

Commentary

Regulation of gene expression and its role in long-term memory and synaptic plasticity

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Histories of science yet to be written will view the latter half of this century as the Age of Molecular Genetics. From a flash of insight that yielded the double helix (1) to the first genetic clone of a mammal (2), molecular genetics has invaded every aspect of biological research. Initially, this molecular-genetic onslaught was limited to species, such as bacteria, yeast, nematodes, and fruit flies, whose size and life cycle constituted an economy of scale that was advantageous to breeding (3). With the introduction of gene-knockout techniques to mice (4), however, molecular genetics now is storming mammals (5, 6). In the broadest sense, the recent paper by Guzowski and McGaugh (7) represents a vanguard of this invasion. By using antisense oligonucleotides as “pharmaceutical” disruptors of gene expression, they have liberated molecular genetics from breeding. Endogenous regulation of gene expression has been outflanked by exogenous control.

In a narrower sense, Guzowski and McGaugh’s work comprises the latest installment in an emerging theme in molecular biology. Basic molecular and cellular processes appear to have evolved early in the animal kingdom, and they underlie more complex functions from development to behavioral plasticity. Time and again, biologists working on similar problems in diverse species have stumbled upon homologous genetic mechanisms—leading neurogeneticist J. C. Hall at Brandeis University to remark, “We’re all working on the same genes, we just don’t know it yet.”

Molecular-genetic studies of behavioral plasticity began more than 25 years ago with Seymour Benzer and coworkers (8), who identified *dunce* and *rutabaga* from forward-genetic screens for single-gene mutants with defective associative learning. Biochemical experiments established, and molecular cloning later confirmed, that these two genes encoded a cAMP-specific phosphodiesterase and adenylyl cyclase, respectively. Subsequent reverse-genetic experiments then demonstrated similar behavioral defects from gene disruptions of an α subunit of G protein, a catalytic subunit of cAMP-dependent kinase (PKA), and a regulatory subunit of PKA. Thus, cAMP signaling was implicated in *Drosophila* learning, as it also was in *Aplysia* (7).

Across the animal kingdom, long-lasting memory is dependent on protein synthesis and, for most tasks, is stronger and longer lasting after spaced training (multiple sessions with a rest interval between each) rather than massed training (multiple sessions with no rest interval between each; ref. 9). In flies, spaced training, in fact, is required to induce protein synthesis-dependent long-term memory (LTM). Conversely, inhibition of protein synthesis completely blocks LTM after spaced training without affecting learning, early memory, or long-lasting memory after massed training (10).

From a molecular perspective, protein synthesis often can involve regulation of gene expression by nuclear transcription factors—some of which are known to be cAMP-responsive (11,

12). Thus, Yin *et al.* (13) cloned *dCREB2*, a fly homolog of rat CREB that is alternatively spliced to yield one protein isoform that acts as a cAMP-responsive activator of (CRE-mediated) gene expression and another isoform that acts as a repressor of the activator. Transgenic flies then were bred that inducibly express either CREB repressor or CREB activator. As they had hoped, Yin *et al.* (14) found that expression of CREB repressor blocks the protein synthesis-dependent LTM induced by spaced training, without affecting learning or early memory formation. This result prompted Bourchouladze *et al.* (15) to look at memory formation in mutant mice homozygous for a partial knockout of CREB (16, 17). Here too, LTM of cued or contextual footshock conditioning was found to be disrupted, with no apparent effect on learning or early memory formation. These behavior-genetic studies thus established a conserved role for CREB and the regulation of gene expression in the formation of long-term memory.

The integrative use of psychological manipulation (training protocols), pharmacology, and genetics in the study of fruit fly memory formation led to proper interpretation of results from experiments on transgenic flies carrying CREB activator. Memory formation after spaced training was normal in these flies; induced expression of CREB activator neither enhanced nor suppressed LTM. Instead, maximal LTM was formed after only one training session—the functional equivalent of a “photographic memory” (18).

In molecular-genetic terms, these opposing effects of CREB activator and repressor indicated that CREB acts as a “molecular switch” during the induction of LTM. This notion led Yin *et al.* (18) to propose a (Δ C) model of LTM formation, in which the ratio of CREB activators to repressors sets the switch to one of three functional states (Fig. 1). If activator levels predominate, then the switch is “on” and LTM ensues after one training session. If repressor levels predominate, then the switch is “off” and LTM is blocked. If activator and repressor levels are equal, then a single training session or massed training produces only a transient increase in CREB activator, which is insufficient to induce (maximal) LTM. Consequently, spaced training is required to induce LTM. In this state, then, the CREB switch acts as an information filter; the only new experience stored in LTM is that which recurs at discrete intervals (19).

