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Location, location, location: The many addresses of memory formation

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ABSTRACT **Memory formation, like real estate, can be summarized succinctly—location, location, location. It is an emergent property involving different anatomical regions in the brain, sets of neuronal circuits, and cellular and molecular interactions between and within those neurons. At each of these levels of description, location continues to be a major organizing principle guiding researchers. The difficulty in the field is the integration of information between the various levels of analyses, and it is proposed that molecular reporters may help to fill that void.**

Anatomical/Systems Level. Lashley (1) pioneered the experimental approach of using anatomical lesions to search for the "engram," the physical locus of long-term memory. Over 30 years of ablations led to the disappointing view that no single, well defined lesion could totally disrupt learning and memory formation. This resulted in the general hypothesis that memories are distributed. Penfield (2) electrically stimulated the temporal cortex of patients, causing them to experience very vivid "memories." These studies led him to conclude that memories are localized. Modern brain imaging, coupled with refined ablation studies, contributes to the contemporary view that interacting networks of neurons, which are widely distributed, participate in memory formation. This interpretation is complicated further by functional redundancy (involving "backup" circuits) and the possibility that different anatomical regions may be used at different times after memory formation.

Cellular Level. At the cellular level, neurophysiologists have been building on the conceptual framework of activity-dependent strengthening of neuronal connections. The search for the loci of memory formation has become reduced to a search for mechanisms that strengthen synaptic connectivity. The current favorite cellular model for learning and memory formation is long-term potentiation (LTP), a physiological description of increased synaptic efficacy after high-frequency stimulation (3). A long-standing controversy is whether the primary locus of change is on the pre- or postsynaptic side of synapses. Presynaptic proponents suggest that potentiation results from changes in the amount of transmitter release through one of many possible mechanisms (4). Postsynaptic advocates favor changes in the efficiency of reception, perhaps modulated through unmasking "silent" synapses (5).

Molecular Level. At the molecular level, insights have been made into key molecules whose activity affects the process of memory formation. These studies highlight two different uses of the word location—the subcellular compartment, where important molecules reside, and the amino acids on which posttranslational modifications occur. Both issues can affect the activity and interactions of important proteins.

There are at least four major kinase systems that are believed to be involved in memory formation: (*i*) the cAMP-dependent protein kinase (protein kinase A), (*ii*) the calcium-calmodulin kinases, (*iii*) the protein kinase C family, and (*iv*) the MAP kinase pathway. The subcellular localization of these kinases (and their opposing functions, the protein phosphatases) are all exquisitely regulated through interactions with other proteins. There is a large family of anchoring proteins for the RII regulatory subunit of protein kinase A, and these anchoring proteins tether other important signaling molecules (6). AKAP79, one such anchor, which is located near the postsynaptic density (PSD), binds protein kinase A and calcineurin (a Ca^{+2}/calmodulin-stimulated phosphatase), as well as a subunit of protein kinase C (7). On cAMP stimulation, the protein kinase A catalytic subunit (which is bound to, and inactivated by, the regulatory subunit) can be freed from this interaction, allowing phosphorylation of nearby substrates. Similarly, Ca^{+2}/calmodulin stimulation allows release and activation of calcineurin phosphatase activity. Therefore, subcellular localization probably tethers enzymes near their substrates, and changes in localization may accompany changes in activity. These anchoring proteins also seem to function as "signaling scaffolds", binding proteins that represent different transduction pathways, perhaps representing points where "cross-talk" occurs.

Recently, Shen and Meyer (8) described the subtle regulation of Ca^{+2}/calmodulin kinase II subcellular location and activity. This protein exists in three subcellular pools–cytosolic, attached to F-actin, and bound to the PSD. When there is a local increase in Ca^{+2} concentration, Ca^{+2}/calmodulin binds the protein, releasing it from its interaction with F-actin and allowing binding to the PSD. Phosphorylation of Thr-286 also occurs, which prolongs the binding to the PSD. Thus, the activating signal, Ca^{+2} (acting together with calmodulin), affects both the persistence of the active enzyme and its location. It is hypothesized that many of the important actions of this enzyme in memory formation involve phosphorylation of substrates that are also bound to the PSD (including *N*-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors).

The involvement of the mitogen-activated protein kinase pathway in memory formation has recently been demonstrated in *Aplysia* and in rats (9, 10). These results show that a cascade of phosphorylation (activation) events are involved in transducing certain cytoplasmic Ca^{+2} signals into the nucleus. This occurs through the sequential phosphorylation and activation of a series of kinases and their subsequent nuclear translocation. The protein kinase A catalytic subunit, when freed from its regulatory subunit, can also translocate into the nucleus (11, 12). Thus, subcellular compartmental boundaries (cytoplasm, nucleus) are

Abbreviations: LTP, long-term potentiation; PSD, postsynaptic density; CREB, cAMP response element binding protein.

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broken down by phosphorylation cascades and/or direct movement of the activated kinase.

