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Dendritic Protein Synthesis, Synaptic Plasticity, and Memory

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Considerable evidence suggests that the formation of long-term memories requires a critical period of new protein synthesis. Recently, the notion that some of these newly synthesized proteins originate through local translation in neuronal dendrites has gained some traction. Here, we review the experimental support for this idea and highlight some of the key questions outstanding in this area.

"…it is possible that synthesis of specific proteins is the essential physical phenomenon paralleling memory, fantasy, and intuition. This hypothesis is supported by the fact that protein synthesis occurs in strongly stimulated neurons and that cells are able to 'learn' to synthesize new specific proteins…." (Monné, 1948).

Nearly 60 years ago, this cryptic statement was put forward to suggest that new protein synthesis might represent a critical step in the establishment of long-term memories in the brain. The ability to directly test the role of protein synthesis in memory had to await the discovery of protein-synthesis inhibitors in the late 1950s (Yarmolinsky and De La Haba, 1959). In 1963, the first direct support for this idea was provided by the work of Louis and Josefa Flexner. They demonstrated that temporal lobe injections of the protein-synthesis inhibitor puromycin from day 1 to 3 after learning were effective in blocking long-term memory in mice for the location where an electric shock was received in a Y-shaped maze (Flexner et al., 1963). Injections that were made later than 3 days after training did not result in any consistent memory deficit. Subsequent work over the last 40 years in a variety of species and learning tasks has solidified the idea that the storage of long-term memories requires a critical period of new protein synthesis shortly after the relevant experience. Although protein-synthesis inhibitors do have pleiotropic effects, work subsequent to the initial observations of the Flexners has provided compelling evidence that new protein synthesis is required for animals to form enduring memories (e.g., Agranoff et al., 1965; reviewed in Davis and Squire, 1984). More recently, genetic approaches have provided further support for the role of protein synthesis in memory formation (Kelleher et al., 2004a; Costa-Mattioli et al., 2005; Banko et al., 2006). Although this volume of work argues strongly for a critical role of translational control in memory processing, we still lack a clear understanding of "why" protein synthesis is so important—how does this newly synthesized pool of proteins alter the functional capabilities of synapses, neurons, or circuits to enable the storage of long-term information?

A Brief History of Local, Dendritic Protein Translation

Until recently, most neuroscientists assumed that all of the proteins required for neuronal function were made in the cell body (with the exception of mitochondria). In 1965, Bodian published his observations of ribosome particles in proximal dendrites adjacent to synaptic "knobs" in monkey spinal cord motoneurons (Bodian, 1965). He speculated that the "…selective establishment of synaptic contacts may be determined by specific proteins synthesized at the synaptic membrane" and that local synthesis might also participate in "…the adaptive adjustments of synapses." In 1982, Steward and Levy detected polyribosomes in the distal dendrites of dentate granule cell neurons in electron micrographs (Steward and Levy, 1982), pointing out that the local synthesis of proteins could allow for the specific modification of synapses. Following these anatomical observations came a series of studies demonstrating that biochemical fractions enriched for synapses could, indeed, incorporate radiolabeled amino acids into protein (Rao and Steward, 1991; Weiler and Greenough, 1991; Torre and Steward, 1992). Feig and Lipton (1993) then showed similar incorporation of radiolabeled amino acids in the dendrites of hippocampal slices from the guinea pig brain, arguing that the early detection of radiolabeled protein in dendrites was too quick (within minutes) to be explained by synthesis in the cell body and transport. In 1996, the first functional role for dendritic protein synthesis was discovered: local protein synthesis in dendrites is required for the rapid enhancement of synaptic transmission induced by exposure to the growth factor BDNF (Kang and Schuman, 1996).

It is now clear that dendritic protein synthesis is required for many forms of long-term synaptic plasticity, raising the possibility that similar local translational control contributes to various aspects of memory processing. We review an emerging set of experiments that examine the role of local dendritic protein synthesis in

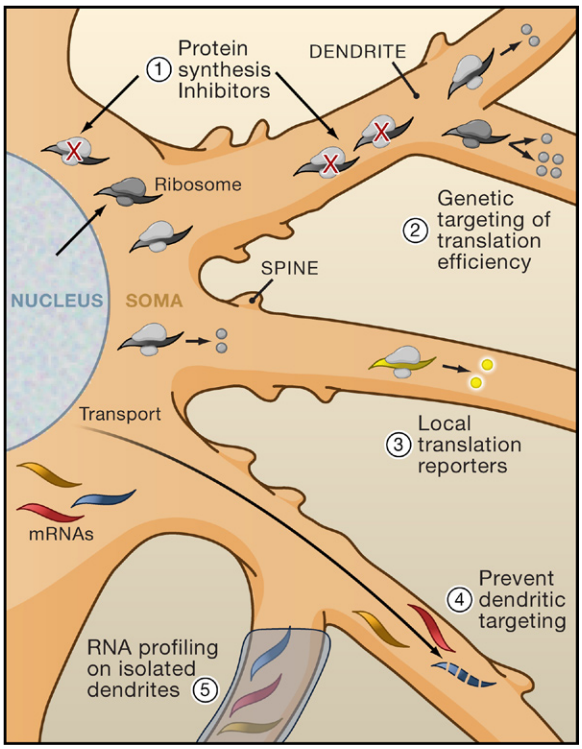

Figure 1. Strategies Used to Determine the Role of Dendritic Protein Synthesis in Memory

