Mating and Memory: An Educational Primer for Use with “Epigenetic Control of Learning and Memory in *Drosophila* by Tip60 HAT Action”

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SUMMARY An article by Xu *et al.* in the December 2014 issue of *GENETICS* can be used to illustrate epigenetic modification of gene expression, reverse genetic manipulation, genetic/epigenetic influence on behavioral studies, and studies using the *Drosophila* model organism applied to human disease. This Primer provides background information; technical explanations of genetic, biochemical, and behavioral approaches from the study; and an example of an approach for classroom use with discussion questions to aid in student comprehension of the research article.

Related article in *GENETICS*: Xu, S., R. Wilf, T. Menon, P. Panikker, J. Sarthi, and F. Elephant, 2014 Epigenetic control of learning and memory in *Drosophila* by Tip60 HAT action. *Genetics* **198**: 1571–1586.

KEYWORDS Tip60; histone acetylation; courtship memory; *Drosophila*; amyloid precursor protein; mushroom body; epigenetic modification

Background

In a recent article published in *GENETICS*, Xu *et al.* (2014) use the fruit fly *Drosophila melanogaster* to study the effect of epigenetic modification by the Tip60 histone acetyl transferase on learning and memory. Using reverse genetic gain-of-function and loss-of-function transgenic techniques, Xu *et al.* (2014) relate changes in Tip60 expression to anatomical changes in the fly brain and to alterations in memory related to courtship behavior. The role of Tip60 in learning and memory is further connected to a *Drosophila* model of Alzheimer’s disease.

D. melanogaster Model System

The fruit fly *D. melanogaster* has long been a powerful and elegant genetic model organism (Rubin and Lewis 2000). *D. melanogaster* (*Drosophila*, or “the fly”) progresses through the egg, larval, pupal, and adult stages of its life cycle in only 10–12 days. The fly is an incredibly useful genetic model because it is easily genetically manipulated. By injecting genetic constructs into eggs, gain-of-function and loss-of-

function gene studies can be performed. The genome of a fruit fly is simpler than mammalian genomes, and yet as a multicellular invertebrate the fly has complex neural organization. *Drosophila* have basic neural molecular mechanisms in common with mammals, and several simple but effective memory studies have been used reproducibly in the fly to better understand how our own memory works (Kahsai and Zars 2011).

Histone Modification

In Xu *et al.* (2014), the authors investigate whether epigenetic modifications can affect memory-mediated behaviors. “Epigenetics” broadly refers to information in the genome that is not coded by the DNA but refers to certain genomic states and how these states can be influenced by the environment. Examples of epigenetic states include gene silencing, imprinting, X-chromosome inactivation, and chromatin modification. Whether inherited or affected by the environment, epigenetic modification of chromatin significantly influences gene expression.

Eukaryotic genomes consist of chromatin, ~40% DNA, and 60% protein (Gupta 2004). In humans, chromatin organizational proteins help condense nearly 2 m worth of DNA (*in every cell*) into a length that is only ~20 millionths of a meter (Alberts *et al.* 2002)! At the most basic level, DNA molecules are typically found in the classic double helix.

Figure 1 Histone acetyltransferases and deacetylases modify lysines on histone tails. (A) The basic unit of chromatin organization is the nucleosome, consisting of the DNA double helix wrapped around a histone core composed of two subunits each of the histones 2A, 2B, 3, and 4. One histone tail is shown, enriched in lysine (K) residues. Lysines may be modified in several ways, including acetylation. HATs covalently link acetyl groups to lysine residues, while HDACs remove the acetyl modifications. (B) The R group of the amino acid lysine consists of a four-carbon chain ending in an amino group, which is positively charged at cellular pH (top). The addition of an acetyl group (COCH₃) covalently linked to the R-group nitrogen neutralizes the amino acid (middle). Alternatively, a methyl group (CH₃) may be covalently added to the R-group nitrogen (bottom).

These strands are next organized into nucleosomes by wrapping the DNA around histone protein complexes (Figure 1A). Nucleosomes are often referred to as “beads on a string” and are somewhat analogous to coiling the cords of earbuds to prevent tangling. The nucleosome consists of ~150 nucleotides of DNA wrapped twice around the histone core and a final histone protein (H1) securing the wrapped DNA molecule (Luger *et al.* 1997). Four types of histone proteins form the core: H2A, H2B, H3, and H4. Two tetramers of these four histones dimerize to form an eight-histone octamer. Each of the histone proteins includes a “tail” that extends from the tightly associated core and can make contact with nearby nucleosomes (Bannister and Kouzarides 2011). These tails are highly basic because they are rich in lysine and arginine amino acids, and these amino acids can be covalently modified. The tails of histones H3 and H4 are the longest, and the most is known about modifications to these tails.