Amino acid residues on important molecules are often post-translationally modified. These modifications occur primarily by phosphorylation, although other modifications such as nitrosylation and oxidation/reduction have been found. Multiple modifications introduce another potential regulatory mechanism: the order-dependence of the modifications. One clear example of this occurs on calcineurin, which normally exists in an inactive conformation that is activated when Ca^{+2}/calmodulin bind to it. This activation step exposes a part of the protein that is susceptible to oxidation-mediated inactivation (13). Oxidation does not affect the unstimulated enzyme. Order-dependent regulation by two different signaling pathways is likely to be quite common among proteins that integrate multiple signals.

Integration. How can the experimental results gathered from different levels of analysis of learning and memory formation be integrated? One possibility is through the use of "reporters," and a recent pioneering example from Impey *et al.* (14) illustrates this point. A large amount of data, collected from *Drosophila*, *Aplysia,* mouse, and rat have converged on the demonstration of the importance of the transcription factor cAMP-response element-binding protein (CREB) (and possibly its related family members) in the process of consolidating long-lasting plastic changes (15). This body of data, collected from a variety of behavioral tasks and models for plasticity, supports the hypothesis that learning-induced changes in gene transcription, at least partially initiated through the activation of CREB family members, are critical in the process of long-lasting changes in plasticity. CREB acts by binding to DNA sequences (cAMP response element sites) that are normally located in upstream promoter regions of certain genes. It is generally believed that CREB-responsive transcription initiates a cascade of gene transcription, which ultimately results in the synthesis of genes whose products are responsible for the structural changes that underlie long-lasting changes in plasticity (16).

Impey *et al.* (17) created a transgenic mouse that contained a cAMP response element-responsive promoter driving expression of a lacZ reporter gene. Hippocampal slices from this mouse were analyzed for their responsiveness to LTP- and late-LTP-inducing stimuli . LTP is typically induced by a single train of high-frequency stimulation, whereas induction of late-LTP usually requires spaced, repetitive trains (18). Late-LTP induction requires transcription and activation of the cAMP pathway (19). By using slices from the reporter mouse, it was shown that stimulation that generates LTP does not induce reporter gene induction, whereas repetitive trains produce late LTP and reporter activation (17). This mouse was then trained in a fear-conditioning task, where the mouse learns to associate the environmental cues of a box with an unconditioned stimulus, electric shock (14). Experimental (paired) animals are placed into the box for 3 min, after which an electric shock is delivered. Control (unpaired) animals receive a shock 24 hr after placement in the box. When experimental and control mice are returned to the box 24 hr after receiving the shock, the experimental mice display high levels of freezing to context, whereas the control mice do not. Freezing is an indicator of fear, presumably reflecting memory of the previous pairing of that environment with electric shock. When experimental and control brain sections are analyzed for β-galactosidase immunoreactivity 8 hr after shock, there were statistically significant increases in the CA1 and CA3 regions of the hippocampi from experimental mice. This change in gene expression correlates with the difference in behavioral freezing. There is also an increase in immunoreactivity by using a CREB Ser-133 phospho-specific antibody. Phosphorylation of this residue is necessary, but not sufficient, for CREB activation. These results demonstrate that CREB activity is increased after paired presentation of the stimulus in many neurons in CA1 and CA3, implying that the transcriptional response is specific. Because partial knockout mice missing the predominant α/δ isoform of CREB are deficient for fear conditioning, these experiments support the hypothesis that CREB-responsive transcription in CA1 and CA3 is important for memory formation of fear conditioning (20).

Reporters can reflect other basic molecular processes, besides transcription, that are involved in memory formation. It is possible to fuse green fluorescent protein with molecules involved in synaptic release, signal transduction, translation, or receptor mobilization, allowing visualization of other steps in memory formation. These types of approaches are quite common at the cellular level but remain very rare at the organismal level. The key experimental breakthrough of Impey *et al.* is that a transgenic reporter allows analysis at the anatomical and systems level and (using transgenic hippocampal slices and primary neuronal cultures) at the cellular and molecular levels. This integration of analyses can be done for any important molecule by using a transgenic reporter that reflects its activity.

Where Is the Street Address? The identification of CREB as a key player in long-term memory formation immediately raised another real estate issue—how synapse specificity could be preserved when cellwide changes in gene expression occurred. Because neurons can have many thousands of synapses, how can gene expression contribute to strengthening only the recently active synapse?

One emerging hypothesis is that recently active synapses are "marked," perhaps partly through a mechanism involving local translation of dendritically located mRNA. Kang and Schulman (21) demonstrated that growth factors can stimulate local (non-cell body) translation. Further corroboration has come from experiments in hippocampal slices and cultured *Aplysia* neurons (22, 23). Most recently, it has been shown that activity-dependent translation of the α-Ca^{2+}/calmodulin kinase II mRNA can occur through a mechanism involving poly(A) tail lengthening (24). These results highlight a possible cellular-level solution to the problem of synapse specificity. The relevance of this mechanism for memory formation needs to be demonstrated. However, once the appropriate transgenic reporter animal is made, it should be possible to verify the use of this mechanism in behavior, which will also yield information about its usage at the anatomical, neural network, cellular, and subcellular levels. It is this type of integration that is needed to unravel the many layers of real estate involved in memory formation.

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