(1) Pharmacological inhibitors of protein synthesis have been widely used to study the role of protein synthesis in plasticity. Although these agents target neuronal protein synthesis globally, rather than specifically in neuronal dendrites, their use has contributed greatly to our current understanding of translational control in memory processing. Spatially restricted perfusion of protein-synthesis inhibitors implicates dendritic translation in synaptic plasticity (e.g., Bradshaw et al., 2003; Sutton et al., 2006). (2) More recently, genetic approaches have been harnessed to alter translational efficiency by manipulating components of the translational machinery itself (e.g., Costa-Mattioli et al., 2005; Banko et al., 2006). These studies also target neuronal protein synthesis globally, though they offer unique insights by providing more subtle alteration of translation efficiency, as opposed to the all-or-none translational response in studies using protein-synthesis inhibitors. (3) The use of fluorescent translation reporters has been used to monitor changes in dendritic protein synthesis (e.g., Aakalu et al., 2001; Ashraf et al., 2006), using mRNAs that encode for inherently fluorescent proteins (such as GFP). Continued use of this strategy will provide much-needed information on how translational control is regulated spatially during memory encoding. (4) The contribution of local translation in dendrites versus global translational control can also be assessed by interfering with normal dendritic targeting of specific mRNAs (e.g., α-CAMKII; Miller et al., 2002). This approach will prevent local dendritic synthesis of the affected gene product but can also alter constitutive synaptic expression of the protein as in the case of α-CAMKII (Miller et al., 2002). (5) Attempts to identify the population of resident dendritic mRNAs have used RNA profiling strategies on mechanically isolated dendrites (or neurites in the case of *Aplysia*) (e.g., Miyashiro et al., 1994; Mocca et al., 2003; Zhong et al., 2006). It is still difficult to authenticate these lists, though further studies using this approach and detailed in situ hybridization studies will help in validating this "local" mRNA population.

memory and synaptic plasticity (Figure 1). We then highlight what we consider to be key issues for future studies considering a role for local protein synthesis in these areas. Note that we confine our discussion to potential functional roles of translation in dendrites and do not elaborate on axonal protein synthesis or the signaling mechanisms that regulate local translation. For a discussion of molecular mechanisms of local translational control, we refer the reader to several recent reviews (Kelleher et al., 2004b; Klann and Dever, 2004; Sutton and Schuman, 2005).

Dendritic Protein Synthesis in Learning and Memory

Associative Learning in Drosophila

Olfactory conditioning in *Drosophila* has offered unique insights into the molecular basis of memory storage, due to the power of genetics in this system. In the olfactory learning paradigm, odorants are used as conditioned stimuli (CS) and are either paired (CS^+) or unpaired (CS^-) with an electric shock. Learning is usually measured on a population level. After conditioning, flies are introduced to a T-chamber and the number of flies that avoid the CS^+ or the CS^- arm of the maze is measured. In fact, the performance index used to measure the strength of learning indicates that if an equal number of flies are distributed in both arms of the T-maze then a "zero" learning score is applied. As the fraction of flies that avoid the CS^+ increases, so does the performance index. An important point here is that a random sampling of the flies that avoid the CS^+ will include flies that exhibit nonassociative avoidance of the odor as well as associative avoidance.

As with other species, the requirement for new protein synthesis distinguishes long-term from short-term memory in *Drosophila* (Tully et al., 1994). Using a fluorescent reporter of local translation (as in Aakalu et al., 2001), Ashraf et al. (2006) examined dendritic protein synthesis in the antennal lobe of *Drosophila* during olfactory learning. The antennal lobe represents the first sensory relay for olfactory input, where the olfactory receptor neurons form synapses with projection neurons in stereotyped glomeruli. The projection neurons, together with local interneurons, process the sensory information and then send axonal projections to the mushroom bodies where they make synapses with Kenyon cells. Most studies have focused on the mushroom bodies as a potential site of memory formation because many of the proteins implicated in memory formation are easily visualized in the mushroom bodies (Margulies et al., 2005), and ablation of the mushroom bodies leads to a learning deficit but no sensory deficit (deBelle and Heisenberg, 1994).

Ashraf et al. (2006) trained flies expressing a yellow fluorescent protein (YFP) reporter of dendritic protein synthesis (fused to the 3′UTR from CAMKIIα, which contains dendritic targeting and translational regulatory elements) and compared the YFP levels in different antennal lobe glomeruli following the above-described olfactory training procedure. To analyze dendritic protein synthesis, they chose flies from the CS^- arm of the maze, analyzed the levels of YFP signal in 6 of 30 antennal lobe glomeruli, and compared the antennal lobe YFP levels to untrained animals. The authors reported that two different odors (octanol and methylcyclohex-

anol) resulted in stimulation of YFP synthesis in some glomeruli of trained relative to untrained flies, implying that local translational activation in the antennal lobes specifically accompanies experiences that are encoded into long-term memory. However, given that flies present in the CS^- arm exhibit both associative and nonassociative avoidance, it is not clear that the observed changes represent learning-related changes or whether they are in fact necessary for long-term memory storage.