The histone tails can be modified by a variety of covalent modifications (Kouzarides 2007) including acetylation of lysine, methylation of lysine or arginine, ubiquitinylation and sumoylation of lysine, and phosphorylation of serine and threonine. The most common and well studied of these modifications are acetylation and methylation (Figure 1B). These modifications are highly dynamic and can change in response to stimulation in a matter of minutes (Kouzarides 2007). Researchers in the field are attempting to understand the “histone code” of different DNA states and activities associated with patterns of histone modifications. Studies show that histone modifications largely influence transcription, although repair, replication, and chromatin condensation can also be affected. By correlating histone states to the expression or silencing of a particular gene under specific conditions, the histone code may be cracked.

Histone modification is thought to affect transcription through two mechanisms (Kouzarides 2007; Bannister and Kouzarides 2011). The first mechanism is the overall compaction of the chromatin itself. Second, modifications on histone tails can recruit and bind specific protein domains,

such as those found in particular families of transcription factors or chromatin-binding proteins. Often both mechanisms work in tandem, whereby distinct histone modifications act as docking sites, recruiting other chromatin-remodeling enzymes that serve to condense or decondense chromatin structure. The most common chromatin state is euchromatin, which is less tightly compacted and correlates with genome regions that are accessible for gene expression—“active” regions where transcription and repair can take place. A gene in euchromatin is not automatically transcribed, but is available to be activated in the presence of the appropriate transcription factors. Alternatively, chromatin can be found in a more compact state, called heterochromatin, which is associated with genome regions that are not currently being accessed and transcribed—“silent” regions such as centromeres and telomeres. High levels of histone tail methylation are commonly associated with “silenced” heterochromatin in mammals, while increased levels of acetylation are frequently associated with “active” euchromatin. Lysine residues have a positive charge and help organize DNA by interacting with the negatively charged phosphate-sugar backbone of DNA molecules. It is thought that the addition of an acetyl group to lysines neutralizes the positive charge and can affect the interaction of histones with DNA and other histones, which leads to “loosening up” the nucleosome and making the DNA more open and accessible to transcription factors, repair proteins, and chromatin-remodeling proteins. Despite these generalizations, the histone code is quite complex. Both acetylation and methylation can be associated with either gene activation or gene repression, depending on specific cellular conditions. Still, acetylation is far more frequently associated with transcription activation (Kouzarides 2007; Bannister and Kouzarides 2011).

The acetylation state of histones is mediated by enzymes that add or remove acetyl groups from lysine residues on histone tails. Enzymes that add an acetyl group are called histone acetyltransferases (HATs). HATs use acetyl-CoA to transfer the acetyl group to the amino group of lysine side

chains. There are three main families of nuclear HATs (Hodawadekar and Marmorstein 2007; Bannister and Kouzarides 2011). The Tip60 protein investigated in Xu *et al.* (2014) is a member of the MYST family, named for the initial members MOX, Ybf2/Sas3, Sas2, and Tip60. The human Tip60 complex is thought to be involved in DNA-damage responses (Grant 2001). Reversal of acetylation is accomplished by histone de-acetylases (HDACs). HDACs are usually associated with repression of transcription (Bannister and Kouzarides 2011).

D. *melanogaster* Brain Anatomy

Invertebrate brains, though simpler than vertebrate brains, are capable of directing complex behaviors, learning, and memory. This study focuses on one particular neuronal grouping of the *Drosophila* brain called the mushroom body (Figure 2A). The mushroom body is a collection of small neurons called Kenyon cells (Heisenberg 2003; Campbell and Turner 2010). In *Drosophila*, the Kenyon cells comprise ~2% of the total neurons of the brain. The Kenyon cell bodies are found on top of the calyx, the site of sensory input (Figure 2B). Sensory inputs leading to the mushroom body come from smell, taste, vision, and hearing, while the Kenyon cell axons carry information out of the mushroom body through the stalk. Connections between the insect mushroom body and behavior date back to 1850 when Felix Dujardin correlated mushroom body size with the complexity of social behavior (reviewed in Campbell and Turner 2010). Later studies showed that the mushroom body is important for learning and memory associations between a stimulus, such as an odor, and a particular outcome, such as electric shock (Heisenberg *et al.* 1985; Heisenberg 2003). Connections between olfaction and memory are the most studied in mushroom body research, but mushroom bodies are involved in a variety of interesting behaviors and memory formation. In some insects, when part of the mushroom body is ablated or destroyed, the insects lose the ability for spatial navigation and memory (reviewed in Strausfeld *et al.* 1998, 2009).

