classical kinases share essentially no sequence similarity with LTRPC7 or other atypical α-kinases. Several amino acid residues known to be crucial for catalysis in PKA are conserved in LTRPC7, and the locations of these residues within the kinase domains of PKA and LTRPC7 are similar. There are, however, structural differences between the two kinase domains. For example, LTRPC7 contains a zinc-binding motif not present in classical kinases, which is predicted to be important for the structural stability of the kinase catalytic domain. Mutations in this motif markedly reduce LTRPC7 kinase activity, consistent with the structural evidence that it is important for catalysis. A key question is whether kinase activity is important for operation of the LTRPC7 channel. Although Runnels *et al.* (*1*) present evidence that kinase activity is essential for channel gating, this conclusion has been challenged by Nadler *et al.* (*2*), and the controversy has yet to be resolved.

Ion channels, like so many other cellular proteins, are regulated by protein phosphorylation and dephosphorylation (*7, 8*). The idea that protein kinase and phosphoprotein phosphatase activities might be associated with ion channels is not new. Kinase activity has been detected in some purified ion channel preparations (*9, 10*), and functional experiments also suggest that kinase and phosphatase activities are intimately associated with the gating of certain channels (*11–15*). More recent biochemical and molecular approaches have made it clear that channels can associate with protein kinases and phosphatases, either with the help of anchoring or scaffolding proteins (*16–19*) or directly (*20–24*). What is most exciting about the new studies is the possibility that channel and kinase activities are intertwined because they are both encoded in the sequence of a single protein (see the figure). It would appear that evolution has chosen a variety of ways to ensure that these activities remain together.

Apparently, LTRPC7 is not the only example of an ion channel with enzymatic activity. LTRPC2, another member of the long TRP channel family, contains ADPR pyrophosphatase activity (*4*) and its gating is controlled by intracellular ADPR (*4, 5*). Like LTRPC7, LTRPC2 is a cation channel expressed by many different tissues. Here again, there is some controversy about channel gating. Both groups studying the gating of LTRPC2 agree that ADPR activates (opens) the channel. On the other hand, nicotinamide adenine dinucleotide (NAD), a precursor of ADPR, is described by Perraud *et al.* (*4*) as having no effect on channel activation, but by Sano *et al.* (*5*) as causing delayed channel activation when included in the whole-cell recording pipette. The interpretation of these results is complicated by the fact that NAD can be metabolized to ADPR, and hence the exact effect of NAD on LTRPC2 channel activation remains unclear. The identification of pyrophosphatase activity specific for ADPR within the carboxyl-terminal region of LTRPC2 suggests that this channel may have evolved an enzymatic activity that limits the availability of its physiological activator, rather than one that promotes its activation, as may be the case for LTRPC7.

Where does the field go from here? These are unlikely to be the last reports of enzymatic activity in ion channel proteins. For example, where there is a protein kinase there is often a phosphatase lurking nearby, and it would come as no surprise to find that some ion channels have phosphatase activity. Finally, the intimate association of enzymatic activities with ion channels raises the intriguing question of whether the ions that flow through the channel—or the conformational changes associated with channel gating—may contribute to the regulation of the enzyme. In other words, are the long TRP channels examples of ion channels with their own personal modulatory enzymes, or are they enzymes that are regulated by their own personal ion channels? Perhaps they are both. Stay tuned for the answer.

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PERSPECTIVES: NEUROBIOLOGY

Learning How a Fruit Fly Forgets

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Whether in humans or fruit flies, memories must be acquired, stored, and retrieved. Although these three stages are separate, distinguishing between them experimentally is tricky. A report by McGuire *et al.* on page 1330 of this issue (*1*) and another published in *Nature* by Dubnau *et al.* (*2*) provide a convincing dissection of olfactory memory in the fruit fly *Drosophila*. With the help of a temperature-sensitive *shibire*^{ts1} (*shi*^{ts1}) transgene, both groups conclude that synaptic output from structures in the fly brain called mushroom bodies (MBs) is required for recall of olfactory memory but not for its acquisition or storage.