Spatial Learning and Contextual Conditioning

Several groups have looked for learning deficits commensurate with altered synaptic plasticity in transgenic mice in which protein translation is altered. As is the case with memory, distinct phases of synaptic plasticity can be isolated based both on their persistence as well as their dependency on new protein synthesis. For example, studies of long-term potentiation (LTP) and long-term depression (LTD) in mammalian brain have distinguished at least two distinct phases—an early phase (typically lasting 1–3 hr) that is independent of new protein synthesis and a late phase that is more persistent (lasting >8 hr) and is dependent upon protein synthesis (e.g., Stanton and Sarvey, 1984; Frey et al., 1988). Mice with conditional expression of a dominant-negative regulator of MAP kinase (MEK1) expressed in the forebrain exhibited an inhibition of protein translation, late-phase LTP, as well as deficits in spatial learning and contextual fear conditioning (Kelleher et al., 2004a). Three groups have recently examined plasticity and memory in mice that lack molecules key to the regulation of protein translation. Klann and colleagues (Banko et al., 2006) examined both LTP and spatial learning in mice that had a constitutive deletion of eIF4E binding protein 2 (4E-BP2). 4E-BP2 normally inhibits translation through its phosphorylation-sensitive binding to the cap binding protein, eIF4E. In hippocampal slices prepared from these knockout mice, stimuli that normally lead to early LTP resulted in long-lasting LTP, whereas stimuli that normally lead to late LTP led to reduced LTP. The authors hypothesized that an enhanced basal rate of translation in the knockout was responsible for these changes. The animals also exhibited impaired spatial learning and long-term contextual fear conditioning. In a similar study, Sonenberg and colleagues (Costa-Mattioli et al., 2005) examined plasticity in mice lacking GCN2, a protein kinase that inhibits translation initiation by phosphorylating eIF2α (eukaryotic initiation factor 2α). They observed the same plasticity phenotype as above. In addition, they observed an enhancement of learning in the Morris water maze following "weak" training but a reduction in learning when intense training was used. In another study, mice that possess a knockout of cytoplasmic polyadenylation element binding protein (CPEB) exhibited blunted LTP when theta burst stimulation was used to induce plasticity (Alarcon et al., 2004). These animals also exhibit normal spatial learning but impaired extinction (Berger-Sweeney et al., 2006), which is the normal reduction in the frequency or intensity of a learned response when reinforcement is no longer provided. In the above studies, because the gene deletions were not temporally controlled, we cannot discern whether the observed deficits are due to the requirement for the molecules during learning versus alterations in brain systems as a result of the long-term loss of the molecule. The possibility that the deficits are due to other, translation-independent functions of these molecules also cannot be ruled out. For example, as Costa-Mattioli et al. (2005) point out, the *GCN2* knockout mice exhibit altered CREB-dependent transcriptional control, which could account in whole or in part for the observed phenotype. Finally, these studies do not distinguish between a general (e.g., somatic) versus local requirement for protein synthesis.

There is one study where the role of dendritic protein synthesis in learning and memory has been specifically examined. Miller et al. (2002) tested spatial and contextual conditioning in mice that lacked the 3′UTR dendritic targeting element of the *CAMKII*α mRNA. These animals, which possess a near elimination of synaptic CAMKIIα (see below), exhibited diminished performance in the Morris water maze and contextual fear conditioning. These results raise the possibility that local synthesis of CAMKIIα is necessary for late LTP as well as the establishment of long-term hippocampal-dependent memories. However, since the modified CAMKIIα mRNA was present throughout development, it is unclear whether the impairments reflect an acute requirement for CAMKIIα synthesis versus a constitutive, long-term knockdown of synaptic CAMKIIα.

Dendritic Protein Synthesis and Synaptic Plasticity

Although direct behavioral evidence for the contribution of local translation in memory storage is limited, there is clear evidence that local translation plays a key role in synaptic plasticity. Given that transcriptional activation at the cell soma is required for late-phase LTP in normal hippocampal slices (Nguyen et al., 1994), initial ideas regarding the sites of translational control naturally focused on the cell soma, and early studies provided some support for this idea (Frey et al., 1989). More recent studies have suggested, however, that in some circumstances, translation in the dendrites themselves is critical, and even that somatic translation may be dispensable. For example, the neurotrophin BDNF induces potentiation of CA3-CA1 synaptic transmission in hippocampal slices where the CA1 dendrites have been surgically isolated from their cell bodies, and this effect still requires new protein synthesis (Kang and Schuman, 1996). In a similar slice preparation, activation of group 1 metabotropic glutamate receptors (mGluRs) or paired-pulse low-frequency stimulation can induce a form of LTD that requires dendritic, but not somatic, protein synthesis (Huber et al., 2000). Hippocampal slices prepared from the *CAMKII*α 3′UTR deletion described above exhibited diminished late-phase LTP (Miller et al., 2002). Moreover, several recent studies have also demonstrated that isolated hippocampal dendritic fields

can support protein-synthesis-dependent forms of LTP (Cracco et al., 2005; Vickers et al., 2005; Huang and Kandel, 2005), and that focal dendritic application of protein-synthesis inhibitors in intact slices inhibits late LTP (Bradshaw et al., 2003).

