Photographic memory in flies

A new study of olfactory memory formation in *Drosophila* shows that a delicate balance of CREB transcription factor activity may play a decisive role in triggering long-term memory.

Memory comes in many forms. How is it that some memories last a lifetime, while others seem to fade as soon as they are formed? The physiological basis of memory formation and retention is of great interest to many of us, whether we are motivated by the personal desire to have a 'good memory' or are simply reflecting the increasing public concern about the memory loss that accompanies dementia.

A universal feature of long-term memory is that it displays distinct temporal phases. Soon after learning occurs, memory is highly labile and sensitive to disruption; the administration of anesthetics or protein synthesis inhibitors selectively erases recent memories, while sparing those acquired a long time ago. The conversion of a recent memory into a long-lived form, a process referred to as memory consolidation, is thus thought to involve structural changes in neuronal circuitry that are brought about by the synthesis of new proteins [1]. Very little is known, however, of the underlying molecular machinery of memory consolidation. The identification of molecules that regulate memory consolidation would be greatly facilitated by the availability of experimental systems amenable to genetic and/or biochemical analysis. Though many organisms, including humans, have been the subject of learning and memory studies, it may be surprising to some that the fruitfly *Drosophila melanogaster*, an organism not renowned for its ability to learn and remember, is providing us with much information about memory mechanisms [2].

A commonly used behavioral paradigm for assessing learning and memory in flies involves classical conditioning of the odor-avoidance response. In this paradigm, the flies are consecutively exposed to two distinct odors, one of which is accompanied by an electric shock; subsequently the flies remember to avoid the odor that had previously been coupled to the shock. With repeated training, it is possible to induce long-term memory (LTM) for the odor–shock association that is dependent on new protein synthesis. Tully and colleagues have carefully analyzed the requirements of LTM formation, and their recent results bring a surprising insight into the molecular basis of memory consolidation [3–5].

A peculiarity of LTM formation is that it not only requires multiple reinforcement of training, but each training session must be followed by a rest period ('spaced training'). In the absence of rest intervals ('massed training'), another distinct form of memory is produced that decays much sooner than LTM and does not require protein synthesis. How, therefore, does spacing facilitate the establishment of LTM? A first key to this question was provided by the observation that overexpression of a dominant-negative form of cAMP responsive element binding protein (CREB), a transcription factor, specifically blocks LTM formation [4]. Gene expression mediated by the CREB family of proteins is therefore necessary for LTM induction; the sensitivity of LTM to protein synthesis inhibitors is perhaps reflected by the ensuing translation of CRE-driven transcripts.

How then does spacing affect CREB's ability to trigger LTM? Yin *et al.* [5] have now created a strain of transgenic fly that carries the gene for the activator isoform of CREB under the control of an inducible promotor. Surprisingly, overexpression of the activator isoform of CREB enhanced LTM, abolishing the requirement for multiple training sessions for induction of LTM [5]. That is, in the presence of excess CREB activator, a single training session alone yielded a long-lasting olfactory memory comparable to the LTM produced by spaced training in wild-type flies. In effect, the mutant flies have gained an olfactory equivalent of photographic memory under these conditions.

Taken together, these results indicate that LTM formation requires a threshold or critical concentration of the activator CREB isoform, moreover, only spaced training can, in usual circumstances, allow sufficient levels of this isoform to accumulate to trigger LTM. What is the molecular basis for this dependence of CREB activator production on training intervals? Based on their observations, Tully and colleagues [5] have suggested the following interesting hypothesis. A single training session activates both activator and the inhibitor isoforms of CREB; the activator isoform is assumed to accumulate preferentially because the inhibitor isoform undergoes functional inactivation more rapidly than the activator isoform. Intervals between training, therefore, allow sufficient time for the concentration of the activator to increase relative to that of the inhibitor.

