Published in final edited form as:

Neurobiol Learn Mem. 2008 March ; 89(3): 247–259. doi:10.1016/j.nlm.2007.08.009.

Altered Protein Synthesis is a Trigger for Long-term Memory Formation

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Summary

There is ongoing debate concerning whether new protein synthesis is necessary for, or even contributes to, memory formation and storage. This review summarizes a contemporary model proposing a role for altered protein synthesis in memory formation and its subsequent stabilization. One defining aspect of the model is that altered protein synthesis serves as a *trigger* for memory consolidation. Thus, we propose that specific alterations in the pattern of neuronal protein translation serve as an initial event in long-term memory formation. These specific alterations in protein read-out result in the formation of a protein complex that then serves as a nidus for subsequent perpetuating reinforcement by a positive feedback mechanism. The model proposes this scenario as a minimal but requisite component for long-term memory formation. Our description specifies three aspects of prevailing scenarios for the role of altered protein synthesis in memory that we feel will help clarify what, precisely, is typically proposed as the role for protein translation in memory formation. First, that a relatively short initial time window exists wherein *specific* alterations in the pattern of proteins translated (not overall protein synthesis) is involved in initializing the engram. Second, that a self-perpetuating positive feedback mechanism maintains the altered pattern of protein expression (synthesis or recruitment) locally. Third, that other than the formation and subsequent perpetuation of the unique initializing proteins, ongoing *constitutive* protein synthesis is all that is minimally necessary for formation and maintenance of the engram. We feel that a clear delineation of these three principles will assist in interpreting the available experimental data, and propose that the available data are consistent with a role for protein synthesis in memory.

Keywords

translation; protein kinase; learning; PKM zeta; positive feedback; knockout mouse; mTOR; GCN2; eIF2; 4E-BP; S6K

Introduction

In this review we propose a model whereby changes in protein synthesis contribute to long-term memory formation. The model specifies that *altered* translation of *specific* proteins is a

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critical component for triggering the consolidation of long-term memory. The mechanism we propose entails a process whereby a transient memory trace, involving the triggering of translation initiation and the subsequent synthesis of new proteins, is stabilized and perpetuated by a positive feedback mechanism requiring minimally only ongoing constitutive protein synthesis. Simply put we propose that, mechanistically, altered protein synthesis triggers the formation of long-term memory.

The model we describe is not a new model, but rather synthesizes and summarizes longstanding thinking among many molecular neurobiologists (Kelleher et al, 2004b; Klann and Dever, 2004; Bailey et al 1996; Steward and Schuman, 2001; Blitzer et al 2005). We feel it is appropriate at this point in time to reiterate this way of thinking about the role of protein synthesis in memory formation because of the recent emergence of alternative, non-protein synthesis-dependent models for long-term memory formation such as are discussed elsewhere in the literature and in this special journal issue. Our goal is to present what we think is a minimal “consensus” model among those scientists who are proponents of a role for protein synthesis in memory formation and consolidation. We present it in order to provide a contrasting model for comparison to non-protein synthesis-dependent models in the literature.

Thus, this review will take the following form. First, we will present an overview of a model positing a role for altered protein synthesis in memory formation and stabilization. Then we will present a summary of the types of experimental results available in the literature that support the general hypothesis that altered protein synthesis contributes to long-term memory formation, and specifically the model we describe. We also will present a discussion of the model in the context of several criticisms of protein synthesis-dependent models for memory formation that have been presented recently in the literature. In this section we will present a specific comparison of our model to the recently described model by Routtenberg and Rekart (2005) proposing a purely post-translational mechanism for long-term memory. Following that, we will describe in more detail specific experimental findings concerning two modes of translation control that we believe are particularly compelling results that support our model for the role of altered protein synthesis in memory formation. Finally, we will editorialize concerning the importance of the ongoing debate concerning a role (or lack thereof) for altered protein synthesis in memory.

The Model

Our model presupposes that memory is in significant part a molecular process through which learned information is stored, and that newly formed memories are susceptible to disruption and must be stabilized for long-term storage through a process referred to as memory consolidation (1). Consolidation of explicit long-term memory requires a series of hippocampus-dependent processes including altered protein translation, and we propose that the molecular processes we describe in our model participate in the consolidation of this specific type of memory as well as other types of memory in the CNS.

What follows is what we consider to be a minimal model for altered protein synthesis as a *trigger* for long-term memory formation. We use the term trigger in order to emphasize several important and defining aspects of the model. First, the altered protein synthesis need only be transient and thus is a triggering as opposed to a constitutive event. Altered rates of synthesis of specific proteins might also be perpetual, but that is not absolutely required by the model. Second, the existence of a trigger implies a specific set of mechanisms that operate to pull the trigger. This is important because in designing experiments one can very selectively eliminate the trigger-pulling mechanism as well as the trigger itself in order to test the validity of the model. Third, “trigger” mechanistically is an appealing term because a trigger is only a small

part of a much larger overall mechanism. The altered synthesis of only a very small subset of the milieu of cellular proteins is all that is minimally required in the model.

The trigger analogy also requires a very important distinction that we would like to emphasize. A trigger is pulled and released. However, in our model the proteins synthesized as a result of activating the trigger continue to be involved in the maintenance mechanism through a self-reinforcing positive feedback loop. The *alteration* in protein synthesis is the trigger. The proteins whose synthesis is altered are not the trigger – they are the readout of the synthetic event. Moreover, in one variation of the model the trigger analogy also breaks down significantly. One possibility is that the proteins whose synthesis is altered feed back upon themselves, perpetually and specifically, increasing their own rate of synthesis and replenishing themselves as they are turned over. Thus, in this case at least part of the “trigger” would have been perpetually incorporated into the maintenance mechanism.