A recent study has demonstrated a rapid form of synaptic plasticity, elicited by dopamine agonists, that requires local protein synthesis (Smith et al., 2005). Restricted application of a dopamine D1/D5 receptor agonist to a dendritic segment led to an increase in endogenous protein synthesis in cultured hippocampal neurons. In addition, D1/D5 receptor activation led to a rapid, protein-synthesis inhibitor-sensitive increase in the frequency of spontaneous miniature excitatory postsynaptic currents (mEPSCs or "minis"), likely mediated by an increase in the number and size of synaptic GluR1 particles. These data suggest that local protein synthesis may be important for the conversion of synapses from a silent to an active state.

This emerging role for local protein synthesis in long-term plasticity is not limited to the hippocampus. Facilitation of *Aplysia* sensory neuron-motor neuron (SN-MN) synapses by serotonin (5HT) is a cellular model of sensitization, a form of learning where behavioral responsiveness is enhanced following the presentation of a noxious stimulus. Work over the last decade has highlighted the role of local protein synthesis in multiple phases of SN-MN synaptic facilitation that have been described, ranging from short-term (<30 min) to long-term (LTF; >24 hr). Using a cell-culture system in which a single SN makes synaptic connections with two MNs, Martin et al. (1997) demonstrated that repeated application of 5HT to one SN branch induced LTF selectively at that site, whereas the other (nonstimulated) branch did not change (Martin et al., 1997). This branch-specific LTF required local protein synthesis in the SN neurite, a finding that would seem to implicate local protein synthesis at the synapse as a necessary requirement for long-term synaptic plasticity in this system. However, this is not always the case. 5HT application exclusively to the SN cell body can be sufficient for inducing LTF lasting longer than 24 hr (Emptage and Carew, 1993; Casadio et al., 1999; Sherff and Carew, 1999), but LTF induced in this fashion does not require local translation at the synapse (Casadio et al., 1999). Taken together, the above studies indicate that the site where plasticity is initiated can determine the cellular location where protein synthesis is required.

Local Protein Synthesis and "Normal" Synaptic Function

Is the regulation of local translation only engaged during the (perhaps contrived) experimental regimens that neuroscientists use, or might local translation be sensitive to the ongoing levels of synaptic activity present in neural networks? Sutton et al. (2004) explored this question by blocking the effects of action potential-driven as well as spontaneous neurotransmission (minis) in cultured hippocampal neurons. The inhibition of minis resulted in rapid enhancement of dendritic protein synthesis, indicating that minis normally inhibit local translation (Sutton et al., 2004). More significantly, minis appear to act, via this translational inhibition, to stabilize synaptic function (Sutton et al., 2006). The blockade of axon potentials alone is known to induce compensatory "scaling" up of postsynaptic responsiveness to glutamate (O'Brien et al., 1998; Turrigiano et al., 1998), but this homeostatic response is slow to develop (typically >12 hr). However, blocking the NMDA receptor (NMDAR) component of miniature neurotransmission markedly accelerates synaptic scaling (<1 hr; Sutton et al., 2006). This rapid scaling is protein synthesis dependent, suggesting that minis normally provide a signal for synaptic stability by tonically repressing the dendritic protein-synthesis machinery. This rapid scaling induced by NMDAR mini blockade is mediated by the synaptic incorporation of new AMPA receptors (AMPARs) with a unique subunit composition. Unlike the pre-existing complement of receptors that contain the GluR2 subunit and are Ca^{2+} impermeable, the novel AMPARs lack the GluR2 subunit and are Ca^{2+} permeable (Sutton et al., 2006). When the NMDAR mini-blockade was restricted to a $\sim$40 μm stretch of dendrite by local perfusion, incorporation of these Ca^{2+}-permeable AMPARs was implemented locally. The initial incorporation/stabilization of these receptors requires local dendritic protein synthesis, yet these receptors are replaced by conventional Ca^{2+}-impermeable (GluR2-containing) receptors over the next 24 hr. Despite the near-complete removal of the novel receptors at synapses, the increase in synaptic strength they initially conferred is still preserved. These results are consistent with a role for local protein synthesis in driving the formation of receptor "slots" in the membrane that can accommodate dynamic trafficking of AMPARs while still preserving the original increase in synaptic efficacy. These data show that local synthesis stabilizes the synaptic expression of GluRs, perhaps allowing for conversion of a short-lived transient type of slot into a stable one that can persist >24 hr.

