of CRL4–Cdt2 recruitment onto PCNA (by a point mutation in PCNA) both abolish damage-induced Spd1 turnover. The authors identify a somewhat degenerate PIP (PCNA Interaction Protein)-degron in Spd1, and provide genetic evidence that this region is important for Spd1 turnover. Finally, by means of bimolecular fluorescence complementation (BiFC) it is shown that Spd1 and PCNA can interact *in vivo*, in a PIP-sequence-dependent manner. Taken together, these observations demonstrate that Cdt2 induction is necessary but not sufficient for Spd1 proteolysis. In addition, interaction of Spd1 with PCNA is required to activate the CRL4–Cdt2 E3 ligase, and this is only possible when active replication causes loading of PCNA onto DNA.

What is the biological function of Spd1? We cannot exclude that the protein is merely ensuring repression of total dNTP production when DNA synthesis is not taking place, but perhaps Spd1 plays an important role in targeting dNTP production to replication and repair factories. In general, the phenotypes associated with excess Spd1 are much more severe than those caused by Spd1 loss. Elevated Spd1 levels (observed e.g. in *cdt2*-deleted cells) cause a strong checkpoint activation, presumably signalled by single-stranded DNA formation due to lowering of dNTP pools upon RNR inhibition (although it cannot be excluded that elevated Spd1 levels may also cause checkpoint activation by jamming other processes at PCNA). In view of the new findings of Salguero *et al.* [16], it is tempting to speculate that an initial increase in Spd1 concentration caused by blocked replication, e.g. after damage, might contribute to checkpoint activation. The activated checkpoint then would cause Cdt2 induction [6] and repair synthesis, which depends on PCNA loading. The combination of the two would drive Spd1 degradation to provide dNTPs for repair synthesis [9,16]. It is intriguing that S phase in the presence of Spd1 in fission yeast resembles replication stress imposed by certain oncogenes in mammalian cells, and it will be interesting to learn if metazoan cells also modulate their RNR activity by small IDPs. Yet, the limited sequence conservation of this interesting protein family has thus far prevented their identification.

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Mushroom-Body Memories: An Obituary Prematurely Written?

Studies on insect olfactory learning have established the mushroom bodies as key brain structures for the formation of long-term memory (LTM). Two new neurons in the fly brain are reported now as essential sites for LTM formation, while mushroom bodies are claimed to be unnecessary to this end.

Ronald L. Davis[1] and Martin Giurfa[2,3]

Insects, with their remarkable learning capacities and relatively simple and accessible nervous systems, provide powerful models for studying associative learning and memory [1–4]. The combination of procedures for classical conditioning with the disruptive methods of genetics has made it possible to identify cellular and molecular substrates of memory in some species, such as the honey bee *Apis mellifera* and the fruit fly *Drosophila melanogaster*. These studies established the mushroom bodies, paired central structures in the insect brain, as a key site for the formation of long-term memories. A recent study [5] of fruit fly learning has attributed this role to two neurons external to mushroom bodies and

questioned the involvement of mushroom bodies in the formation of long-term memory (LTM). We discuss these findings in the light of methodological and conceptual views on LTM formation.

The fruit fly has played a pivotal role in unraveling the molecular genetic bases of memory [3,4,6]. Flies can be trained to associate an odor (the conditioned stimulus, CS) with an aversive electric shock (the unconditioned stimulus, US) in the laboratory. Typically, groups of flies are presented with two different odors, one paired with electric shock (CS+), and another unpaired with shock (CS-) [7]. Retention is afterwards measured in a T-maze where conditioned flies must choose between the CS+ and the CS- (Figure 1A). LTM, which lasts for days and requires protein synthesis, is generated by repeating the training cycles 5–10 times and spacing them by 15 minutes ('spaced conditioning'). If training cycles are spaced by only 30 seconds ('massed conditioning'), the memory created is independent of protein synthesis [8] (Figure 1B).