Alzheimer's Disease and Amyloid Precursor Protein

Alzheimer's disease (AD) is the most common form of dementia, associated with progressive memory loss and impairment of language and cognitive function (Lam *et al.* 2013; Alzheimer's Association 2014). The symptoms associated with AD are caused by specific accumulations of protein in the brain that disrupt the structure and function of neurons. There are two common types of damage associated with the brains of AD patients: tangles of neurofilaments associated with a protein called tau and plaques of deposited amyloid- β protein. Mutations in amyloid- β protein predispose the protein to misfolding with a significant amount of β -pleated sheet protein structure exposed. The β -sheet regions of amyloid proteins tend to stick together, resulting in large aggregations of the amyloid- β protein into filaments called amyloid fibrils. These fibrils are extremely insoluble

and therefore accumulate and form into the "senile plaques" associated with AD (Lam *et al.* 2013).

Amyloid- β protein is processed from amyloid precursor protein (APP) (O'Brien and Wong 2011). APP is a membrane protein in which the N terminus is situated in the membrane with the C terminus located outside the cell. Membrane-localized enzymes called secretases process APP by cleaving at defined sites and releasing a C-terminal fragment into the extracellular space. If APP is cleaved by α -secretase, then a nonamyloid-forming fragment is released as part of the normal physiological process. However, if APP is cleaved by both β - and γ -secretases, then the disease-associated secreted amyloid- β peptide of ~40 amino acids is formed. Certain familial mutations increase the risk of amyloid- β plaque formation from the secreted C terminus due to minor differences in cleavage sites and misfolding of the processed peptide fragment. In this study by Xu *et al.* (2014), the effects of loss-of-Tip60 and gain-of-Tip60 function are compared in the context of mutant flies that are also transgenic for the human APP protein, either with or without the disease-associated C terminus.

Techniques Used by Xu and Colleagues

GAL4-UAS expression

In *Drosophila*, the use of the GAL4-UAS system can be used to specifically control the expression of transgenes in certain cell types or times during development, as reviewed in Duffy (2002). The system relies on a transcription factor, GAL4, and the DNA sequence to which it binds, the UAS gene promoter sequence. To control transgenic expression in flies, the transgene, such as Tip60, is placed in a construct with the UAS sequence as its promoter. The UAS-Tip60 transgenic fly thus carries the Tip60 transgene but does not express it. A second transgenic fly is made in which the GAL4 transcription factor is controlled by promoter elements for a gene that is expressed in one cell type or at one time in development, such as the promoter for the neural protein OK107. The OK107-GAL4 transgenic fly makes GAL4 in all of its neuronal cells, but not in any other cells of the body. Since GAL4 has no DNA sequences to activate, it has no presumed effect. When the OK107-GAL4 fly is crossed to the UAS-Tip60 flies, the offspring that carry both transgenes will therefore express the Tip60 construct only in neural cells where GAL4 is also being expressed, thanks to the interaction of the GAL4 transcription factor and the UAS promoter. Xu *et al.* (2014) use this method to express the wild-type Tip60 histone acetyltransferase, as well as a dominant negative version of Tip60, designated Tip60^{E432Q}. Transgenic expression of wild-type Tip60, in addition to the fly's normal endogenous Tip60, results in overexpression and a *gain-of-function* effect of Tip60. In contrast, the Tip60^{E432Q} mutation is a dominant negative mutation that prevents Tip60 from successfully transferring the acetyl group from acetyl-CoA to lysines on histone tails. Since the amount of acetyl-CoA