The model is summarized in Figure 1. In describing the model we will make a large number of assumptions for the sake of simplicity. First, we will assume that these events occur locally at a dendritic spine, and that the process is NMDA receptor-dependent. The model that we present also does not specify molecules or mechanisms, although we will give several illustrative examples in a later section of this paper. As a final simplifying assumption the model ignores the need for altered gene expression as a component of the triggering mechanism.

The model is as follows. A memory-causing event such as a set of appropriately contingent environmental signals leads to NMDA receptor activation and the subsequent formation of a memory engram. As mentioned above we will describe this as happening at a dendritic spine of a hippocampal pyramidal neuron. The NMDA receptor activation recruits signal transduction mechanisms to precipitate an altered rate of synthesis of a specific subset of synaptic proteins. This altered protein synthesis is in addition to “housekeeping” protein synthesis that contributes to ongoing maintenance of the dendritic spine (the blue pathway in Figure 1). Also, the model specifies that the spine maintain constitutive synthesis of effector proteins that, when in a complex with the appropriate partners, can increase synaptic strength (the green pathway in Figure 1). The “appropriate partners” are the targets of the signal for altered protein synthesis (the red pathway in Figure 1). Thus, the induced proteins interact with a subset of constitutively synthesized proteins in order to increase synaptic strength.

In this model the altered synthesis of a specific subset of proteins in the dendritic spine is the trigger for synaptic potentiation and memory formation, and the “new” proteins interact with other proteins already present to effect the change. The effector protein complex is the readout of the altered protein synthesis.

How is the elevated level of the effector complex maintained? An absolute requirement is the need for a self-reinforcing positive feedback of some sort. In one alternative the elevated synthesis of the red (potentiating) pathway becomes quasi-constitutive by a positive feedback mechanism. In this alternative the triggering mechanism perpetuates itself by promoting its own re-synthesis at a new, higher rate locally. This allows the mechanism to perpetuate the altered spectrum (or rate) of synthesis of the new subset of local effector proteins. Alternatively and minimally, the effector complex might perpetuate itself by an increased rate of recruitment to the local subcellular compartment of other copies of itself synthesized elsewhere in the cell. The positive reinforcement mechanism in this second scenario is that a protein, once synthesized in the specific compartment, is able to subsequently capture others of its own species that are synthesized globally in the cell.

Note the mechanistic distinction between the first and second alternatives. The second scenario does not require any long-lasting increase in protein synthesis anywhere in the cell – as long

as the relevant protein species are synthesized at an adequate overall basal rate for the entire cell the changes will be persistent. The first scenario involves an increased rate of synthesis of specific proteins and necessitates that the altered rate of synthesis continues perpetually.

In our model one of these two alternative mechanisms is the maintenance mechanism at the molecular level. The self-perpetuating structural/functional change is a component of the engram, and serves as a molecular basis for memory storage.

The altered pattern of proteins translated can be anything from one specific protein species to the entire complement of proteins necessary to form a dendritic spine. Our model does not specify the details of this aspect of the mechanism. Indeed, identifying the spectrum of proteins involved in the triggering event is a topic of contemporary research in this field, and the particulars are unclear at this time.

However, what is more clear are several of the specific molecular processes involved in changing the spectrum of proteins that are being translated. These potential regulatory mechanisms are described in more detail in several sections below. For now we have treated these mechanisms as a black box and discussed the model for altered protein synthesis in memory initialization in general terms. This permitted us to provide the broad outlines and fundamental principles of the model without getting bogged down in mechanistic details.

The current model in the context of prior discussions in the literature

Some of the terminology commonly used in the literature, and ambiguity in that terminology, likely has led to some misunderstandings of what actually has been previously proposed as a role for protein synthesis in memory consolidation. This is nicely illustrated by consideration of the use of the term “new” protein synthesis, which of necessity is used quite frequently in the memory literature. Interpretation of results of experiments using general protein synthesis inhibitors like anisomycin led to the conclusion that “new” protein synthesis is necessary for memory formation. Making this type of conclusion is a fundamental limitation based on the types of experiments that can be designed – anisomycin is a general inhibitor of all protein synthesis and it blocks the translation of newly synthesized protein. However, “new” is not a very precise descriptor. After all, all proteins are “new” just after they are synthesized, so saying that new protein synthesis is required is essentially redundant with saying protein synthesis is required. The basic point that was being made was that something *unique* with protein synthesis was happening immediately after stimulation or training. Something that could be blocked by inhibiting protein synthesis immediately after training, but not when protein synthesis was blocked a few hours later (post-consolidation).

Unfortunately, some of this ambiguity of word usage was deliberate. In the early studies of protein synthesis and memory, it was not clear whether the memory-associated alterations in protein synthesis needed to be long-lasting or perhaps perpetual. In addition, a possibility that was commonly proposed was that there were “magic bullets” for memory – unique protein species whose expression might be turned on and stay on as part of the memory process. These considerations also gave rise to the use of some admittedly but necessarily ambiguous terms like new or de novo (used as synonyms for novel), altered, inductive, etc. versus ongoing, constitutive, and baseline protein synthesis.

As our understanding of the role of protein synthesis in synaptic plasticity and memory has increased, these types of earlier “magic bullet” models have been rendered untenable. It appears clear at this point that there are not unique proteins expressed in the cell whose synthesis is changed in an all-or-none fashion to serve as a maintenance or expression mechanism for memory. In contrast, reasonably subtle, local changes in the rates of synthesis of whole batteries of proteins are likely to serve as the triggering event for memory consolidation. There is no

simple and clear single term that can capture this distinction, unfortunately. However, it is an important component of our model that we wish to delineate, and we have tried to capture it in the description we have presented.

