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Minireview

Cell Adhesion Molecules, CREB, and the Formation of New Synaptic Connections

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As in other areas of biology, the study of behavior has relied on model organisms that lend themselves primarily to either a genetic or a cell biological approach, but not both. Yet, by themselves, each of these approaches has limitations. Genetic approaches allow an unbiased search for new genes and a delineation of genetic pathways. These advantages carry with them the limitations that the function of the gene so identified may not be well understood and may therefore be only peripherally involved in the behavior of interest. By contrast, cell biological approaches are useful for analyzing how a gene works. But these analyses are often carried out in artificially reduced systems that may not allow the establishment of a causal relationship between the cellular function of that gene and the consequences of perturbing that function for behavior in the intact animal. In the past, the limitations of each of these approaches restricted the depth to which any model organism could be used for exploring behavioral mechanisms. But now, as a result of the ability to clone genes from any species and to recognize the homology of genes identified independently in different contexts, the information obtained in a cell biologically tractable system can be advanced by findings emerging from genetically tractable systems and vice versa.

This complementarity has been routine in most areas of biology. What is new is that the molecular study of behavioral plasticity is becoming sufficiently mature so that it too can now exploit this complementarity. As evidenced from three papers in this issue of *Neuron* from Davis, Schuster, Fetter and Goodman, we are gaining a deeper insight into how a long-term change in synaptic strength can be stabilized by combining the power of genetic analysis with earlier cell biological studies. In so doing, we are also furthering our understanding of the molecular biological bridges between learning and development.

In these papers, the Goodman lab has undertaken a genetic approach to the question of how activity stabilizes synapse formation. They used the nerve muscle synapse of Drosophila, a system in which individual motor cells can be identified and studied at both a structural and physiological level. In addition to providing a model system for studying early events in axon guidance and target recognition, this synapse exhibits activity-dependent alterations in the size and complexity of the larval motor endings (Keshishian et al., 1996). Finally, this synapse also undergoes activity-dependent increases in synaptic growth and effectiveness in the adult fly in two well-characterized mutants, *ether-a-go-go Shaker* (*eag Sh*) and *dunce* (*dnc*; Budnik et al., 1990; Zhong et al., 1992).

In their first paper, Schuster et al. show that although Fasciclin II (Fas II, the Drosophila homolog of the vertebrate immunoglobulin-related cell adhesion molecule, NCAM) is present on both the pre- and postsynaptic elements of the synapse, it is not required for initial, activity-independent synapse formation. However, Fas II is required later for synapse remodeling and for the sprouting of additional synaptic contacts, processes that are thought to be activity dependent. The finding that activity-independent processes are normal in Fas II null mutants, whereas activity-dependent ones are not, led Schuster and his colleagues to ask: what role does Fas II have in this activity-dependent synaptic growth? They found that hypomorphs expressing half the protein have a large increase in the number of synaptic boutons as compared with wild-type animals, suggesting that the concentration of Fas II regulates sprouting and the ability of neurons to form new synapses.

The importance of cell adhesion molecules for activity-dependent synapse formation may prove to be quite general. Analogous events have previously been demonstrated in the development of the vertebrate neuromuscular junction. There, Landmesser et al. (1992) and Tang et al. (1992) found that removal of polysialic acid from NCAM (a procedure that is functionally equivalent to reduced expression of Fas II) causes misrouting of motor axons and inhibits sprouting following curare-induced paralysis.

These findings are not only important from a developmental point of view; they are also interesting for learning-related synaptic plasticity in the adult organism. One of the defining features of long-term memory, first characterized in studies of long-term facilitation of sensory and motor neurons of the gill-withdrawal reflex of Aplysia, is the growth of new synaptic connections. This growth parallels long-term memory and requires new protein synthesis. A search for proteins in Aplysia whose expression is altered during long-term facilitation revealed that not only are some proteins increased in expression with learning, but some proteins are actually decreased in their level of expression. Among proteins so decreased are several isoforms of apCAM, an adhesion molecule homologous to NCAM and Fas II (Bailey et al., 1992; Mayford et al., 1992). In addition to the reduction in newly synthesized protein, there also was a decrease in the expression of the transmembrane apCAM isoform at the cell surface of sensory neurons (but not the motor neurons) due to internalization. This internalization required new protein synthesis and was mediated by elevations in intracellular cyclic AMP (cAMP) (Bailey et al., 1992). An antibody recognizing apCAM caused defasciculation when added to cultured sensory neurons (Keller and Schacher, 1990). Together, these findings suggested that apCAM may normally act to restrain growth by causing processes of the sensory neurons to fasciculate through homophilic interactions. Training for long-term memory leads to internalization of apCAM, which causes defasciculation of the presynaptic preterminal processes of the sensory neurons.