Figure 2 Mushroom bodies in *Drosophila* brain are sites of sensory processing, behavior, and memory associations. (A) Frontal view of the mushroom bodies, composed of Kenyon cells. The calyx, α -, α' -, β -, β' -, and γ -lobes are labeled. (B) The cell bodies of the Kenyon cells are situated in the calyx, which is located posteriorly and dorsally in the *Drosophila* brain. A pedunculus projects anteriorly before dividing upward into the dorsal α - and α' -lobes and the β -, β' -, and γ -lobes toward the midline (Jenett *et al.* 2006; Kaun *et al.* 2011; Zhou *et al.* 2012). Xu *et al.* (2014) demonstrate an effect of Tip60 on the α/α' - and β/β' -lobe development.

substrate in a cell is limited, expression of the inactive Tip60^{E432Q} protein results in an overall *loss-of-function* effect on Tip60 histone targets.

Mushroom Body Imaging

Xu *et al.* (2014) examine the effects of Tip60 expression on mushroom body anatomy using immunohistochemistry (IHC). IHC is based on antibody interactions with a target protein of interest (Watkins 2009). The antibodies have binding sites that specifically recognize the structure of the protein target within preserved tissue. Either the antibodies used for detection can be directly linked to a fluorescent chromophore for visualization by fluorescent microscopy or the signal can be amplified, as in this study, using secondary antibodies that highlight the antitarget antibody to result in a stronger fluorescent signal. In this study, entire brains dissected from larvae or adult flies with the wild-type or transgenic Tip60 genotypes were preserved and stained for mushroom body markers. At the same time, they were also stained for Tip60 localization to determine whether or not Tip60 is present in the *Drosophila* neural organ involved in courtship memory, as seen in figure 1, figure 2, figure 3, figure 5, and figure 7 in Xu *et al.* (2014). In figure 1, Tip60 localization is compared to localization of ELAV (embryonic lethal abnormal visual protein), a protein found in all neurons. In Xu *et al.* (2014), figure 1 also utilizes confocal fluorescent microscopy to better determine the location of Tip60 inside cells. Confocal microscopy produces an image from a single focal plane without light interference from cells or proteins above or below that plane (Smith 2011). Such a narrow section of collected fluorescence data produces a very accurate localization image. For example, in figure 1F of Xu *et al.* (2014), Tip60 (in red) can be seen precisely surrounded by the GFP marking the cell membranes. In figure 2 of Xu *et al.* (2014), Tip60 localization is compared to expression of Fascillin II and with Trio, two proteins involved in the axonal growth of neurons and with cell adhesion.

D. melanogaster Courtship and Memory Assay

Xu *et al.* (2014) test the memory of flies with different genotypes by assaying their response to a virgin female following an unsuccessful courtship of an unreceptive female. During courtship, *D. melanogaster* males go through an elaborate rit-

ual that prepares the female to be receptive and provide feedback to the male of whether or not he is courting an appropriately receptive female rather than an already mated female, a male, or an inanimate object (Ejima and Griffith 2007; Griffith and Ejima 2009). After identifying a potential mate by visual and olfactory cues, the male begins pursuit while performing intermittent bouts of a courtship song composed of wing vibrations. The pursuit and courtship song are continued while the male extends his proboscis to occasionally lick the genitals of the female. If the female is receptive, she signals by lifting her wings, the male mounts her, and copulation takes place (Sturtevant 1915). Video examples of courtship behavior can be found in Ejima and Griffith (2007).

The success of male courtship depends upon both his behaviors and the receptiveness of the female. Virgin female *Drosophila* are unreceptive upon eclosion from a pupa, but become receptive to courtship within 2 days. However, upon mating, female *D. melanogaster* become immediately unreceptive and remain that way for 1–2 days (Manning 1967). In turn, previous failure during courtship influences the fervor with which a wild-type male *D. melanogaster* will court a subsequent female. Males who have recently been unsuccessful during courtship of unreceptive females spend less time pursuing other females over a period of 2–3 hr (Siegel and Hall 1979). This process is known as conditioned courtship suppression because over the period of the hour with the mated female the male does not get “rewarded” for his courtship advances to the already mated female, who is not receptive to his efforts. If a male reduces the number of courtship advances that he makes to the mated female, then he can be said to have learned to suppress his courtship advances. If the male still suppresses his courtship behaviors when he is newly placed with a virgin female (who would be more likely to accept his advances), then the male fly is showing associative memory of the courtship suppression, while a fly with a memory defect is likely to court the second female vigorously. Because the learned courtship suppression lasts for only 2–3 hr, it is an example of short-term memory, which does not involve changes in gene expression, in contrast with long-term memory, which requires transcriptional changes in addition to multiple training periods separated by rest (McBride *et al.* 1999; van Swinderen 2009).