The *shibire* gene encodes dynamin, a mictrotubule-associated guanosine triphosphatase that is important for synaptic vesicle recycling in neurons and hence for synaptic transmission (*3*, *4*). The temperature-sensitive allele *shi*^{ts1} encodes a version of dynamin that is defective if the temperature is raised above 29°C (the restrictive temperature). When *shi*^{ts1} flies are exposed to the restrictive temperature, synaptic vesicles can no longer be recycled and synaptic transmission throughout the central nervous system ceases (*5*). The *shi*^{ts1} allele was originally identified in flies that became paralyzed at the restrictive temperature (because neural activity was blocked) but moved normally at the permissive temperature (20°C) (*6*). If the cause of paralysis is restricted to a known subset of neurons, then the involvement of these neurons in specific behaviors can be tested (*7*). For example, expression of a *shi*^{ts1} transgene in fly photoreceptor neurons causes blindness at elevated temperature (30°C) and expression of the same transgene in cholinergic neurons causes paralysis at this temperature. These handicaps are quickly reversed if the flies are returned to a lower temperature (20°C).

Fruit flies learn to avoid an odor that is administered in association with an electric shock (*8*), and such learning depends on the MBs. Flies that lack MBs can smell odors and sense electric shocks, but they

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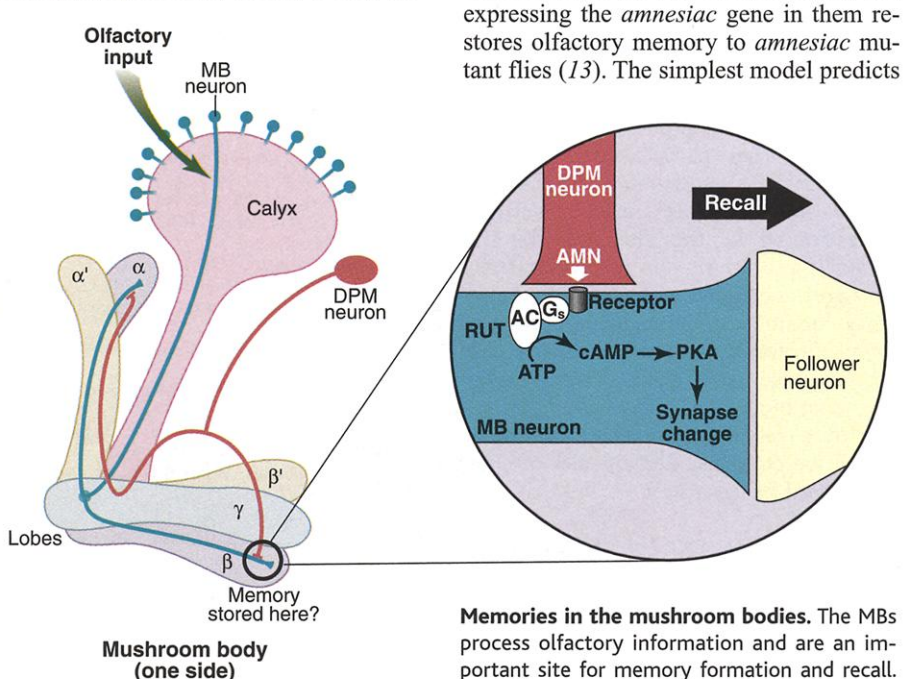
cannot form an association between the two cues (*9*). McGuire *et al.* (*1*) and Dubnau *et al.* (*2*) engineered flies so that they expressed *shi*^{ts1} in MB neurons and then taught these transgenic flies to avoid an odor associated with an electric shock. By changing the temperature at which the flies were learning or performing olfactory memory tasks, the investigators were able to switch off MB synaptic output during learning (acquisition), during testing (retrieval), or between training and testing (storage). With this approach, they could identify which stages of memory—acquisition, storage, or retrieval—depended on synaptic transmission from MB neurons.