Subsequent findings in vertebrate systems strengthened the notion that cell adhesion molecules may play

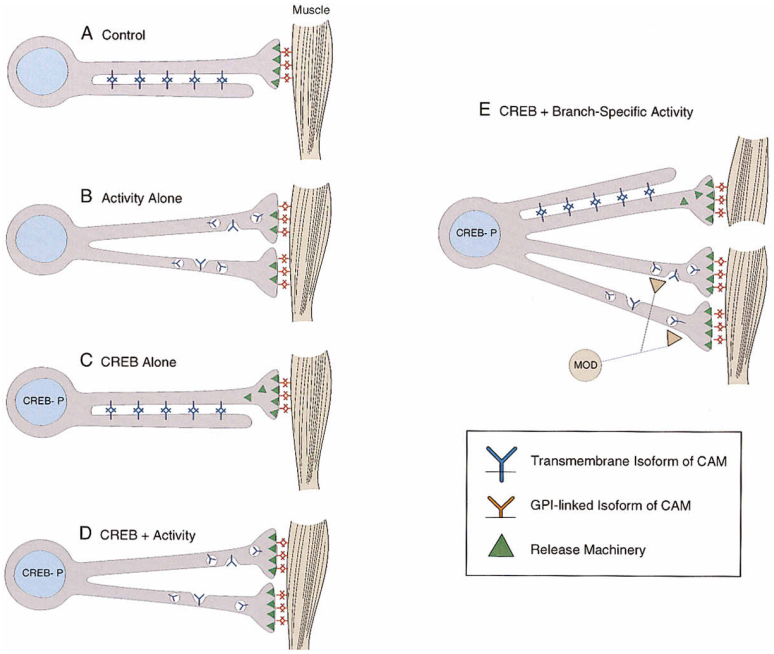

Figure 1. A Potential Mechanism Whereby Activity-Induced Down-Regulation of Cell Adhesion Molecules and CREB-Mediated Gene Expression Lead to Increased Synaptic Strength

(A) Initial, activity-independent state of synapse formation.

(B) Phosphorylation of CREB activates CREB-mediated transcription, which increases the amount of release machinery available in the cell. However, the newly synthesized components of the release machinery are not incorporated into pre-existing synapses. Therefore, without the growth of new synaptic contacts (which requires cell adhesion molecule [CAM] internalization), there is no increase in synaptic strength.

(C) Activity or an increase in cAMP signals the internalization of the transmembrane form of CAM from sites of homophilic interaction and removes a constraint on growth, thereby signaling synaptic sprouting. The internalized transmembrane CAM is then targeted for degradation and the membrane is recycled to sites of new synaptic growth. By contrast, GPI-linked CAM stabilizes pre-existing synapses. However, this synaptic sprouting is not accompanied by the recruitment of additional components of the release machinery. As a result, the existing components of the release machinery are redistributed among all the synaptic boutons, such that there is no overall functional increase in synaptic strength.

(D) CREB activation and activity-induced internalization lead both to synaptic sprouting and to the synthesis of rate-limiting components of the release machinery, with a resultant increase in synaptic strength.

(E) Local activity, as might be produced by a modulatory input (MOD) important for learning, can trigger branch-specific internalization of CAM, thereby marking the local postsynaptic site. Activation of CREB-mediated transcription leads to the synthesis of rate-limiting components of release machinery. As products of CREB-mediated transcription are only functional at sites of new synaptic growth, synaptic strengthening occurs in a branch- and synapse-specific manner.

a role in long-term synaptic plasticity of adult organisms. Exogenously added fragments of either L1 or NCAM were found to block LTP in rat hippocampal slice cultures (Lüthl et al., 1994). Moreover, in the chick, memory for passive avoidance, a form of associative learning, is blocked by intracerebral injection of antibodies against NCAM or L1 (Rose, 1995).

These earlier studies were correlative; they did not show a causal relationship between the down-regulation of cell adhesion molecules and growth of new synapses. Now, in their second paper, Schuster et al. have returned to this problem and provided a causal link. They took advantage of the increase in synaptic growth and effectiveness observed in the mutants *eag Sh* and *dnc*. *eag Sh* has mutations in two potassium channels resulting in increased neuronal excitability; *dnc* has a mutation in a cAMP-specific phosphodiesterase resulting in elevated intracellular cAMP concentrations. In both *eag Sh* and *dnc*, the increase in synaptic sprouting is suppressed by transgenes that maintain concentrations of Fas II, demonstrating that the down-regulation of Fas II is not only sufficient but also necessary for activity-dependent synaptic growth. However, despite a large increase in the number of boutons, synaptic effectiveness is not increased in Fas II hypomorphs. Indeed, evoked release from single boutons has a reduced quantal content, suggesting that the wild-type amount of release machinery was distributed throughout more boutons. Then what is required for the increase in synaptic transmission observed with activity-dependent synaptic plasticity?