Xu and colleagues used females from the Canton-S strain (one of the “wild-type” strains of *D. melanogaster*). The

virgin females, collected and isolated before they had a chance to mate, composed one experimental group, while mated 5-day-old females formed the second group. During the training/learning phase of the experiment, wild-type or transgenic males were placed with mated females for 1 hr. The amount of the males' courtship activity was measured using a courtship index, and the courtship index during the first 10 min was compared to the courtship index during the final 10 min of the hour, as shown in figure 3 of Xu *et al.* (2014). If the courtship index in the final 10 min is lower than in the initial 10 min, then the males have learned, showing courtship suppression conditioning. The males were then separated from the mated females and placed with virgin females. The courtship indices of the trained males were compared to the courtship indices of males that had not been exposed to mated females, as a control. Males whose courtship index remained low remembered the courtship suppression, while males that initiated courtship at the same level as their untrained counterparts did not. Note that in this experiment the learning of courtship suppression (reduction of behavior with mated females) is separate from the memory of courtship suppression (reduction of behavior with new, virgin females), as shown in figure 3b of Xu *et al.* (2014).

ChIP-Seq

Chromatin immunoprecipitation (ChIP) uses antibody–protein interactions to investigate proteins that are bound to DNA. The process can be combined with sequencing to determine the DNA patterns associated with the protein of interest in a process called ChIP-Seq (Barski and Zhao 2009). In a ChIP experiment, cell or tissue lysates are collected under the desired conditions, and a reversible cross-linking reagent is used to form covalent bonds between proteins and the DNA regions to which they are bound. The chromatin is isolated and sheared into small, individual pieces of DNA and attached proteins. Then antibodies are used to bind to and identify a protein of interest, such as Tip60. A bead with an affinity for the antibody will then collect the antibody–protein–DNA complexes and “precipitate” them, bringing them out of the general lysate solution. The cross-linking can then be reversed so that the DNA and the protein collected may be studied separately. In ChIP-Seq, the DNA is sequenced. This is a common way to find DNA recognition sites for transcription factors.

When Xu and colleagues performed a ChIP-Seq experiment using Tip60 as the target protein, they analyzed the sequences that were associated with Tip60 protein. If a sequence is discovered in this Tip60 ChIP-Seq, then the sequence is likely part of a gene for which the histones were being acetylated by Tip60 at the time of the experiment. The authors investigated what types of genes were being influenced in this way by Tip60 association by using a database that correlates genes with their known functions. The genes that were identified in this Tip60 ChIP-Seq were then sorted by their functions into major groups, as shown in figure 7A of Xu *et al.* (2014).

Xu *et al.* (2014) next used FlyAtlas (<http://flyatlas.org>), a database that reports where certain genes are known to be expressed in the fly. The authors show whether Tip60-associated genes are known to be up-regulated or down-regulated for a variety of tissue locations (figure 7B in Xu *et al.* 2014). Finally, the authors performed a ChIP-Seq experiment using RNA Poly II as the target protein. Since RNA polymerase II is required for gene transcription, the presence of RNA Pol II with a gene sequence indicates that sequence was being transcribed and expressed at the time of the experiment (figure 7C, Xu *et al.* 2014).