Generalization of this Δ C model of LTM formation to mammals led to the speculation that the activator/repressor ratio might reflect the net action of protein isoforms from CREB, CREM, and possibly ATF1, all of which are cAMP-responsive CREB family members (20, 21). In this context, the CREB[−] knockout in mice might simply have reduced the activator/repressor ratio rather than blocked CREB-mediated gene expression altogether—leading to the prediction that spaced training (but not massed training) would “rescue” the LTM deficit in CREB[−] mutant mice. Kogan *et al.* (22) largely have confirmed this prediction for three different tasks (con-

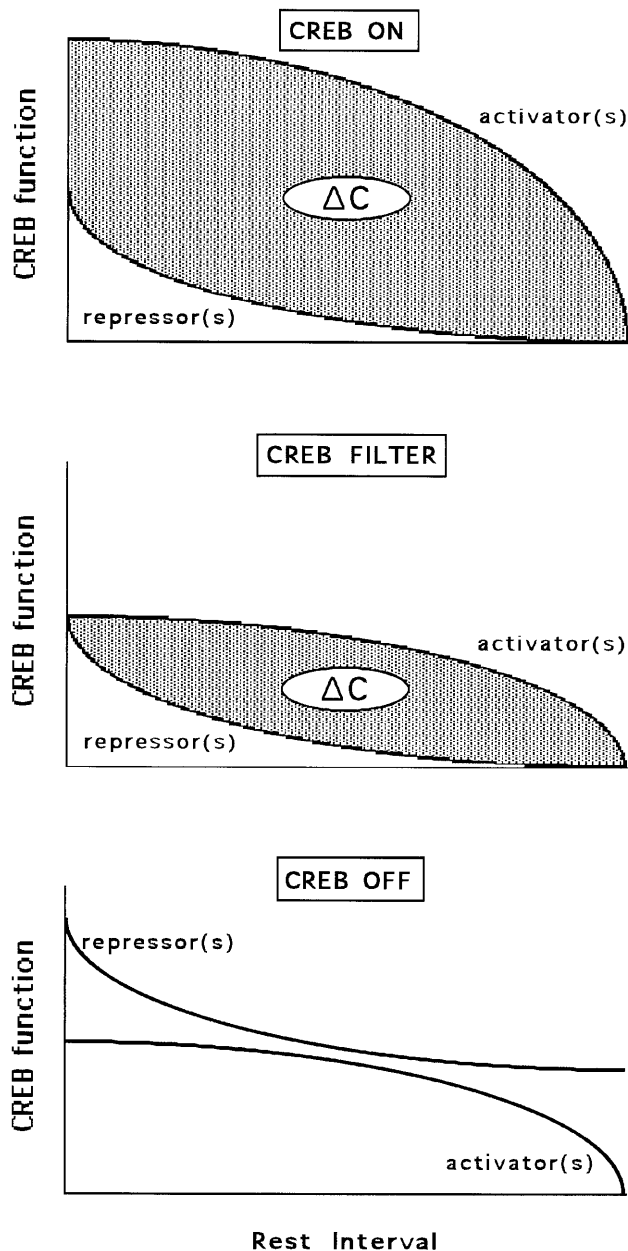


FIG. 1. Functional model for CREB as a molecular switch controlling the induction of LTM formation. (Top) When CREB activator(s) predominate over repressor(s), the CREB switch is "on" and LTM is induced after just one training session (which is normal for some tasks). (Middle) When CREB activator(s) and repressor(s) are equal, the CREB switch acts as an information filter and induction of LTM requires spaced training (which is the case for most tasks). (Bottom) When CREB repressor(s) predominate over activator(s), the CREB switch is "off" and LTM, even after spaced training, is blocked.

textual footshock conditioning, Morris water maze, and food preference).

Guzowski and McGaugh (7) have extended to rats the general observation that altered expression of CREB specifically disrupts long-term memory. Moreover, by injecting antisense oligos directly into the dorsal hippocampus just 6 or 26 hr before (spaced) training in Morris water maze, they have gained exquisite spatial and temporal control over these genetic manipulations (cf. ref. 6). Thus, transient disruption of CREB in the dorsal hippocampus of adult rats is sufficient to suppress LTM. Maldevelopment is not an issue.