There are differences between the two studies. Dubnau *et al.* (*2*) used less-selective promoters to drive transgene expression in MB neurons and tested immediate and 30-minute memory, whereas McGuire *et al.* (*1*) used more-selective promoters and tested immediate and 3-hour memory. Nonetheless, the take-home result is the same—MB synaptic output is required for olfactory memory recall, but not for its acquisition or storage.

The cAMP second messenger pathway is required for learning in flies (*10*). Several genes encoding components of the pathway are expressed at high levels in the MBs (*10*). Importantly, deregulated cAMP signaling in the MBs abolishes learning in flies (*11*), whereas restoring normal cAMP signaling selectively to the MBs restores olfactory learning (*12*). A study of the forgetful fly mutant *amnesiac* also implicates the MBs in memory. The *amnesiac* gene is highly expressed in two dorsal paired medial (DPM) neurons that specifically innervate the axon terminals of the entire MB lobe ensemble and are thought to modulate MB activity (see the figure). These neurons are critical for memory, and expressing the *amnesiac* gene in them restores olfactory memory to *amnesiac* mutant flies (*13*). The simplest model predicts that important learning-related synaptic modifications occur in the presynaptic termini of MB neurons and that they are mediated, at least in part, by cAMP signaling. The results of McGuire *et al.* (*1*) and Dubnau *et al.* (*2*) tell us that output from these putatively modified MB synapses is indeed essential for the recall of learned olfactory information.



Memories in the mushroom bodies. The MBs process olfactory information and are an important site for memory formation and recall. The MB axon terminals (blue) are innervated by a modulatory DPM neuron (red). It is likely that memory is encoded as a cAMP-dependent modification of MB presynaptic termini. Release of AMN neuropeptide (encoded by the *amnesiac* gene) by the DPM neuron may contribute to adenylyl cyclase (AC) stimulation and cAMP signaling. Synaptic transmission at these modified MB synapses is required for the recall of olfactory memory. The alpha and beta lobes may be the most important part of the MB for memory recall. G_s, an adenylyl cyclase–stimulatory G protein; RUT, the adenylyl cyclase encoded by the *rutabaga* gene.

It is possible that converging sensory pathways carrying information about the odor and electric-shock stimuli trigger cAMP signaling in the MBs through the coincident activation of the calcium-stimulated adenylyl cyclase encoded by the *rutabaga* gene. However, we know only that olfactory sensory information reaches the MBs through their dendrites, which reside in a part of the MB called the calyx. We do not yet know where the electric shock pathway intersects the olfactory pathway or whether it ever reaches the MBs.

In MB-*shi*^{ts1} transgenic flies at the restrictive temperature, the MBs cannot pass on information through chemical synapses (*1*, *2*). However, presumably they are still electrically active (they may have electrical synapses). In addition, the cAMP cascade and other biochemical pathways (that may mediate plasticity) should be intact. These two new papers tell us that learning occurs without MB *output*—not that learning is independent of the MBs. Therefore, odor and shock stimuli may be associated within MB neurons, or earlier in the olfactory circuitry before the olfactory stimulus reaches the MBs. Extensive work in honeybees implicates the antennal lobes as well as MBs in olfactory learning (*14*).

Where in this neural ensemble are specific memories encoded? It seems unlikely that all 5000 MB neurons are required for the fly to distinguish between two odors. McGuire and colleagues expressed the *shi*^{ts1} transgene in subsets of MB neurons and from their findings predict that the MB alpha and beta lobes may be the crucial players in olfactory memory recall. Further refining the involvement of particular MB neurons in memory should be forthcoming.

These two papers highlight the benefits of studying learning and memory in the fruit fly. In addition to the advantages that the fly offers—forward genetics, mutant selection, and modest brain complexity—techniques to express genes in defined brain regions of the fly currently surpass those available for mammals. Ultimately, we wish to understand how and where memories are stored. Further molecular analysis of *Drosophila* learning mutants, coupled with studies expressing powerful effector-transgenes such as *shi*^{ts1} in more defined sets of MB neurons, should help to identify the exact neurons and synapses from which individual memories are read out.

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