The processes of learning and then remembering odors are both mediated by the olfactory nervous system. Odor processing starts at the olfactory receptor neurons located on the antennae and maxillary palps, with olfactory information being conveyed through the glomeruli of the antennal lobe to the mushroom bodies and lateral horn via projection neurons (Figure 2A). At the level of the antennal lobe, each odor is encoded as a specific pattern of glomerular activation that is conserved between flies [9]. The mushroom bodies contain about 5000 intrinsic neurons called Kenyon cells. The response of Kenyon cells to odors is highly selective and each odor is represented sparsely among the cells [10]. Kenyon cells are not identical: α/β, α′/β′, and γ Kenyon cells can be distinguished by functional and anatomical criteria (Figure 2A). The α/β and α′/β′ axons divide in two branches, with the α and α′ branches constituting the vertical lobes of the mushroom bodies while the β and β′ branches constitute the horizontal lobes. The γ neurons project only to a horizontal lobe.

At least six memory traces have been localized at different levels of the olfactory circuit of the fly, including the projection neurons of the antennal lobe and all three classes of Kenyon cells [11]. These memory traces have been identified using functional imaging to visualize and measure calcium influx or synaptic release in response to the CS+ after learning. The memory traces that occur in the α/β and γ Kenyon cells appear to represent LTM, as they form only many hours after spaced and not massed conditioning, and are disrupted in parallel with LTM by feeding inhibitors of protein synthesis to the flies before training (for α/β Kenyon cells) or by expressing a dominant negative of the transcription factor dCreb2 in these neurons [12,13]. Furthermore, the formation of the α/β neuron memory trace is blocked in parallel with LTM in 26 different LTM mutants [14].

Despite these and other cumulative findings showing that Kenyon cells host long-term memory traces, a recent paper by Chen *et al.* [5] has provided a dissenting view on the role of mushroom bodies for LTM formation in the fly. At the same time, the authors identified a new neuronal substrate for LTM formation provided by two dorsal-anterior-lateral (DAL) neurons, which are located outside the mushroom bodies but are nevertheless connected to them (Figure 2A).

Chen *et al.* [5] used KAEDE, a green fluorescent protein (GFP) variant that changes its structure irreversibly to a red fluorescent protein (RFP) upon ultraviolet irradiation, and a temperature-sensitive ribosome-inactivating toxin that inhibits protein synthesis ($RICIN^{cs}$) (Figure 2B). Combining both tools and keeping flies at 18°C allows visualizing the synthesis of new proteins based on the accumulation of new GFP on the background of previously photoconverted RFP. In flies kept at 30°C after photoconversion, $RICIN^{CS}$ is activated and thus blocks the synthesis of new proteins. Consequently, new green KAEDE is not synthesized, and only red KAEDE is observed in neurons (Figure 2B).

Using these tools and spaced olfactory conditioning, Chen *et al.* [5] performed a behavioral screen searching for neurons that are involved in the formation of memories that are protein synthesis-dependent. The disruption of this process with $RICIN^{CS}$,