RT-PCR of Tip60 Targets

Xu *et al.* (2014) observed a memory deficit and abnormal mushroom body structures associated with alterations in Tip60 function. To determine how these phenotypes may be related to changes in gene expression due to alterations in Tip60 HAT activity, they used RT-PCR to analyze the messenger RNA (mRNA) levels of sequences that they associated with Tip60 based on the ChIP-Seq experiments. In RT-PCR, RNA is collected from cells or tissues (in this case adult fly heads), and the polymerase reverse-transcriptase is used to make a DNA copy of an RNA transcript—the opposite process to that of an RNA polymerase (Nolan *et al.* 2006). By using poly(T) as a primer against the poly(A) tails found on eukaryotic mRNA transcripts, DNA copies of the mRNA transcripts will be present in amounts relative to the number of mRNA transcripts for each gene present. The reverse-transcriptase procedure is followed with a PCR reaction using primers for specific genes, and the reaction includes a dye that specifically binds to double-stranded DNA. Thus, as copies of the specific gene transcript are made (making double-stranded copies), fluorescent dye levels increase and can be detected and quantified. In this way, quantitative estimates of the level of original mRNA transcripts can be measured. Transcript levels of target genes are usually compared to transcript levels of constitutive, “housekeeping” genes, as a control. The expression level of Tip60-associated genes are examined in flies that are transgenic for the dominant negative Tip60 construct; therefore, gene expression under the loss of Tip60 function is assessed (Xu *et al.* 2014). Since the dominant negative Tip60 could potentially affect transcription levels throughout the organism, comparison of transcript levels of the Tip60 target genes to the housekeeping genes is normalized to the difference between the housekeeping genes under wild-type and the transgene conditions to account for any global gene expression changes. Differences are expressed as “fold change,” as seen in figure 8A of Xu *et al.* (2014).

APP Expression Model of AD in *Drosophila*

Several studies from the Elephant lab have utilized the *Drosophila* model of AD in which the human APP transgene is expressed in the fly. In this fly model of AD, APP can be expressed in its full-length form, which, when

cleaved by certain secretases, can accumulate as plaques and promote neurological damage. Additionally, Tip60 has been shown by the Elefant lab and other labs to form a complex with the secretase-cleaved extracellular C terminus of APP (AICD). AICD translocates into the nucleus where it forms a transcriptionally active complex with Tip60. Loss or inappropriate recruitment of this complex to cognition-linked genes leads to epigenetic misregulation of these genes that is associated with AD phenotypes. The phenotype of flies with the full-length human APP protein can be compared to the phenotypes of control flies expressing a mutant variant in which the APP gene lacks the C terminus of the protein, so that, while the APP is expressed, no cleavage of the C terminus can occur and thus a transcriptionally active Tip60/AICD complex does not form. These AD model flies show apoptosis (programmed cell death) of neurons, defects in the outgrowth of neuron axons, and behavioral defects in sleep and movement (Pirooznia *et al.* 2012a,b; Johnson *et al.* 2013; Pirooznia and Elefant 2013a,b), and these AD phenotypes are each dependent upon the presence of the APP C terminus. The Elefant lab has shown that increased Tip60 histone acetyl-transferase levels can protect against certain AD-like phenotypes that occur in this APP over-expression fly AD model, and this was shown by comparing the phenotypes of APP AD flies that were co-expressing the transgene either for the wild-type Tip60 or the dominant negative Tip60^{E432Q} mutation. To determine whether the phenotypes observed can be related to the human AD problems stemming from epigenetic misregulation of Tip60/AICD genes, the full-length APP/Tip60 wild-type or Tip60^{E432Q} flies were compared to APP C-terminal deletion (APP-CT)/Tip60wt or Tip60^{E432Q} flies using courtship suppression assay for the memory phenotype measurement. In figure 9 of Xu *et al.* (2014), the defects in both learning and memory shown are distinct. If flies show defects in the courtship suppression with a *new female*, but not the initial courtship suppression, then the defect is in memory. However, if the flies never show courtship suppression to begin with, then the defect is in learning, and these flies would have no learned behavior to remember in the company of a new female. Students may analyze which fly phenotypes caused by full-length APP expression are due to learning or memory, and whether these behavioral phenotypes can be affected by the expression of wild-type or dominant negative Tip60 (Tip60^{E432Q}). It is especially interesting to consider the results of figure 9, the disease model, compared to the effects of Tip60 expression on its own in the nondisease model in figure 3 of Xu *et al.* (2014).

Connection to Genetic Concepts

The article by Xu *et al.* (2014) nicely illustrates several important concepts of genetics. As Tip60 is a histone acetyl-transferase affecting the state of euchromatin, this article focusing on the effect of Tip60 expression on memory

reminds students that chromatin involves more than just the DNA; the state of the histones as protein components is important as well. In addition, histone modifications constitute epigenetic changes that can influence gene expression in the absence of DNA mutations. By comparing the effect of transgenic expression of wild-type Tip60 and the dominant negative Tip60^{E432Q}, the article demonstrates both gain-of-function and loss-of-function investigations. The study also highlights the powerful GAL4-UAS system and the genetic tractability of *Drosophila* (Duffy 2002). Students can relate to the behavioral phenotypes studied here, including learning, memory, and courtship behavior. Finally, the Xu *et al.* (2014) article uses a common genetic model organism to relate the basic biology of epigenetics to a significant human illness, Alzheimer's disease (Jakovcevski and Akbarian 2012). As individuals can be predisposed to developing AD based on genetic differences in the amyloid precursor protein, discussion of this article can serve as an introduction to considering human genetic disorders.