Mounting evidence suggests that the regulation of AMPAR subunit composition may represent a common plasticity motif. For example, other reports where activity blockade is achieved, in whole or in part, by targeting the postsynaptic receptors themselves have similarly found new expression of Ca^{2+}-permeable AMPARs at synapses (Ju et al., 2004; Thiagarajan et al., 2005). Moreover, high-frequency activation of excitatory synapses on cerebellar stellate cells induces a switch of AMPAR phenotype—from GluR2-lacking to GluR2-containing receptors (Liu and Cull-Candy, 2000). Moreover, Plant et al. (2006) have recently documented the converse type of regulation during LTP: GluR2-lacking AMPARs are rapidly recruited to hippocampal CA1 synapses, and their presence is required to maintain plasticity (Plant et al., 2006). These findings thus suggest that the regulation of AMPAR subunit composition at synapses is a common mechanism contributing to both activity-induced and homeostatic forms of synaptic plasticity. Although

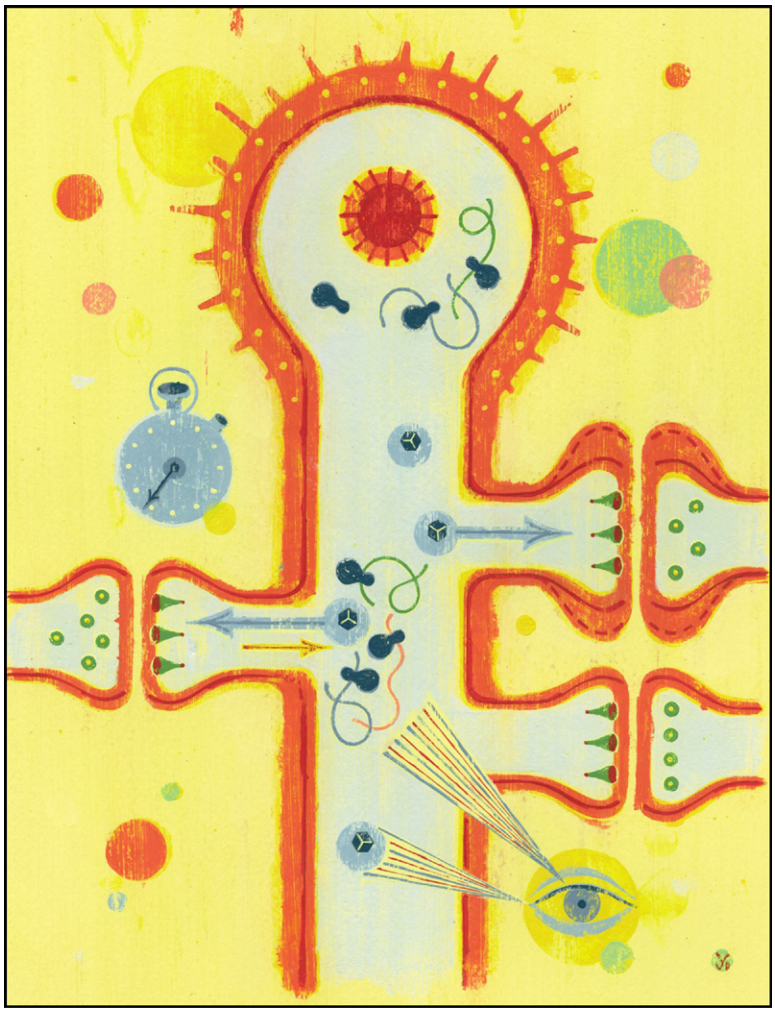

Figure 2. Local Protein Synthesis and Plasticity: Unresolved Issues

Shown is a neuron with a cell body and three synapses. Local protein synthesis at the left-hand synapse has recently been stimulated (red arrow). The eye signifies the importance of determining the local mRNA population (colored squiggly lines) and local proteome (cubes floating on circles). The ribosomes are depicted in dark blue and highlight the uncertainty in ribosome number per synapse, as well as the possibility of ribosome sharing between synapses, or ribosome biogenesis. Our uncertainty about the spatial specificity of locally synthesized proteins is depicted by the proteins entering both the left-hand synapse as well as the top right-hand synapse. The question of the time at which new protein synthesis occurs and contributes to synaptic function is illustrated by the stop watch. The putative role of local translation in morphological remodeling of synapses is shown by the orange expansion of the top right-hand synapse. Illustration by Brad Yeo.

it is clear that local synthesis is coupled to GluR subunit plasticity initiated by mini-blockade, it remains to be determined whether the other examples of GluR subunit plasticity also use a similar mechanism.