Another point related to this deserves emphasis. The targets of the protein trigger do not have to be uniquely synthesized in the cell only in response to a plasticity-inducing event. The protein trigger need only alter protein synthesis above some threshold locally in a specific compartment of the cell. For example, “new” synthesis of a protein at a particular dendritic spine may be involved in memory formation. The new expression of this protein locally could then trigger its own perpetual re-synthesis and stabilization at this spine – this is the first scenario for self-perpetuation described above. However, in terms of global protein synthesis in the cell, causing only a very small fractional change would be sufficient as a triggering event. Moreover, in the second alternative self-perpetuation mechanism the fractional change in any individual protein is transient, and the ongoing baseline translation rate of the protein in the cell body is sufficient to maintain and perpetuate the dendritic change over time. Thus in either scenario, when looking at the overall neuron, memory-associated changes in protein synthesis would be barely discernable quantitatively. All that is necessary is a small but functionally effective localized change in specific protein translation.

Finally, please keep in mind that this simplified model does not incorporate a need for altered gene transcription as part of the trigger. We have not left out altered gene expression as part of the model because we think it is not involved, indeed the opposite is the case. The available literature makes clear that altered transcription is also a necessary component of the trigger, at least for the longest-lasting forms of synaptic plasticity and memory. Nevertheless, we have left out altered gene expression as part of the model because this keeps things simpler to explain, and because this special issue of *Neurobiology of Learning and Memory* is focused on protein synthesis specifically. Theoretically, altered transcription need not be necessary for long-term memory – as our simplified model makes clear, alterations in protein synthesis could be sufficient. It is not clear *why* altered transcription is necessary for long-term memory, just that it is. Perhaps a necessity for altered transcription is an evolutionary leftover from metabolic signaling in prokaryotes, for whom there is no functional compartmentalization of the genome from the protein translation machinery.

Neuromodulation of protein synthesis

In a recent publication our colleague Paul Gold commented upon a number of issues concerning the importance of neuromodulatory mechanisms in memory formation, re-formation, and recall (Gold, 2006). We share many of his views concerning the importance of these types of mechanisms in memory, but would like to comment upon a few specific statements from this recent publication, as we were indeed invited to do by the Editor-in-Chief of this journal. Specifically we would like to rebut his general assertion that consideration of neuromodulatory mechanisms provides an alternative viewpoint that allows one to circumvent the apparent need for protein synthesis for memory formation and storage.

In his recent manuscript Gold states that “The findings that the effects of the protein synthesis inhibitors can be rescued by a wide range of (neuromodulatory) treatments indicate that protein synthesis is not necessary for memory formation.” (Word in parenthesis added by DS and EK.) We would like to offer an alternative viewpoint for explaining the types of observations that Gold refers to, i.e. that neuromodulators can rescue memory in the face of protein synthesis inhibition. We will pursue this specific point in a small degree of detail because we find it very interesting to incorporate the role of neuromodulators into our model wherein altered protein synthesis serves as a trigger for memory formation. We interpret the findings described by Gold et al not as a refutation of the idea that altered protein synthesis contributes to initial

memory formation, but rather as a compelling set of findings that drive refinement of the model such that it incorporates an important role for neuromodulation in controlling the protein synthesis-dependent mechanism of triggering long-lasting memory.

We would like to specify four different scenarios that are consistent with both the observation that neuromodulators can rescue memory in the face of protein synthesis inhibition and our overall model that altered protein synthesis is a necessary trigger for memory formation. 1) Experimentally, protein synthesis inhibition is likely never complete. Indeed, as a working approximation most investigators assume that inhibition is 90% complete, that is, that 10% of protein synthesis capacity remains. Neuromodulators could increase the sensitivity to triggering stimuli, or the efficacy of the protein synthesis apparatus, in order to allow the remaining protein synthesis capacity to cross the threshold necessary to trigger memory formation. 2) A variation of this idea is that neuromodulators could amplify downstream effects of a small (10% of normal) triggering change in protein synthesis to allow for robust memory formation. Even in the context of neuromodulators allowing the recovery of memories that had been “lost” due to protein synthesis inhibition during memory formation, one must keep in mind that the recall of the memory presumably involves mechanisms that can be modulated. A memory trace formed under conditions of 90% synthesis inhibition might still be dependent on alterations in protein synthesis for its formation, be sub-threshold for recall under normal conditions, but be available for recall if the readout were suitably amplified. Indeed, even the neuromodulatory effects to allow recall of lost memories might act via translation-dependent mechanisms. 3) Neuromodulators could recruit additional alternative mechanisms for altering protein synthesis that do not contribute to “baseline” memory formation, but rather are specific amplifying mechanisms associated with neuromodulator-enhanced memory formation. These alternative mechanisms might be able to substitute for the missing protein synthesis capacity (remember we are talking about a specific subset of proteins being the trigger, so the effects on protein synthesis of neuromodulators would not have to be global). This alternative requires that these mechanisms would not be susceptible (or 100% susceptible) to the protein synthesis inhibitor being utilized in the experiment. 4) Finally, as Gold points out, neuromodulators could indeed recruit a protein synthesis-independent mechanism that operates to allow memory formation. However, the existence of this mechanism does not necessitate its involvement in non-neuromodulator enhanced memory formation. In other words, the neuromodulator-dependent mechanism might only be relevant under specific circumstances.

We consider the first three alternatives tenable because neuromodulators, which can enhance memory formation and even retrieval after apparent “loss” of memory, interact with and modulate the protein synthesis machinery. This neuromodulation of protein synthesis happens via post-translational mechanisms, some specific ones of which we will comment upon later. Indeed, viewed from our perspective, observations that neuromodulators can rescue memory in the face of protein synthesis inhibitors is entirely consistent with the overall hypothesis of a role for altered protein synthesis in triggering memory formation.

Finally, we would like to point out that this consideration does not negate the many valid points raised by Gold. For instance, we completely agree with his assertion that experimentally manipulating protein synthesis or gene expression may cause secondary effects, likely altering neuromodulatory mechanisms indirectly, and that this mechanism potentially contributes to amnesia produced in a wide variety of experiments.

