the proteasome. One caveat to this study, however, is the duration of proteasome inhibitor treatment. Prolonged exposure to proteasome inhibitors leads to an accumulation of polyubiquitinated proteins, which depletes the cellular pool of free ubiquitin (Schubert et al., 2000). The ubiquitination of proteins does not always target them for degradation. Monoubiquitination, representing the attachment of a single ubiquitin to a target protein, is involved in many cellular functions including the endocytosis of plasma membrane proteins (reviewed in Hicke, 2001). As such, additional experiments are required to firmly establish a role for the proteasome.

Recent work has identified a mechanism by which the ubiquitin-proteasome pathway can acutely modulate synaptic transmission, by regulating the ligand-induced endocytosis of mammalian AMPA receptor subunits (G.N. Patrick et al., 2003, Soc. Neurosci., abstract). Patrick et al. demonstrated that inhibition of proteasome activity blocked the agonist-mediated internalization of both GluR1 and GluR2 in hippocampal neurons. Pretreating neurons with proteasome inhibitors for as little as 5 min was sufficient to block GluR endocytosis, suggesting that agonists actively regulate the proteasome. In addition, expression of a ubiquitin K48R mutant, in which polyubiquitin chain formation is inhibited, also blocked GluR1 and GluR2 endocytosis. As K48R permits monoubiquitin-dependent processes, these data indicate a role for the proteasome and polyubiquitination in the agonist-induced internalization of GluRs.

Emerging Issues

The above studies indicate that protein synthesis and degradation occur locally at synapses on mature neurons and within growth cones. In mature neurons, synaptic signals modulate both protein synthesis and protein degradation in order to alter the ensemble of synaptic proteins and change synaptic strength (see Figure 1). Signals mediated by NMDA receptor activation seem especially important. Protein synthesis is critical for establishing enduring changes at synapses induced by synaptic activation, and local proteasome activity may either facilitate or oppose these plastic modifications. Although protein synthesis is usually viewed as being "constructive" (activity induces synthesis of proteins that are necessary for synaptic modification), it is interesting to consider whether synaptic signaling might also decrease the synthesis of certain proteins that normally limit synaptic strength. In growth cones, signals are gen-

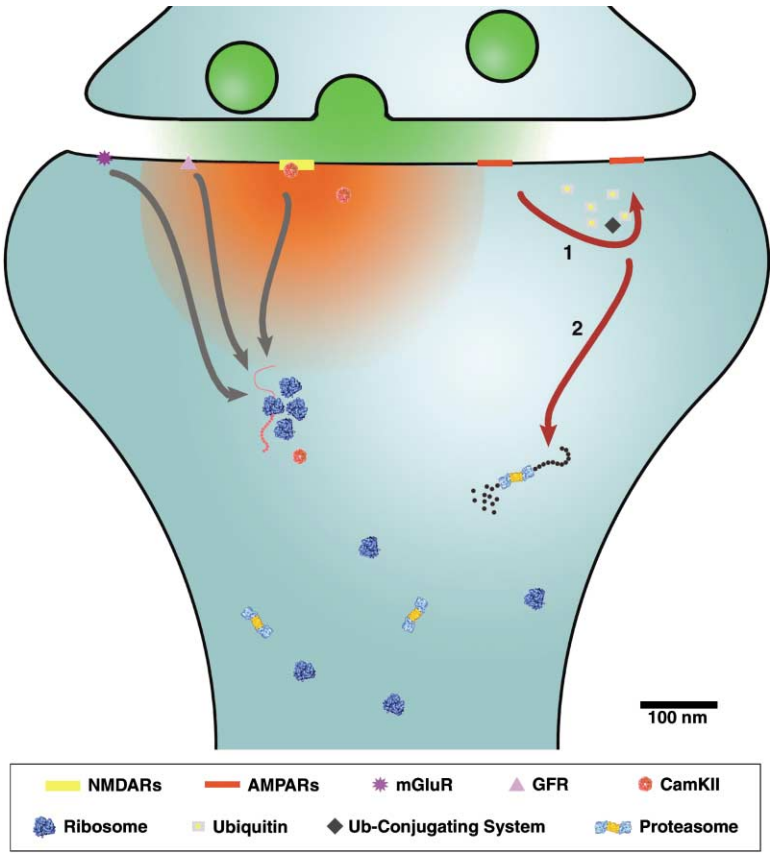

Figure 1. Synaptic Protein Synthesis and Degradation

An individual synapse is shown with protein and membrane components roughly to scale. As illustrated by the green gradient in the synaptic cleft, the presynaptic terminal (top) is releasing a single quantum of neurotransmitter onto an individual spine. Three pathways leading to increased protein synthesis at the synapse are shown (gray arrows): activation of metabotropic glutamate receptors (mGluRs), growth factor receptors (GFRs), and NMDA receptors (NMDARs). The orange gradient near the top of the spine is an NMDAR-mediated Ca^{2+} transient. Signals generated by synaptic activation also trigger the translocation of ribosomes and mRNA from the dendrite into the spine. Putative roles for the ubiquitin-proteasome system are also illustrated: activity-induced ubiquitination of synaptic protein (1) and ubiquitin-dependent protein degradation (2). For the sake of clarity, only a small fraction of synaptic proteins are shown in the figure. Artwork courtesy of Bryan Smith.

erated by receptors for growth factors and guidance molecules, and there is at least one example (Campbell and Holt, 2001) where the same extracellular signaling molecule (netrin) requires both protein synthesis and protein degradation. Together, these lines of evidence have brought us to a concept of the neuron in which key aspects of gene expression are controlled locally at thousands of individual cytoplasmic microdomains rather than centrally in the nucleus and cell body. An important issue for the future is the elucidation of the signal transduction pathways that locally regulate protein synthetic and degradative processes.

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