Unresolved Issues

The Local mRNA Population

It is difficult to evaluate the full spectrum of synaptic functions that local translation might regulate because it is still unclear how many different mRNAs are present in dendrites (Figure 2). Currently, lists range from 40–400 candidates (e.g., Eberwine et al., 2001; Steward and Schuman, 2001; Zhong et al., 2006) depending on the methods employed for mRNA detection. There are two basic approaches: one that is candidate based (mRNA detection via in situ hybridization) and one that is unbiased (mRNA detection in dendritic fractions using cDNA microarrays or PCR-based strategies). There are two main preparations: one involving dissociated neurons and one involving tissue sections or brain slices. Clearly, studies examining mRNA localization in dissociated cultured neurons are useful because the spatial resolution in these cells can lead to intriguing observations, like Eberwine's report that distinct mRNAs are differentially distributed within the neuritic arbor of a single neuron (Miyashiro et al., 1994). It seems imperative, though, to verify the localization of any bona fide dendritic or axonal mRNA in tissue sections from whole brain. It has become clear that viewing in situ hybridization images from earlier published papers won't suffice: the experimental parameters optimized to visualize an intense in situ hybridization signal that occupies a dense layer of cell bodies or a nucleus are likely to be insufficient to discern a perhaps less intense, but nevertheless real, signal in the dendrites. The alternative approach, using microarray analysis of microdissected dendritic or somatic regions from brain slices, has recently provided new data. Bloch and colleagues describe 154 mRNAs that are enriched in dendrites, including some of the usual suspects as well as others encoding proteins involved in membrane trafficking, protein synthesis, posttranslational protein modification, and protein degradation (Zhong et al., 2006). Another local mRNA population identified indicates that some of the locally translated proteins might include proteins involved in the process of local protein synthesis. A cDNA library of mRNAs generated from isolated neurites of *Aplysia* sensory neurons included a large proportion of mRNAs from the 5′ TOP family, encoding translation-related proteins, including ribosomal proteins and canonical translation factors (Moccia et al., 2003). Some of these factors, including the ribosomal protein S6 (Khan et al., 2001), CPEB (Si et al., 2003), and the elongation factors eEF1α (Giustetto et al., 2003; Huang et al., 2005) and eEF2 (Elvira et al., 2006), can

be translated outside of the cell body in neuronal processes. Lastly, we note that the potentially important area of mRNA stability, and its potential regulation by plasticity, remains completely unexplored.

The Local Proteome

What proteins are synthesized in dendrites? Is there a logic that underlies the synthesis of proteins in the cell body versus dendrites and if so, does synaptic signaling or plasticity alter this logic? We know that some synaptic proteins can be synthesized in the cell body whereas some synaptic proteins appear to have a local source. There is only one case we know of, in which contribution of dendritic versus somatic protein synthesis has been addressed for a particular protein. The mRNA for CAMKIIα is one of the most prominent dendritic mRNAs identified to date and rapid synthesis of CAMKIIα has been observed following synaptic plasticity (Ouyang et al., 1999). Mayford and colleagues deleted the dendritic targeting element in the 3′UTR of the *CAMKII* mRNA and then assessed the synaptic mRNA and protein levels (Miller et al., 2002). The dendritic *CAMKIIα* mRNA was almost completely abolished (1.4% of wild-type levels) and the levels of CAMKIIα protein in the postsynaptic density were reduced by 83%. Given that the mRNA and protein levels were only modestly altered in the cell body (85% of control levels), these data suggest that most of the CAMKIIα protein is made locally in the dendrites during normal brain function.

Although the Miller et al. (2002) study represents a good example of a candidate-based approach to understanding the impact of local synthesis on synaptic composition, there have been no systematic and unbiased inquiries to determine the local proteome. This is a tougher problem because identification of proteins in dendrites does not indicate their site of synthesis. A method for selectively identifying newly synthesized proteins is clearly required. How does the local proteome change during synaptic development and plasticity? Do plastic synapses simply alter the numbers of proteins they already possess, or is there a fundamental restructuring of the synaptic proteome, either by changing the abundance of some proteins relative to others or adding new proteins or protein isoforms? To achieve a global understanding of this problem, we need to have knowledge of these proteomes (somatic versus dendritic) and how they are modified by plasticity. It is important to consider the classes of proteins that could lead to synaptic change including neurotransmitter receptors, enzymes, and scaffolding molecules. The particular class of protein(s) that give rise to the local proteome sets limits on the duration of the plasticity that can be affected by protein synthesis.

Local Protein-Synthesis Machinery

Just how many ribosomes and polyribosomes are available to make protein for synapses? If proteins are to be made available to individual synapses, then one might expect that each synapse would possess its own supply of protein-synthesis machinery. Thus far, all of our information on the presence of polyribosomes near synapses comes from electron micrographs (Steward and Reeves, 1988; Ostroff et al., 2002), in which it is possible to identify polyribosomes by their somewhat stereotyped morphology (clusters, spirals, or staggered lines; e.g., Ostroff et al., 2002) but difficult to identify free ribosomes. The absolute density of polyribosomes in dendritic shafts and spines is about 1/μm (Ostroff et al., 2002). Thus, assuming a synaptic density of the same frequency (1/μm), each spine could possess on average a single, dedicated polyribosome. In the young (postnatal day 15) hippocampus, only 12% of spines on CA1 pyramidal neurons contained a polyribosome; following potentiation, this number increased to ∼35% (Ostroff et al., 2002). Given that each polyribosome is thought to translate a single mRNA this puts constraints on the timing and diversity of locally synthesized proteins. These observations raise the possibility that there could be local assembly of polyribosomes or the "biogenesis" of ribosomes with unique protein composition. Alternatively, the data derived from electron micrographs may underestimate the number of polyribosomes present.