A comparison with the post-translational model of Routtenberg and Rekart

Routtenberg and Rekart have recently proposed that the only role for protein synthesis in memory formation and storage is replenishment of cellular protein constituents (Routtenberg and Rekart, 2005). Their alternative model is that the mechanisms for memory formation and

maintenance are completely post-translational – a model that starkly contrasts with the model we have proposed here. We certainly agree with Routtenberg and Rekart that there is a prominent role for post-translational mechanisms in memory formation, and that exclusively post-translational mechanisms are capable of long-term information storage and indeed likely to maintain memory in certain cases. We also note that Routtenberg and Rekart make a number of valid points concerning direct and indirect side effects of protein synthesis inhibition. In particular, we reiterate the warning by these authors that protein synthesis inhibition certainly has secondary effects on post-translational mechanisms. Finally, we note that both our model and that of Routtenberg and Rekart propose a necessity for protein replenishment as a component of memory storage.

However, we wish to take issue with three aspects of the Routtenberg and Rekart paper. First, that a broad review of the available literature allows the conclusion that memory is by-and-large independent of instructive protein synthesis. We obviously disagree with this overall theme of their paper, and will discuss the experimental results supporting the protein synthesis-dependence of memory in the second half of this article. Second, in Routtenberg and Rekart's description of the two alternative models (translation-dependent versus exclusively post-translational, Fig 1 in their 2005 TINS review) they mischaracterize the current protein-synthesis-dependent models as being independent of a need for positive feedback. It is *not* the case that current protein synthesis-dependent models are proposed to be independent of a requirement for positive feedback. We emphasize this specifically, because it captures a common misperception about protein synthesis-dependent memory mechanisms that involve structural changes – erroneously thinking that they are somehow intrinsically stable. Even long-term structural changes whose initiation requires altered protein synthesis require an ongoing reinforcement mechanism that is self-perpetuating. This principle is discussed in more detail by Roberson and Sweatt (1999), and Sweatt (2003). Thus, there is no distinction along this line between protein synthesis-dependent models and the model of Routtenberg and Rekart.

This brings us to the third and likely most important disagreement that we have with the “exclusively post-translational” model of Routtenberg and Rekart. The ongoing self-reinforcement mechanism for protein synthesis-dependent memory storage almost certainly requires post-translational modifications as well. The known sites at which post-translational mechanisms regulate protein synthesis are so extensive as to defy brief summary. Thus, the experiment that Routtenberg and Rekart describe as the “unique prediction” of their model, that kinase inhibitors, etc will block memories long after they are established, does not distinguish between the two alternative models. Protein synthesis-dependent models of memory also predict that inhibitors of post-translational modifications will reverse established memories, because of the intertwining of post-translational mechanisms with the regulation of protein synthesis.

For a particularly compelling example of this type of interplay in memory storage, we encourage the reader to examine the PKM-zeta model of memory storage proposed by Sacktor and colleagues (Pastalkova et al 2006; Kelly et al 2007). Their model invokes mechanisms involving positive feedback, protein synthesis, and post-translational modifications, in an integrated fashion whereby the interplay of the three mechanisms allows memory storage. We highlight this model as a specific example where inhibitors of post-translational processes have been used experimentally to test a protein synthesis-dependent model of memory storage.

The non-specificity of anisomycin and similar inhibitors of general protein synthesis

In this review we will not comment on a role for protein synthesis in memory reconsolidation. We are focusing instead in this review on the concept that specific alterations in protein

synthesis are a trigger for the initial formation of a lasting memory trace. Whether similar mechanisms are involved in memory re-consolidation is a fascinating question, but beyond the scope of what we choose to address in this particular commentary.

However, a recent clear and succinct paper by Rudy and colleagues that dealt principally with memory reconsolidation makes note of the many possible side-effects of anisomycin, a sub-topic of clear relevance to our discussion (Rudy et al 2007). The non-specificity of anisomycin is certainly well established and we would like to amplify further some of the issues raised by Rudy and his colleagues. As Rudy et al emphasize, anisomycin is non-specific in several important respects and indeed is an activator of the mitogen-activated protein kinase (MAPK) superfamily pathways as part of its mechanism of action as an antibiotic. Specifically, anisomycin activates stress-associated MAPKs (also known as SAPKs), which have already been directly implicated as suppressors of synaptic plasticity in the mammalian CNS. Also, SAPKs are in general functional antagonists of the ERK MAPKs, the proper function of which is critical for plasticity and memory formation. Thus, there is no doubt that anisomycin and other protein synthesis inhibitors of this category are very blunt tools in terms of trying to parse out molecular mechanisms contributing to memory formation.

We point this out as but one specific consideration that compels the need for an alternative approach that goes beyond the use of anisomycin and drugs of its ilk. In our opinion, that alternative approach is genetic engineering of mice to selectively eliminate specific mechanisms of translational control. Not to eliminate protein synthesis, or translation generally, or to modify the basic machinery of translation *per se*. But rather, to selectively and subtly manipulate the modulatory mechanisms that influence ongoing protein synthesis. To specifically manipulate those mechanisms that operate to shift the specific patterns of translational readout and bias the machinery to one set of proteins versus another. We will provide several specific examples of this type of approach in the next section wherein we identify some of the existing data supporting a role for altered protein synthesis as a trigger for memory formation.

However, before proceeding, we note that this approach also has its caveats – altering the modulatory machinery may alter overall synthesis as well. Altered synthesis of specific proteins may have general effects in the cell as well – for example, altered synthesis of specific proteins may secondarily affect post-translational mechanisms. Moreover, in the case of non-inducible knockouts, secondary developmental effects are a consideration. Nevertheless, specifically engineering decrements or enhancements of the mechanisms modulating ongoing protein synthesis is a quantum step forward versus using manipulations designed from the outset to eliminate >90% of the synthesis of every protein species expressed in the cell.