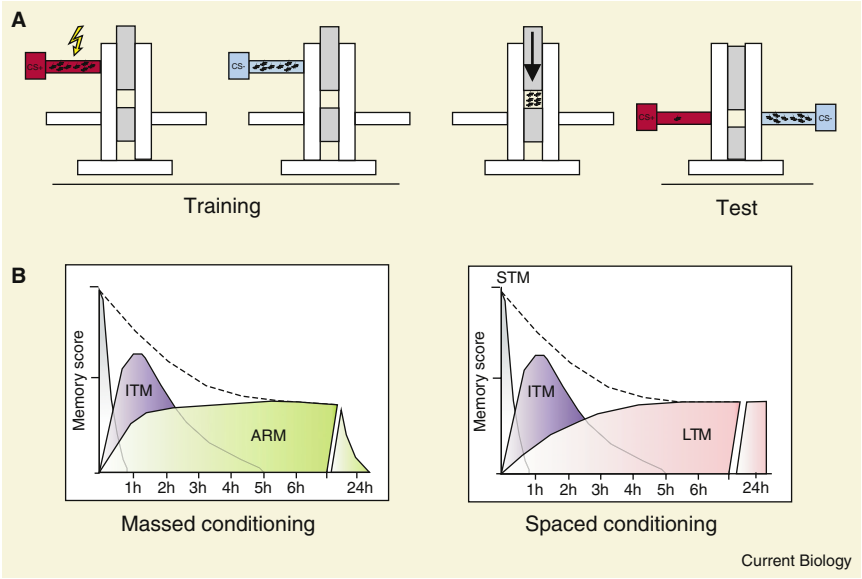

Figure 1. Olfactory learning and memory in *Drosophila melanogaster*.

(A) Olfactory conditioning. A group of flies is presented with an odor paired with electric shocks (CS+; red), and then with another odor not paired with shocks (CS-; blue). In a retention test, flies must choose between the CS+ and the CS- in a T-maze in the absence of shock. (B) One model for memory phases after aversive olfactory conditioning. Adapted from [18]. The dashed line represents the memory score of flies at different times after conditioning. Left panel: *memory phases after massed conditioning*; STM, short-term memory (0–30 min); ITM, intermediate-term memory (0.5–6 h); ARM, anesthesia-resistant memory, which is protein-synthesis-independent and decays in the day-range; right panel: *memory phases after spaced conditioning*; LTM, long-term memory, which is protein-synthesis-dependent and is maintained in the day-range.