Spatial Specificity

A major advantage of local protein synthesis is the potential to make proteins available to specific synaptic sites. This is based on the assumption that specificity at the level of individual synapses is required for both neural and behavioral plasticity. But, what do we actually know about the degree of specificity required for these changes? It is unclear what level of specificity is required for the neural circuits that underlie behavioral learning. If the neural representation for learning is stored at a distributed network of synapses, how much overlap is there in the representation of different learned events?

What degree of spatial specificity can local translation confer? Clearly, local translation restricts the potential supply of new products to sites close by, but whether local protein synthesis can give rise to specificity between neighboring synapses is still an open question. This has proven to be a very difficult question to address on the single synapse scale. The fact that polyribosomes tend to cluster beneath synaptic sites has been perhaps the strongest evidence for the ability of local translation to alter the composition of individual synapses independently. However, as noted above, the predominant localization of polyribosomes in dendrites is in the dendritic shaft beneath spines, not in the spines themselves (Steward and Levy, 1982; Ostroff et al., 2002). If devising a system for controlling the composition of individual synapses were the true logic underlying dendritic protein synthesis, why isn't the translational machinery compartmentalized the way that synapses are? Moreover, it is important to keep in mind that spatial specificity has two components—the capacity for activity at individual synapses to stimulate local translation and the resulting fate of locally synthesized products. It is possible that local translation confers specificity in only one of these domains. For example, localizing the machinery

immediately beneath synapses may enhance sensitivity sufficiently that localized activity at single synaptic sites can drive local synthesis, but whether those products are then dedicated to that synapse or other sites may be subject to additional regulatory mechanisms such as translation-independent recruitment processes (for instance, synaptic tagging/capture; Frey and Morris, 1997; Martin et al., 1997).

At the level of synaptic changes it has not been, in our opinion, convincingly demonstrated that two adjacent synapses can be independently modified either via morphological changes or changes in synaptic strength. The earliest demonstrations of "specificity" of long-term potentiation involved a comparison of synapses in two different laminae in area CA1: stratum oriens and stratum radiatum, separated by hundreds of microns (Andersen et al., 1977). The specificity established in these experiments pertains to inputs, rather than synapses, though often people erroneously claim that LTP is synapse specific. On the contrary, there are several studies that have shown that LTP can "spread" to neighboring synapses (e.g., Bonhoeffer et al., 1989; Schuman and Madison, 1994; Engert and Bonhoeffer, 1997). A recent study reported "synapse-specific" morphological changes (Matsuzaki et al., 2004), but close examination of the figures shown suggests that nearby synapses also change. As such, we believe it is still an open question as to what spatial scale (e.g., adjacent synapses versus groups of synapses) at which specificity is required. It is clear, however, from the early Andersen work, and other studies (e.g., Clark and Kandel, 1984; Frey and Morris, 1997; Martin et al., 1997), that specificity can be achieved at the level of different inputs. At both *Aplysia* and hippocampal synapses, appropriate stimulation of neighboring synapses can also lead to a "capture" of plasticity, which is the expression of synaptic enhancement at weakly stimulated inputs by a strong inducing stimulus applied to a different set of inputs. In both cases, this capture does not require local protein synthesis. Together, these studies indicate that some input-specific forms of plasticity can use protein-synthesis-independent mechanisms for achieving spatial specificity.

Temporal Windows for Protein Synthesis

The role of protein synthesis in memory storage is time limited. After learning, long-term memory becomes progressively less susceptible to disruption by protein-synthesis inhibitors (reviewed in Davis and Squire, 1984). Most studies have demonstrated that long-term memory is disrupted when protein synthesis is blocked either during training or for the first 1–3 hr following training. This time-limited role for protein synthesis has also been observed for long-term synaptic plasticity, where protein synthesis is required in a brief time window during and shortly after induction (Montarolo et al., 1986; Frey et al., 1988; Frey and Morris, 1997; but see Fonseca et al., 2006). However, it should be pointed out that, unlike behavioral studies of long-term memory, the analysis of long-term synaptic plasticity is usually restricted to <8 hr for technical reasons, although the synaptic changes presumably endure far beyond that. Thus, the possibility that protein synthesis may be required at some later point for long-term maintenance of plasticity cannot be ruled out. In some cases, for example, a second distinct phase of protein synthesis has been shown to be important for long-term memory (as in Grecksch and Matthies, 1980). Even in these cases, however, the requirement for new synthesis is still relatively brief (hours) relative to the persistence of the memory (days to weeks). Taken together, these observations indicate that we need not consider the role of protein synthesis over the entire lifetime of a memory (potentially years) but over the considerably shorter timeframe of a few hours. As we discuss in our conclusions, the more difficult question of how one maintains molecular memory at a synapse need not involve regulation of protein synthesis.