Experimental results consistent with the model that altered protein synthesis is a trigger for memory

By way of introduction to this section we feel it is useful to step back and review the basics of experimental design, because we will use these basics as an organizing platform for our discussion of the experimental results available supporting a role for altered protein synthesis in memory formation. Therefore, in the next section we will consider the basics of hypothesis testing, using a system that has been described in more detail elsewhere (Sweatt, 2003). We then will proceed to considering specific examples of results from each of the basic types of experiments in the context of investigating a role for altered protein synthesis in memory formation. In our view these results are strongly consistent with the hypotheses that altered protein synthesis is necessary for memory formation.

A. The four basic types of experiments

In general there are four basic types of experiments that any scientist can perform, which we refer to as “*block*, *measure*, *mimic*, and *determine*” experiments. What follows is a brief description of each of these four types of experiments.

The *determine* “experiment” is not really an experiment at all; rather, it is an observation or characterization. Examples of this type of pursuit are: determining the amino acid sequence of a protein, sequencing a genome, determining the crystal structure of an enzyme or determining the structure of the DNA double helix. In the context of the present discussion on the role of altered protein synthesis in memory formation, the relevant determination is that training an animal leads to memory formation (see Figure 2).

Block, *measure*, and *mimic* are experiments, and they are all specific types of approaches to test different predictions of a hypothesis. For the following discussion we will address the simple general case of testing the hypothesis “A causes C by activating B”, and the specific case of testing the hypothesis “Training (A) causes Memory (C) by Altering Protein Synthesis (B)” (see Figure 2).

The *mimic* experiment tests the prediction that “if B causes C, then if I activate B artificially I should see C happen as a result”. The mimic terminology arises from the fact that one is trying to mimic with a drug (etc.) an effect that occurs with some other stimulus, e.g. training an animal in the present example. We note before continuing that at the current state of understanding and experimental sophistication, mimic experiments are essentially impossible to execute in the context of mammalian learning and memory. This is because an enormous amount of fundamental understanding of the system is necessary, along with the capacity for very subtle manipulation, in order for the experiment to work. For example, we hypothesize that specific alterations in protein synthesis underlies memory formation. In theory the mimic experiment would be to induce synthesis of the right proteins in the right compartments in the right neurons in the CNS, and then the animal will have an altered behavior identical to that caused by a training session. Of course, doing this experiment requires that one know exactly which proteins and synapses to manipulate so that one can selectively achieve the right behavioral output – this is beyond the level of understanding for all mammalian behaviors at this point. However, a variation of the mimic experiment, that is experimentally tractable at present, is to selectively augment specific protein translation or gene transcription and assess whether memory formation is improved as a result.

The *measure* experiment tests the prediction that “A should cause activation of B”. Using our example of altered protein synthesis as a trigger for memory, the measure experiment predicts that the memory-inducing stimulus should cause an increase in the synthesis of specific proteins in relevant areas of the CNS. This is of course determined by measuring the synthesis of proteins as directly as possible, hence the *measure* terminology.

The *block* experiment tests the prediction that “if I eliminate B then A should not be able to cause C”. In our example, this predicts that protein synthesis inhibition, or more specifically a manipulation to block the cell’s capacity to alter the rates of synthesis of proteins, should block the ability of a training event to cause memory.

In summary, then, the mimic experiment tests sufficiency, the block experiment tests necessity, and the measure experiment tests whether the event does in fact occur. Each type of experiment has its strengths and weaknesses, but positive outcomes in testing each of these three predictions for any hypothesis makes for strong support of the hypothesis.

The case for altered protein synthesis in memory formation

In this next section we will present a very brief overview of the results available in the literature that are consistent with the hypothesis that altered protein synthesis is a trigger for memory formation. We will organize this section according to the broad and basic categories of experimental results that were presented above – the block, mimic, and measure types of experiments. In the interest of keeping the discussion to a reasonable length, we will cite only one or two examples for each category of experiment. Additional specific examples will be provided in the section discussing the biochemical mechanisms for regulating protein synthesis later in the review.

Results from “block” experiments, testing the prediction that disrupting protein synthesis or its regulation should block memory formation.

Example 1 - These types of experiments of course were the first to implicate regulation of protein synthesis in memory formation, as exemplified by Bernie Agranoff’s pioneering experiments demonstrating that protein synthesis inhibitors could block memory formation in goldfish (Agranoff et al 1966). Indeed, experiments wherein protein synthesis inhibitors blocked memory when applied immediately post-training were seminal animal studies leading to the general hypothesis that long-term memory requires “consolidation” (Barondes, 1970; Squire et al 1972). As these studies have been extensively discussed (and critiqued) in the literature and in the first section of this review, we will not reiterate them here.

Example 2 – A second example will be provided in the section on eIF2 α below.

Results from “mimic” experiments, testing the prediction that augmenting protein synthesis will enhance memory formation.

Example 1 – It has been observed that experimentally induced over-expression of the transcription factor CREB augments memory formation. Thus, in another recent example of a “mimic”-type experiment Brightwell et al (2007) used viral vector-mediated gene transfer to determine whether long-term memory for place learning could be facilitated by increasing levels of the cyclic AMP response element-binding (CREB) protein in the dorsal hippocampus. CREB-over-expressing rats showed significant improvements in memory formation, indicating that formation of long-term memory can be facilitated by increasing levels of hippocampal CREB protein. Although these investigators utilized a manipulation of a transcription factor, the effects of this manipulation clearly can be attributed to an alteration in the cell’s capacity for altered synthesis of specific proteins. These results therefore are consistent with the “mimic” experiment prediction that improving the cell’s capacity for altering protein synthesis will lead to an improvement of memory.

Example 2 – A second example will be provided in the section on eIF2 α below.

Results from “measure” experiments, testing the prediction that memory formation will be associated with changes in protein synthesis.