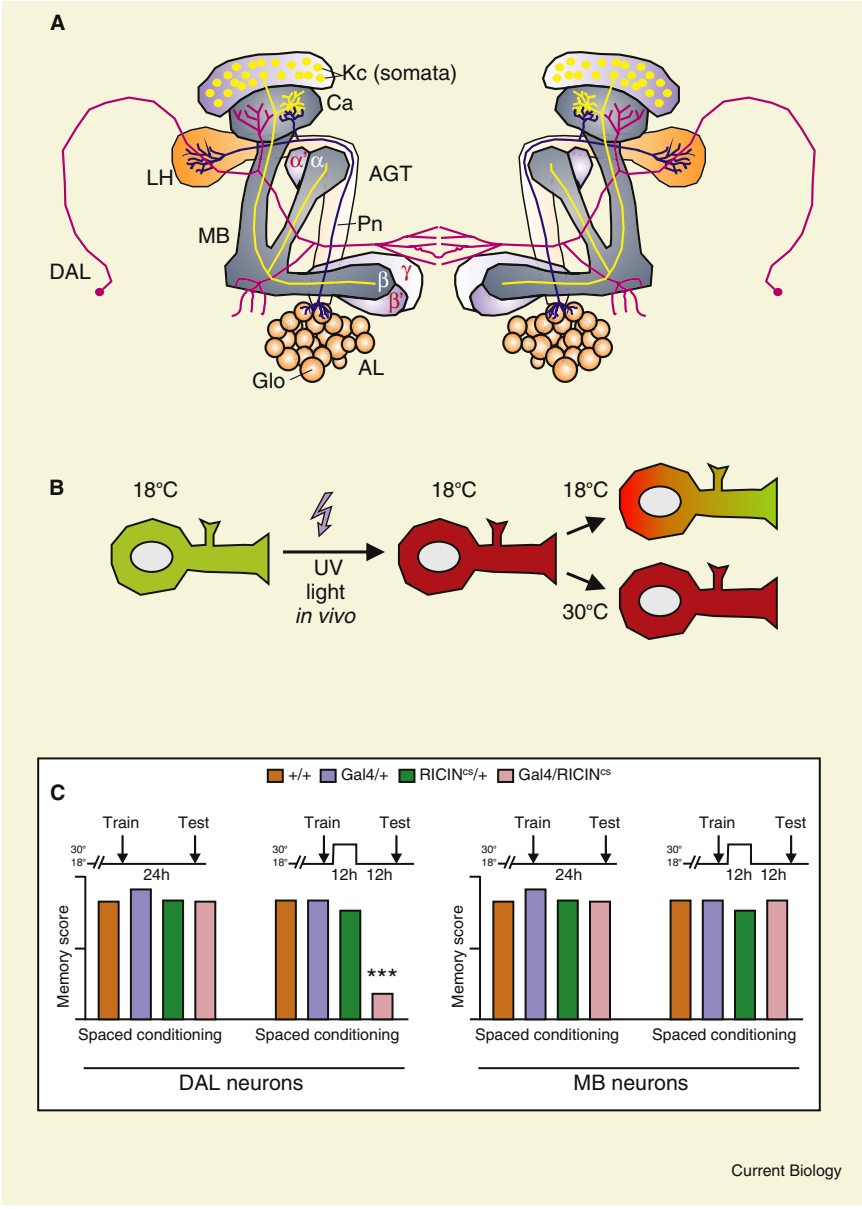

Figure 2. DAL-neuron involvement in LTM formation in the fruit fly.

(A) Schematic frontal view of the *Drosophila* brain showing the two DAL neurons. A projection neuron (Pn) shown as example (blue) projects from the antennal lobe (AL) to the mushroom body (MB) and the lateral horn (LH) via the antennoglomerular tract (AGT); glo, glomerulus; the Pn projects to the calyx (Ca) of the mushroom body where it contacts the dendrites of a Kenyon cell (Kc) shown as example (yellow); α and α′, vertical lobes of the mushroom body; β and β′ and γ, horizontal lobes of the mushroom body; arborizations of the Kenyon cell into the α and β lobes are shown. The DAL neurons are indicated in red. (B) The *KAEDE-RICIN*^{CS} principle. KAEDE is a green fluorescent protein (GFP) variant that changes its structure irreversibly to a red fluorescent protein (RFP) upon ultraviolet irradiation; RICIN^{cs} is a temperature-sensitive ribosome-inactivating toxin that inhibits protein synthesis. In flies raised at 18°C, KAEDE appears as a GFP and RICIN^{CS} is inactive; yet, after UV irradiation, KAEDE is irreversibly photoconverted into RFP. In flies kept at 18°C after photoconversion, RICIN^{CS} is still inactive and new green KAEDE is synthesized and accumulates with time, while the level of pre-existing red KAEDE remains unchanged. In flies switched to 30°C RICIN^{CS} is active and no new KAEDE is produced. (C) Schematic summary of results reported in [5]. Memory score in 24 h retention test for flies in which protein synthesis was inhibited via RICIN^{CS} in DAL neurons (left) and in mushroom body neurons (right). Memory scores were intact in wild-type flies (+/+) as well as in flies with Gal4 or RICIN^{CS} alone, but blocking protein synthesis via Gal4/RICIN^{CS} in DAL neurons significantly reduced memory; the same procedure does not impair LTM in mushroom body neurons. (Adapted from [5].)

if effective, should lead to an impairment of LTM, rather than memories that are independent of protein synthesis. This was what happened when protein synthesis was inhibited in DAL neurons, thus providing the first evidence for the involvement of these neurons in LTM formation. As a control, the authors performed massed conditioning with the expression of activated RICIN^{CS} in DAL neurons and showed that memory remained intact. Further experiments delineated the time that *de novo* protein synthesis in DAL neurons was required for normal LTM formation to the first 12 hours after spaced training. These findings demonstrate that DAL neurons can now be added to the neural system underlying LTM in *Drosophila* (Figure 2C).