One of the hallmarks of long-term synaptic plasticity in Aplysia, Drosophila, and rodents is its dependence on cAMP, protein kinase A (PKA)-mediated transcription, and in particular on cAMP response element–binding protein (CREB)-mediated gene expression. In the early 1970s, cell biological studies in Aplysia implicated cAMP and PKA as an important signaling pathway for simple forms of nonassociative and associative learning and for short-term memory. These studies found that sensitization and classical conditioning of the gill-withdrawal reflex in response to tail shock enhances the reflex response for a period ranging from several minutes to several weeks by recruiting the activity of serotonergic and other modulatory neurons. The modulatory neurons in turn increase the synaptic strength of the connections between the sensory and motor neurons of the reflex by enhancing transmitter release from the terminals of the sensory neurons. This increase is mediated in good part by cAMP and PKA. The same signaling system was soon implicated in Drosophila using a genetic approach. Working with a completely different learning task, olfactory discrimination, Seymour Benzer, Chip Quinn, Ron Davis, Yadin Dudai, and their colleagues identified three major memory mutations, *dnc*, *rutabaga*, and *amnesiac*, each of which involve one or another step in the cAMP pathway (Davis,

1996). Thus, whereas the cell biological studies in Aplysia showed how cAMP and PKA works on the cellular level, producing synaptic plasticity in a neural circuit that participates in learning and short-term memory, the complementary genetic studies in Drosophila showed that this signaling system is causally important for memory in whole animal behavior.

During the past decade, the complementary focus in these two systems turned from learning and short-term memory to long-term memory. And now the plot thickens. Work in Aplysia showed that the long-term memory for sensitization, produced by repeated, spaced tail shocks, is associated with a persistent increase in synaptic strength due to enhanced transmitter release resulting from the growth of new connections between the sensory and motor neurons. This requires new protein synthesis and gene expression involving both the activation of CREB-1 and the removal of a repressor (CREB-2). Parallel studies in Drosophila pinpointed a homologous switching mechanism. Thus, in the odorant-based learning task, expression of a CREB activator converts the need for spaced training to a single training trial (Yin et al., 1995). Mutation of the phosphorylation site for activated CREB abolishes this phenotype, indicating that CREB activation is sufficient for conversion of short-term memory to long-term memory.

In the third paper, Davis et al. (1996) test the hypothesis that CREB-mediated gene expression is required to render the new growth in synaptic structure functionally competent. They found that expression of a CREB repressor in the *dnc* mutant blocks functional, but not structural plasticity. Conversely, expression of a CREB activator in Fas II hypomorphs leads to an increase in both synapse number and synapse function. Expression of the CREB activator in wild-type animals does not increase synaptic function, indicating that CREB and Fas II act in parallel, and suggesting that the products of CREB-mediated transcription are only functional in boutons in which Fas II concentrations are reduced.

These three papers provide further causal evidence for the role of adhesion molecules and of CREB in activity-dependent synaptic remodeling. Moreover, the studies provide support for a model in which elevations in cAMP lead to down-regulation of cell adhesion molecules, producing structural growth, while activation of CREB-mediated transcription produces functional increases in transmission (Figure 1). In addition, these genetic studies from the Goodman lab have dissociated the structural mechanism for synaptic plasticity from the functional mechanism. This seems particularly important. One crucial assumption about the plasticity underlying learning and memory is that it can be synapse specific. The finding that long-lasting forms of synaptic plasticity require gene expression has raised the question of how synapse-specific plasticity can be achieved and maintained. Are the products of gene expression targeted to specific synapses, or are they delivered to all synapses, but only functional at synapses marked by previous activity? The finding that expression of the CREB activator only produces a functional increase in synaptic strength in Fas II hypomorphs where new synapses have sprouted suggests the interesting possibility that Fas II down-regulation may not only relieve a restraint on growth, but may also mark the synapse for structural and functional alterations (Figure 1). This hypothesis raises a further question about the nature of the still unknown but presumably complex signaling processes whereby the removal of cell adhesion molecules leads to the growth of new synaptic connections.

In addition to presenting important new findings, these papers also raise new questions. To begin with, why do Fas II null mutants undergo synapse elimination, whereas hypomorphs expressing 50% of wild-type Fas II concentrations undergo increased synaptic growth? One possible answer is that the different isoforms of Fas II, glycosyl phosphoinositide (GPI) linked and transmembrane, perform different functions. Perhaps the GPI-linked isoforms are activity independent and serve to stabilize synaptic contacts, whereas the transmembrane isoform is activity dependent and stabilizes contacts between fasciculated processes (Figure 1). If a minimal amount of GPI-linked Fas II were required for synapse stabilization, synapses would be eliminated in their complete absence, but a reduction to 50% of wild-type levels might suffice to stabilize synaptic contacts. The idea that Fas II isoforms might be differentially localized gains support from studies of apCAM in Aplysia. Here, the transmembrane isoform is internalized during facilitation, while the apCAM concentration at preexisting synapses, thought to be the GPI-linked isoform, actually appears to show an increase (Zhu et al., 1995; Bailey et al., 1996, Soc. Neurosci., abstract).