Example 1 – Medina and his colleagues have observed increases in specific proteins in association with aversive conditioning in rats (Cammarota et al 1998). Using a one-trial inhibitory avoidance training paradigm they found that memory formation is associated with a learning specific, time-dependent increase in CAMKII (alpha subunit) protein in the hippocampus. In addition, inhibitory avoidance training was accompanied by an increase in glutamate receptor (GluR1) protein expression as well. These data of course suggest that CAMKII and AMPA-subtype glutamate receptors in the hippocampus participate in the early phase of memory formation in this training paradigm. In the context of the present discussion,

they also serve as results confirming that memory formation is in fact associated with alterations in the synthesis (or breakdown) of specific proteins.

Example 2 – In a much earlier set of studies using the Aplysia model system, Barzilai and colleagues (1989) demonstrated increases in protein synthesis as a result of memory-associated synaptic plasticity. In this system, long-term, but not short-term, synaptic facilitation induced by 5-HT in Aplysia sensory neurons is blocked by application of inhibitors of protein synthesis or RNA synthesis during the 5-HT application period. These findings suggested that proteins not needed for the short-term process are selectively required for long-term synaptic facilitation. To identify these proteins, Barzilai and colleagues examined changes in overall and specific protein synthesis in sensory neurons using 2-D polyacrylamide gel electrophoresis. In these experiments, 5-HT initiated pronounced changes in total protein synthesis rates as assessed by 35S-methionine labeling. In addition, 5-HT also produced three temporally distinct changes in the synthesis of individual protein species that could be resolved on 2-D gels.

These findings are consistent with later findings investigating synaptic plasticity in the mammalian CNS, in studies of LTP-inducing stimulation in the hippocampus. In their studies of LTP-associated changes in protein synthesis, Kelleher and colleagues also found increases in global protein synthesis in association with the induction of lasting changes in synaptic strength (Kelleher et al 2004a). One conclusion from these two sets of studies is that altering overall protein synthesis along with augmenting the synthesis of specific proteins may be a common mechanism in memory formation. For the present purposes, these two studies serve as examples of the induction of lasting changes in synaptic strength being associated with alterations in protein synthesis.

Molecular mechanisms for triggering changes in protein synthesis in memory

For the next section of this review we will turn our attention to the question: How is protein synthesis regulated in memory formation? We chose to take the opportunity of this special issue of *Neurobiology of Learning and Memory*, devoted to the question of protein synthesis in memory, to provide a review of a couple of the basic biochemical mechanisms for controlling protein synthesis, typically referred to as translational control by those who work in the field... We hope that this section will be a useful introduction to the topic of protein synthesis and its control, for readers for whom this might be an unfamiliar area.

The mechanisms for regulating protein synthesis are themselves horrendously complicated, even without the added complexity of trying to understand how neuronal activity-dependent mechanisms might impinge upon them. Nevertheless, Figure 3 and the following discussion is a brief summary of some of the signal transduction mechanisms that are hypothesized to operate in neuronal activity-dependent regulation of local protein synthesis in CA1 pyramidal neurons.

Translation typically can be divided into three steps: initiation, elongation, and termination. Although each of these steps requires protein factors that can be biochemically regulated, translational control occurs primarily at the initiation step when the small 40S ribosomal subunit is recruited to mRNA and positioned at the initiation codon. Translation initiation itself can be divided into three steps. The first step is the binding of the initiator Met-tRNA_i^{Met} to the small 40S ribosomal subunit, usually termed the 43S complex. The second step, is the recruitment of the 43S complex to the initiation codon of an mRNA to form the 48S complex. The third step is the joining of the 48S complex to the large ribosomal subunit to generate a translation-competent ribosome. The majority of translation control occurs via changes in the phosphorylation state of initiation factors or their regulatory proteins.

In the context of synaptic plasticity and memory, two major aspects of translational control have been investigated in recent years: 1) the phosphorylation of eIF2 α , and 2) regulation of mTOR and its effector molecules. We will discuss each of these in turn.

Regulation of eIF2 α phosphorylation

The eukaryotic initiation factor 2 (eIF2) is a heterotrimer, consisting of α , β , and γ subunits, that binds the initiator Met-tRNA_i^{Met} and GTP to form the 43S complex. Exchange of GDP for GTP on eIF2 is catalyzed by the initiation factor eIF2B and is required to reconstitute a functional ternary complex for a new round of translation initiation (Hinnebusch, 2000). Phosphorylation of the α subunit of eIF2 at Ser51 decreases the dissociation rate of eIF2 from eIF2B, which blocks the GDP/GTP-exchange and causes a decrease in general translation initiation (Hinnebusch, 2000, Sonenberg and Dever, 2003). Importantly, although phosphorylation of eIF2 α inhibits general translation, it triggers increased translation of specific mRNAs that contain upstream open reading frames (uORFs), including the transcriptional modulator ATF4 (Harding et al., 2000, Vattem and Wek, 2004). Thus, the phosphorylation of eIF2 α can control both general and gene-specific translation. Importantly, ATF4 plays a role as a repressor of synaptic plasticity and memory formation (Abel et al., 1998, Bartsch et al., 1995, Chen et al., 2003).

It has been proposed that L-LTP and long-term memory require increases in general protein synthesis (Kelleher et al., 2004; Govindarajan et al., 2006). If this proposition is correct, in the “measure” type of experiment one would predict that L-LTP and training that produces long-term memory would be associated with decreased phosphorylation of eIF2 α . Recent evidence indicates that L-LTP and contextual fear conditioning are associated with decreased phosphorylation of eIF2 α (Costa-Mattioli et al., 2005; Costa-Mattioli et al., 2006). What about the “block” type of experiment? Although the identity of the phosphatase(s) that dephosphorylates eIF2 α in the brain is unknown, Sal003, a compound that blocks eIF2 α phosphatases (Boyce et al., 2005), prevents induction of both L-LTP and long-term memory (Costa-Mattioli et al., 2006). Thus, regulation of eIF2 α meets the “block” and “measure” criteria for involvement in L-LTP and memory.