Approach to Classroom Use

It is suggested that this Primer article be provided to students concurrently with the Xu *et al.* (2014) article. Students would prepare for discussion of the article by reading the Introduction, Results, and at least the first paragraph of the Discussion in that article. Instructors may apply the C.R.E.A.T.E. approach of Hoskins *et al.* (2011) in which students Consider, Read, Elucidate hypotheses, Analyze and interpret the data, and Think of the next Experiment, to promote critical thinking about the article and further understanding of the experiments. Instructors may choose to provide multiple linear articles to illustrate how studies develop and hypotheses evolve as new data are discovered (Hoskins *et al.* 2011). The article by Xu *et al.* highlighted here is the latest of a number of studies from the Elefant lab linking Tip60 HAT activity to neural anatomical changes and behavioral effects, particularly with the *Drosophila* AD model. Articles to consider leading to the Xu *et al.* (2014) article include Lorbeck *et al.* (2011), Pirooznia *et al.* (2012a,b), Johnson *et al.* (2013), and Pirooznia and Elefant (2013a,b).

An overview of basic mushroom body anatomy and an introduction to fly courtship behavior may be useful to begin discussion of the Xu *et al.* (2014) article. A video of fly courtship, such as can be seen in the open access Movie S1 in Ejima and Griffith (2007) (<http://cshprotocols.cshlp.org/content/2007/10/pdb.prot4847.full>) will help stimulate student interest. A review of the GAL4-UAS transgene system may also be useful before students examine the article in depth (Duffy 2002). Instructors may want to divide the class into small groups to consider individual figures or panels in more detail or be assigned the specific further thought questions listed below and then review the main findings as a class.

Thought Questions: Figures from Xu *et al.* (2014)

- Figure 1. Compare subcellular localization of the two markers. How is Tip60 localization consistent with its HAT function?
- Figure 2. How does Tip60 expression compare to expression of FasII and Trio, two proteins involved in cell adhesion and axon patterning? What might you infer about cells expressing Tip60? In which lobes (α , α' , β , γ) is FasII found? In which lobes is Trio found?
- Figure 3. Describe how the wild-type, OK107-GAL4, UAS-Tip60^{E432Q}, and UAS-Tip60wt flies each serve as controls for the experimental OK1-7-GAL4/UAS-Tip60 flies
- Figure 3. What does the decrease in courtship behavior in the final 10 min with the unreceptive female indicate? Were the GAL4/UAS-Tip60 flies deficient in learning attenuation of courtship behavior? Why or why not? Why is it important to view figure 3A before considering memory in figure 3B?
- Figure 3B. Expression of either dominant negative Tip60^{E432Q} (partial loss of function) or of abnormal levels of Tip60wt (gain of function) result in similar courtship indices in the 3-hr memory test. What is a potential explanation of this? What does this tell you about Tip60 levels in a normal fly?
- Figure 3 vs. figure 4, figure 5, and figure 6. Is it reasonable to attribute the deficits in learning associated with increased or decreased Tip60 activity (as seen in figure 3) to the change in mushroom body morphology? Why or why not? Propose an explanation for how transgenic Tip60wt expression might promote memory deficits.
- Figure 7. Choose three gene groups and speculate why it might make sense for Tip60 HAT to be associated with these genes in the context of this article (Xu *et al.* 2014), the defects in both learning and memory shown are important. Consider using FlyBase (<http://flybase.org>) to look up further information and specific examples from the gene groups listed.
- Figure 8. Based on the expression changes of Tip60 targets with the dominant negative Tip60^{E432Q} construct, how does normal Tip60 HAT activity affect gene expression?
- Figure 9. Compare learning between the flies expressing APP vs. APP lacking the C terminus (APP dCT). Does the C terminus of APP contribute to learning deficit or restoration? How does the co-expression of Tip60wt with APP affect learning? Evaluate the effect on memory when expressing APP in the fly vs. expressing APP with Tip60wt or APP dCT with Tip60. How might you interpret this effect?

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