Interestingly, CREB expression was reduced (as expected) 6 hr after injecting the antisense oligos but was enhanced

14–20 hr later. At the molecular level, this rebound effect reveals autoregulation of CREB (23). At the behavioral level, however, this molecular observation appears confusing. Opposite effects on CREB expression both act to reduce LTM—a result inconsistent with previous studies in flies and mice. Importantly, the anti-CREB antibodies used by Guzowski and McGaugh to monitor levels of expression do not distinguish among the various CREB protein isoforms, which include both activators and repressors (24). Hence, the CREB activator/repressor ratios 6 and 20–26 hr after injection remain unknown, in spite of the overall levels of CREB expression. To this end, the ΔC model predicts that, when specific isoforms are identified, repressors will predominate at both time points.

The evolutionary conservation of molecular genetics dictates that mechanistic insight to behavioral plasticity will be augmented by the "horizontal integration" of data across various animal model systems. Certainly, these studies of CREB in flies, mice, and rats highlight this notion. The power of molecular genetics, however, also lies with "vertical integration." The gene becomes an independent variable, experimental manipulations of which can be used to identify concomitant effects at multiple levels of analysis. This aspect of molecular genetics has been demonstrated elegantly by studies of CREB's role in synaptic plasticity both in *Aplysia* (25–27) and *Drosophila* (28).

In the *Aplysia* sensory motor neuron coculture system, injection into the sensory neuron nucleus of an anti-CREB activator antibody blocks the structural and functional changes associated with the appearance of long-term synaptic facilitation (LTF) after spaced applications of serotonin. Conversely, these structural and functional changes occurred after only one serotonin application when the sensory neuron nucleus was injected with anti-CREB repressor antibody. At the larval neuromuscular junction in fruit flies, induced expression of CREB repressor blocks the increase in synaptic transmission, but not the increase in synaptic arborization, which is usually seen in *dunce* mutants. Induced expression of CREB activator, on the other hand, enhances synaptic function in *fasciclin II* mutants, which usually show increased synaptic arborization without increased synaptic transmission. Thus, molecular-genetic manipulations of CREB activators and repressors in both cellular model systems produce opposite effects on synaptic plasticity (see ref. 29).

Significantly, the key to understanding CREB's role in *Aplysia* synaptic plasticity derived from the critical properties of CREB-mediated LTM formation defined in the fly behavioral experiments. Parametric studies in normal flies revealed two critical behavioral properties of LTM formation: LTM accumulated incrementally as a function of the number of spaced training sessions and as a function of the rest interval between each training session. Yin *et al.* (18) then deliberately designed their experiments on transgenic CREB activator flies to evaluate these two properties. Flies were subjected to 1, 10-massed, or 10-spaced training sessions. Comparisons of the latter two groups and the former two groups assessed the "rest interval" and "number of training sessions" properties, respectively. What Yin *et al.* (18) discovered was that overexpression of CREB activator enhanced LTM by abrogating both requirements for spaced training.

Working at the cellular level, Kandel *et al.* (30) originally injected sensory neuron nuclei with anti-CREB repressor antibody (which presumably results in a relative increase in endogenous CREB activator) and evaluated LTF only after the usual five spaced applications of serotonin. In this context, they observed no effect of their antibody treatment and, thus, concluded that this particular CREB isoform was not involved in LTF formation. After learning of the fly experiments, Bartsch *et al.* (27) then reinjected sensory neuron nuclei with anti-CREB repressor antibody and also discovered that only one serotonin application was sufficient to induce LTF.

This story underscores a more general problem with attempts to identify molecular and cellular substrates of behavioral plasticity. Single genes tend to function in several distinct cellular processes, (i.e., they are pleiotropic). CREB, in fact, is a classical case. It responds to many signals (growth factors, calcium, cAMP, etc.) and functions in many cellular processes (stress, injury, plasticity, etc.), even within neurons (31–37). Hence, one does not necessarily know which particular molecular or cellular function is relevant to behavioral plasticity. The molecular-genetic approach resolved this issue for CREB. By manipulating CREB as an independent variable in behavioral experiments, the critical parameters of long-term memory formation were identified. In the cellular studies, then, analogous genetic manipulations and experimental parameters produced analogous (opposing) effects on synaptic plasticity. The approach of Guzowski and McGaugh (7) puts another twist on this theme. By limiting CREB disruption in time and space, they have unambiguously delimited an anatomical region of the brain (dorsal hippocampus) involved with long-term memory formation of a spatial task (water maze). These are vertical integration at its best.

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