It is perhaps the “mimic” experiment that provides the best evidence that translational control by eIF2 α is required for L-LTP and long-term memory. In experiments with mice containing a genetic deletion for GCN2, one of the kinases that phosphorylates eIF2 α , it was shown that the threshold for inducing L-LTP and long-term memory is reduced (Costa-Mattioli et al., 2005). Moreover, heterozygous mutant mice in which the eIF2 α phosphorylation site has been mutated from a serine to alanine, exhibit a reduced threshold for inducing L-LTP and long-term memory in multiple memory tasks (Costa-Mattioli et al., 2006). Thus, regulation of eIF2 α meets the “block, measure, mimic” criteria to be a signaling molecule involved in translational control in L-LTP and memory formation.

Interestingly, there appears to be a limit to how much eIF2 α dephosphorylation is conducive to long-lasting synaptic plasticity and memory. Although the threshold for L-LTP and long-term memory is reduced in GCN2-deficient mice, normal L-LTP inducing stimulation fails to result in L-LTP, suggesting that additional inhibitory factors are synthesized due to the lack of translation repression and block L-LTP in these mice. Moreover, although the threshold for inducing long-term memory is reduced in the GCN2-deficient mice, robust training paradigms that normally produce long-term memory in wild type mice fail to elicit long-term memory in GCN2-deficient mice. Taken together, these findings suggest that the regulation of eIF2 α phosphorylation must be tightly controlled for the normal expression of L-LTP and long-term memory.

Regulation of mTOR and its effectors

The recruitment of the 43S complex to the initiation codon of an mRNA to form the 48S complex is facilitated by the 5'-cap structure that is present on nearly all eukaryotic mRNAs (Shatkin, 1985). The cap-structure is specifically recognized by eukaryotic initiation factor 4F (eIF4F) (Gingras et al., 1999), which contains three initiation factors: 1) eIF4E, the cap-binding factor (Sonnenberg et al., 1978); 2) eIF4A, a DEAD box RNA helicase; and 3) eIF4G, a large protein that acts as a scaffold between the ribosome and the mRNA (Imataka et al., 1998). With respect to translation initiation, eIF4E is regulated via phosphorylation and direct interactions with inhibitory eIF4E-binding proteins (4E-BPs). The 4E-BPs specifically inhibit cap-dependent translation initiation by preventing the assembly of the eIF4F complex, and consequently ribosome binding to the initiation codon on mRNA.

The mammalian target of rapamycin (mTOR) is a protein kinase that triggers translation initiation by phosphorylating at least two effectors, p70 ribosomal S6 kinase 1 and 2 (S6K1/2), and the 4E-BPs (Gingras et al., 2001, Hay and Sonenberg, 2004). Typically, the phosphatidylinositol-3 kinase (PI3K) signaling pathway is upstream of mTOR. In most schema, the PI3K catalytic subunit phosphorylates the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP2) to form PIP3, which then recruits Akt to the membrane where it is phosphorylated and activated by PDK1 (Brazil and Hemmings, 2001). Akt activates mTOR through inhibition of the tuberous sclerosis complex (TSC). TSC is a heterodimer which contains TSC1 (hamartin) and TSC2 (tuberin), the latter serving as a GAP (GTPase-activating protein) for the GTPase Rheb. Akt phosphorylates TSC2, decreasing its Rheb-GAP function (Garami et al., 2003, Inoki et al., 2005a, Inoki et al., 2005b) that results in mTOR activation. mTOR exists in two distinct complexes, one of which contains the adaptor protein Raptor that recruits S6K1/2 and 4E-BP, the two mTOR effectors involved in translation control (Beugnet et al., 2003, Choi et al., 2003, Schalm et al., 2003). The drug rapamycin disrupts mTOR-Raptor interactions, thereby preventing mTOR from phosphorylating S6K1/2 and 4E-BP (Kim et al., 2002, Oshiro et al., 2004). The binding and inhibition of eIF4E by 4E-BP is regulated by its phosphorylation (Pause et al., 1994). Unphosphorylated 4E-BP binds with high affinity to eIF4E; phosphorylation of 4E-BP by mTOR inhibits its binding to eIF4E and permits the initiation of translation (Beretta et al., 1996, Pause et al., 1994; Gingras et al., 2001).

A large body of evidence implicates mTOR signaling in long-lasting synaptic plasticity and memory formation across multiple experimental preparations and species (for reviews, see Klann and Dever, 2004; Kelleher et al., 2004), but we will focus on experiments using rodents. First, “block” experiments have shown that the mTOR inhibitor rapamycin blocks late phase long-term potentiation (L-LTP), a long-lasting form of synaptic plasticity often studied as a molecular correlate of long-term memory in hippocampal slices (Tang et al., 2002). Rapamycin also blocks long-term memory formation in mammals in a number of tasks (Tischmeyer et al., 2003; Bekinschtein et al., 2007; Dash et al., 2006; Parsons et al., 2006), as does genetic deletion of S6K1 and S6K2, downstream effectors of mTOR (Antion et al., 2007). Second, “measure” experiments have shown that L-LTP and long-term memory is associated with the activation of mTOR and increased phosphorylation of several downstream effectors (including 4E-BP and S6K), and increased eIF4F complex formation (Kelleher et al., 2004; Banko et al., 2005; Tsokas et al., 2005; Dash et al., 2006; Tskokas et al., 2007). Thus, mTOR-dependent signaling meets the “block” and “measure” criteria for involvement in L-LTP and memory formation.