The finding that CREB activation occurs in parallel with the down-regulation of Fas II in Drosophila differs from the result in Aplysia where the two processes appear to be in series. In wild-type flies, the modest induction of the CREB activator gene that occurs with a mild heat shock used in these experiments is not enough to alter synaptic function. By contrast, strong induction of CREB used in experiments in flies showing that CREB is required for learning does result in increased synaptic function. Perhaps this represents a quantitative difference between learning and developmental plasticity. Alternatively, perhaps the degree of induction achieved in the learning experiments in Drosophila and Aplysia is too high and not completely physiological. For example, the experiments on induction of CREB-1 in Aplysia used bath application of serotonin, whereas the physiological release of serotonin in vivo is far more localized, quite likely restricted to specific synaptic regions. Perhaps in the intact Aplysia, the recruitment of CREB-1 may also be used only for synapse strengthening and not for growth, while a parallel cAMP-dependent pathway, possibly involving other PKA-dependent transcriptional activators, may be recruited for growth per se.

In the broadest sense, the studies from the Goodman lab and the earlier work on snails, flies, chicks, and mice speak to one of the unresolved questions in neurobiology: to what degree do the mechanisms of learning-related plasticity relate to those of developmental plasticity? Two opposing views on this question were clearly framed in the 1950s and 1960s by Paul Weiss and Roger Sperry. Weiss argued that learning-related activity is important in the development of neural circuits, whereas Sperry advocated that developmental plasticity and learning were not related. According to Sperry's view, the connections in the brain are programmed by recognition molecules that are expressed on appropriate pre-

and postsynaptic neurons of each synapse, so that connections form independent of activity or experience.

Most neurobiologists had adopted Sperry's perspective until quite recently. Now that a number of cell recognition and adhesion molecules have been identified, it is clear that Sperry's framework only applies to pathway selection and target region selection, the first two steps of the developmental program important for synapse formation. However, on reaching their targets, there is a third step, synapse selection, whereby each presynaptic axon is matched to its specific postsynaptic target neuron to produce the precise order required for mature function. This last step requires an activity-dependent mechanism. Thus, in a sense, the recent experiments in Drosophila and the earlier experiments in frogs and mammals have reconciled the previously irreconcilable views of Weiss and Sperry. The studies from the Goodman lab further illustrate why Sperry's view seemed so compelling even for the third step of synapse formation. Synapse formation in Drosophila also requires Sperry-like adhesion and recognition molecules, but now the isoforms of these molecules important for the last step of synapse formation are themselves activity dependent!

This argument suggests the interesting possibility that the fine tuning of connections late in development may be achieved by isoform switching (or recruiting) whereby an activity-dependent isoform may be substituted for the activity-independent forms used earlier for recognition events. One consequence of isoform switching is that it allows neuronal activity to modulate cell surface receptors and thereby cell–cell interactions at an appropriate stage of development. There are clear precedents for such developmental regulation of splicing and isoform switching, including the switch from transmembrane to secreted immunoglobulins during B cell development and the developmentally regulated expression of acetylcholine receptors, neurofilaments, and even NCAM in the nervous system.

Although these findings and the possibilities they suggest for the interactive strategies of different cell biological and genetic model systems are encouraging for the molecular study of synaptic and behavioral plasticity, we are only at the beginning of a long journey. The problems of memory storage and of the fine tuning of synaptic connections are deep and have two major components that need to be analyzed: the molecular mechanisms for regulating and stabilizing the strength of synaptic connections and the network properties whereby these mechanisms are distributed through the neural circuits that mediate behavioral plasticity. Although we know very little about the network properties of synapse stabilization, we are beginning to see in outline one mechanism for regulating synaptic strength: activity or modulatory signals increase cAMP and PKA which, perhaps together with other second messenger systems, leads to the activation of CREB and related transcription factors thereby strengthening newly grown synaptic connections. A key component of this mechanism is the regulation through internalization of cell adhesion molecules at the cell surface, leading to removal of a restraint on growth.

These considerations raise a final question: is the stabilization of synaptic connections by the cAMP- and CREB-triggered cascade restricted to development and learning? Or is this set of mechanisms also used more generally and in other contexts to change a transient, short-term functional synaptic change into a stable, self-maintained, long-term structural change? The early results from studies of drug abuse, alcohol addiction, and the action of antidepressant drugs bear watching.

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