Are the proteins made locally necessary and sufficient for bringing about a long-term change, or is the contribution of protein synthesis transient, perhaps necessary, but not sufficient, for plasticity? Some evidence for the first scenario has come from molecular studies of L-LTP. For example, an atypical isoform of PKC, PKMζ, is induced downstream of translational activation (Osten et al., 1996) and is both necessary and sufficient for the maintenance of late-phase LTP (Ling et al., 2002). A similar story has been described for the role of BDNF in late-phase LTP. Application of BDNF to hippocampal slices induces protein-synthesis-dependent late-phase LTP (Kang and Schuman, 1996) and also maintains late-phase LTP in the presence of protein-synthesis inhibitors, implying that new synthesis of BDNF and associated maturation enzymes can sustain changes in synaptic strength (Pang et al., 2004; see also, Kang et al., 1997). In the Pang et al. study, it is unclear whether dendrites are the source of these translation-dependent changes.

In contrast, work in *Aplysia* suggests that the functional changes induced by local translational activation may be time limited. In addition to LTF described earlier, repeated pulses of 5HT also induce a protein-synthesis-dependent intermediate phase of synaptic facilitation (ITF; lasting ~3 hr) at SN-MN synapses (Ghirardi et al., 1995). Unlike LTF, ITF requires neither the SN soma nor transcription (Ghirardi et al., 1995). It can be induced by repeated pulses of 5HT to the synapse, but not the cell body, and is prevented by blocking synaptic protein synthesis selectively (Sherff and Carew, 2004). The studies of ITF as well as an intermediate phase of memory (ITM) in *Aplysia* also address another issue, namely that the persistence of these functional changes is limited. Specifically, both ITF and ITM decay after ~3–5 hr, long before the long-term phase sets in (Mauelshagen et al., 1996; Sutton et al., 2001). These results suggest that local protein synthesis can support lasting forms of synaptic plasticity but alone cannot maintain these changes over the days, weeks, or years required for permanent memory storage.

Morphological Remodeling

Remodeling of synaptic architecture likely contributes to memory storage and thus represents a potential function for locally synthesized proteins. Studies in mammals have demonstrated that bidirectional changes in synaptic growth accompany synaptic plasticity (Engert and Bonhoeffer, 1999; Harris et al., 2003; Matsuzaki et al., 2004; Zhou et al., 2004). Similarly, there is evidence that local protein synthesis in dendrites can interact with these growth mechanisms. For example, activation of mGluRs is known to stimulate dendritic translation (Weiler and Greenough, 1993), produces a form of LTD that requires dendritic translation (Huber et al., 2000), and produces a protein-synthesis-dependent lengthening of spines (Vanderklish and Edelman, 2002). It is unclear, however, whether these changes in spine morphology themselves have a direct functional impact on synaptic transmission or whether they are indicative of a different class of change that is functionally important.

Clearer evidence for this role has come from studies of LTF at *Aplysia* SN-MN synapses. Behavioral training sufficient to induce memory for sensitization lasting weeks is accompanied by growth of the SN synaptic terminal (Bailey and Chen, 1983). Similarly, LTF of *Aplysia* SN-MN synapses is associated with the growth of new SN varicosities. In this case, the synaptic growth has a clear functional impact in strengthening the SN-MN synapse and is coupled to local protein synthesis. LTF induced by synaptic stimulation persists beyond 72 hr and is associated with growth of new SN varicosities, whereas LTF induced by somatic stimulation decays within 48 hr and is not accompanied by synaptic growth (Casadio et al., 1999). Blocking local protein synthesis during restricted synaptic 5HT stimulation completely blocks the long-term synaptic facilitation and growth (Martin, et al., 1997; Casadio et al., 1999). These results suggest that synaptic remodeling may be one class of functional change that is critically dependent on local, as opposed to cell-wide, translational control.

Closing

In 1950, Katz and Halstead expanded on Monne's idea, suggesting that "…genesis of the memory trace is the formation, as a result of individual experience, of geometrically ordered protein molecules in the neurons of the cerebrum" (Katz and Halstead, 1950). In recent years we have elucidated the identification (Cheng et al., 2006), molecular abundance (Chen et al., 2005), and some of the protein-protein interactions that make up the synaptic scaffold that Katz and Halstead refer to. The relative stability of synaptic transmission and spine structure suggests that the molecular composition of individual synapses can be maintained in the face of the addition and removal of individual proteins. Yet we know very little about how the molecular identity of individual synapses is set up or maintained. Although it is likely that there are master scaffold molecules or "slot proteins" for neurotransmitter receptor complexes, it is not yet clear what kind of dynamics or rules govern the maintenance, shrinkage, or growth of the synaptic protein interaction matrix. Once we understand whether the molecular memory is due to a few proteins or an emergent property of the system, many of our current isolated observations on individual protein dynamics, protein synthesis, and degradation will appear more unified. It is possible, for example, that local synthesis (and degradation) can acutely regulate the level of a "master" protein, leading to long-lasting changes in the synaptic protein matrix that far outlast the period of synthesis or degradation. Synaptic modifications such as these are attractive candidates for the molecular mechanisms that may underlie memory formation and storage.

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