“Mimic” experiments with mice that lack the translation repressor 4E-BP2, the predominant 4E-BP isoform in the mouse brain (Banko et al., 2005), have provided further evidence that translational control by mTOR signaling is a critical component for L-LTP. Similar to experiments with GCN2 and eIF2 α mutant mice, the threshold for inducing L-LTP is reduced

in hippocampal slices from mice that lack 4E-BP2 (Banko et al., 2005). The reduction in the threshold for L-LTP induction is correlated with increased eIF4F complex formation (Banko et al., 2005).

As was the case for the GCN2 knockout mice, stimulation paradigms that produce L-LTP in wild type mice resulted in no L-LTP in slices from the 4E-BP2 knockout mice. Consequently, it was shown that enhanced eIF4F complex formation and possibly excessive translation may be detrimental to L-LTP in 4E-BP2 knockout mice (Banko et al., 2005). Consistent with the lack of L-LTP, the 4E-BP2 knockout mice displayed long-term memory impairments in multiple memory tasks (Banko et al., 2005; Banko et al., 2007). All together, these data indicate that proper translational control by 4E-BP2 is critical for L-LTP and long-term memory formation.

Conclusion

We have presented what we hope to be a clear and simple model, summarizing current thinking concerning a role for protein synthesis in long-term memory formation. We also have presented arguments supporting the model based on published experimental results, and placed our discussion in the context of other prior commentaries.

However, in closing we choose to emphasize one additional point. Ongoing discussions of the role of protein synthesis in memory formation are not only subtle academic arguments, nor are scientists working in this area dealing with arcane, esoteric issues. The issues under discussion are of great relevance to understanding human learning and memory disorders. They are particularly relevant to understanding the basis of several forms of human neurodevelopmental disabilities such as Fragile X syndrome and Tuberous sclerosis, and potentially are relevant to a wide variety of human learning and memory disabilities.

This has two implications worth emphasizing. First, discussions and debates of the role of protein synthesis in memory formation, such as is taking place in this special issue of NLM, are important and worthwhile. These debates and intellectual arguments help us convey to the outside scientific world why this topic is interesting. Second, it is important for scientists engaged in these discussions and debates to consider that everything we say has real-world implications. The 40-year-old debate on the role of protein synthesis in memory has generally been dealt with as a fascinating abstract issue by many of us working in the field. This has made for a very pleasant and free-wheeling atmosphere, where we were all free to make statements on the extreme ends of the spectrum for the sake of developing an argument about the molecular basis of memory formation. However, in the current era there is increasing emphasis on the clinical relevance of all basic research. There also have been many recent discoveries about the clinical relevance of genetic mutations directly or indirectly affecting protein synthesis. Thus it is important for the memory scientist interested in the basic molecular and cellular mechanisms of memory formation to remember that our arguments have implications far beyond intellectual give-and-take. This should not diminish our pleasure in engaging in the debate, but rather should compel us to move forward aggressively with testing our ideas in the laboratory.

Acknowledgements

EK - NS034007, NS047384, and the FRAXA Research Foundation; DS - MH57014, NS13546, NS047466, NS057098, and McKnight Brain Research Foundation. The authors wish to thank Felecia Hester for assistance with preparation of the manuscript.

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Figure 1.

A minimal model for protein synthesis as a trigger for long-term memory formation. The model summarized in this figure makes a large number of assumptions for simplicity's sake: that these events occur locally at a dendritic spine, and that the process is NMDA receptor-dependent. The model does not specify molecules or mechanisms, and it ignores the need for altered gene expression. In this model a memory-causing event such as a set of appropriately contingent environmental signals leads to NMDA receptor activation and the subsequent formation of a memory engram. NMDA receptor activation recruits signal transduction mechanisms to precipitate an altered rate of synthesis of a subset of synaptic proteins. This altered protein synthesis is in addition to "housekeeping" protein synthesis that contributes to ongoing

maintenance of the dendritic spine (blue pathway). Also, the spine maintains constitutive synthesis of effector proteins that when complexed with the appropriate partners can increase synaptic strength (the green pathway). The targets of the signal for altered protein synthesis (the red pathway) comprise the appropriate partners for the green pathway, in order to increase synaptic strength. Thus, the altered synthesis of the appropriate proteins is the trigger for synaptic potentiation, and the “new” proteins interact with other proteins already present to effect the change. The effector protein complex is the readout of the altered protein synthesis. The red pathway becomes quasi-constitutive by a positive feedback mechanism. Thus, the triggering mechanism can perpetuate itself by one of two possible mechanisms. First, it might promote its own re-synthesis at a new higher rate locally in order to perpetuate the altered spectrum (or rate) of synthesis of a subset of local effector proteins. This mechanism is what is illustrated in this figure. Alternatively and minimally, the complex might perpetuate itself by an increased rate of recruitment to the locale of a protein synthesized globally throughout the cell. One of these two mechanisms is the maintenance mechanism at the molecular level. The self-perpetuating structural/functional change is a component of the engram, and serves as a molecular basis for memory storage.

The Four Basic Types of Experiments

General Hypothesis: $A \rightarrow B \rightarrow C$

Specific Hypothesis: Training (A) $\rightarrow$ Altered Protein Synthesis (B) $\rightarrow$ Memory (C)

Experiment	General Prediction	Specific Prediction
• Determine	• None (A makes C happen)	(Training makes Memory Happen)
• Block	• Blocking B should block A causing C	Blocking Altered Protein Synthesis should block Memory formation
• Mimic	• Activating B should cause C	Enhancing Altered Protein Synthesis should enhance Memory formation
• Measure	• A makes B happen	Training is associated with Altered Protein Synthesis

Figure 2.

The four basic kinds of experiments to examine the role of altered protein synthesis in memory formation. A hypothesis leads to four fundamental types of predictions (predicted observations or predicted experimental outcomes). In this table, for each category of experiment, both the general form of the predictions (left-hand column) and the specific predictions of the hypothesis that altered protein synthesis is involved in memory (right-hand column), are shown. See text for additional discussion.

Figure 3.
Signaling pathways controlling protein synthesis in neurons.