Yet, Chen *et al.* [5] made a surprising finding: disruption of protein synthesis by RICIN^{CS} in the mushroom bodies after spaced training did not impact LTM (Figure 2C). Thus, the authors discard a role for protein synthesis in the mushroom bodies for LTM. They maintain that the level of inhibition induced by RICIN^{CS} is high enough in the mushroom bodies to see a disruptive effect if protein synthesis was required. Their conclusion is, therefore, very provocative as it would seem to contradict a wealth of literature in fruit flies and honeybees indicating that the mushroom bodies are crucial for LTM formation, which is known to require protein synthesis [1,2,4,11].

Should we then reconsider our current understanding of mushroom body function in memory formation in insects? We believe that, despite the obvious interest of these new results, we should be extremely cautious before joining Chen *et al.* [5] in their exclusion of mushroom bodies as sites for LTM formation for several reasons.

Firstly, the conclusions of Chen *et al.* [5] are based on a negative result — a molecular disruption that did not have a behavioral effect. Negative results are inherently less conclusive, as there is an increased burden on the experimenter to prove that the manipulations employed were effective. Secondly, the RICIN^{CS} transgene employed was a weak blocker of protein synthesis in the mushroom bodies compared to three other, stronger transgenes that were identified. Yet even the strong transgenes failed to eliminate protein

synthesis in the mushroom bodies upon robust and chronic developmental expression as one would expect, although defects in axonal structures were observed. Thus, the RICIN^{CS} transgene used may lack the potency necessary for making strong conclusions about the requirement for normal protein synthesis. A control experiment performed to test the potency of RICIN^{CS} in blocking mushroom-body protein synthesis using the KAEDE reporter is questionable for two reasons. It remains unknown whether KAEDE expression offers a good surrogate for the hoped-for effect on the many endogenous proteins that are involved in LTM. In addition, the inhibition of KAEDE expression was monitored only in the cell bodies of the mushroom bodies. Protein synthesis underlying LTM is complex, being required in multiple cellular compartments, including the cell body, axons and dendrites. Whether the RICIN^{CS} toxin had sufficient efficacy across all compartments is unknown.

Finally, neurons are plastic and redundant. A large body of evidence has indicated that olfactory memory in insects is distributed across multiple nodes of the olfactory nervous system. There exist approximately 5000 mushroom body neurons, but only two DAL neurons. Mushroom bodies are remarkably resilient to insults. A genetic lesion that completely removes the vertical lobes leaves learning and memory after massed conditioning completely intact; one that removes the horizontal lobes leaves learning and memory after both spaced and massed conditioning intact [15]. Redundancy and/or plasticity of the mushroom bodies may allow insults to many of these neurons before phenotypic effects are observed, whereas insults to a node of the system comprising only two neurons would have immediately observable effects.

We believe that past and current evidence from multiple insect species favors a model in which cellular consolidation, including the requirement for new protein synthesis, occurs at multiple nodes within the olfactory nervous system, with systems consolidation — which has not yet been demonstrated in *Drosophila* — overlaying consolidation at the cellular level [16]. The discovery by Chen *et al.* [5] that the DAL neurons comprise one of the nodes in the olfactory nervous system required for olfactory memory in the fly could prove to be an important contribution to our understanding of the circuitry underlying LTM, but prior studies showing that these neurons are important for heat sensation [17] cast doubt on their specificity for memory formation. Most importantly, the negative result with regard to mushroom bodies requires additional experiments. Until then, as the Spanish proverb says, ''those you claim to be dead are in perfectly good health''.

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Homologous Recombination: How RecA Finds the Perfect Partner

How do two identical DNA sequences find each other during homologous recombination, amidst a 'sea' of unrelated DNA? New studies reveal how RecA promotes the search for homology by sampling DNA in three dimensions.

Kevin Hiom

Homologous recombination is an essential cellular process required, amongst other things, for the completion of DNA replication, the faithful repair of DNA damage and, in meiosis, for the genetic re-assortment that occurs during the production of gametes. In humans, defects in homologous recombination are directly associated with diseases, such as early onset breast and ovarian cancer [1], the haematological disease Fanconi's anaemi [2] and the premature ageing disorder Werner's syndrome [3].

The central component in homologous recombination is