Although this model explains beautifully the need for spaced training to trigger LTM, much needs to be learned about the detailed mechanisms by which *Drosophila* CREB regulates LTM formation. Moreover, the *Drosophila* results indicate that activation of CREB, whose overall requirement for consolidated memory formation is highly conserved across animal phyla [6], may be differentially regulated for distinct memory pathways.

We now turn to the sea snail *Aplysia*, an organism in which the role of a cAMP messenger pathway in learning and memory has been well established. The best-studied learning paradigm involves a component of the sensitization of gill withdrawal reflex that has been reproduced in cell culture. In this system, the monosynaptic connection between a sensory and a motor neuron displays both short-term and long-term synaptic enhancement following applications of serotonin, a neurotransmitter released during *in vivo* training. Like LTM formation in flies, long-lasting synaptic enhancement in *Aplysia* requires protein synthesis and is observed only with repeated application of serotonin. A series of elegant experiments has demonstrated that long-term synaptic facilitation is brought about by activation of cAMP-dependent protein kinase (PKA) which, upon translocation into the nucleus, activates CREB [7]. The requirement for CREB in *Aplysia* long-term facilitation is comparable to its role in *Drosophila* LTM.

In the case of *Aplysia*, translocation of PKA into the nucleus was observed only under conditions that induced long-term facilitation [8]. It thus appears that the critical, and perhaps rate-determining, step of long-term facilitation is the appearance of PKA in the nucleus. Extrapolating these observations from *Aplysia* to *Drosophila*, one might have expected that overproduction of the activatable isoform of CREB would have no effect on LTM formation unless there is a concomitant increase in the level of active, nuclear PKA. But such overexpression actually resulted in robust LTM in the absence of multiple training [5]. The stimulatory effect on LTM was nevertheless dependent on the presence of the putative PKA phosphorylation site on this CREB isoform, supporting the notion that, like *Aplysia* long-term facilitation, *Drosophila* LTM is induced by the activation of a cAMP cascade. In *Drosophila*, each training episode must therefore activate a signal transduction cascade that reaches all the way to the nucleus regardless of the outcome of training. In effect, flies have a highly sensitized system to produce LTM that appears to be at the mercy of CREB activity. It remains to be seen whether a cAMP signaling pathway indeed regulates CREB activity for LTM induction *in vivo*.

CREB is a member of the basic region/leucine zipper (bZip) family of transcription factors, and was initially identified as a nuclear factor that promotes transcription through cAMP response elements (CREs) [9]. The bZip domain directs DNA binding as well as dimerization of CREBs, and transcription is activated by phosphorylation of a single serine residue by active PKA. In mammalian systems, there is a subfamily of CREBs with many homologous members, some of which act as negative regulators of CRE-driven transcription. The generation of different forms of each CREB family member by alternative RNA splicing, and the ability of CREBs to form heterodimers through the conserved bZip domain, make CRE-dependent regulation highly complex.

Drosophila is also likely to have a large family of CREBs. Although Yin *et al.* [4,5] have examined the potential roles of particular splice variants of *Drosophila* CREB (see also [10]) in LTM induction, we do not yet know whether these particular isoforms are involved in LTM formation in wild-type flies. For example, overexpression of the CREB activator isoform may have recruited a parallel memory pathway that normally remains silent. Identifying the *Drosophila* neurons involved in LTM production, and finding a physiological correlate of LTM, would facilitate the discovery of not only the specific CREB isomer(s) involved in LTM induction but also the series of events by which active CREB is accumulated to trigger LTM. Inhibitory isoforms of CREB, as suggested by Tully's model, may play a role in suppressing activation of CREB. CREB activity may also be modulated by co-activators [11], and feedback mechanisms may autoregulate CREB production. We are thus beginning to understand the molecular basis of long-term memory formation, but may so far have seen just the tip of the iceberg. The complexity of CREB function alone suggests that future studies would undoubtedly uncover a wealth of interactive regulatory processes that shape our memory.

References

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