Genome Biology

RESEARCH HIGHLIGHT

# A *Drosophila* model for the role of epigenetics in brain function and development

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**Abstract**

A genetic study in *Drosophila* gives important insights into the epigenetic control of gene expression implicated in a human mental retardation syndrome.

Epigenetic mechanisms that regulate gene expression are key processes in the interaction between genes and environmental inputs such as those received by the human brain via the sensory system. Epigenetic control of gene expression has been implicated in learning and memory, for example, and deregulation of epigenome plasticity seems to be an important step in the pathogenesis of neurological diseases [1]. As such, it is not surprising that recent studies investigating the genetic causes of mental retardation have found evidence for the involvement of the epigenetic machinery [2].

Kleefstra syndrome (formally known as 9q34 syndrome) is a clinical condition comprising multiple features, including severe mental retardation. The syndrome is causally linked to mutations or disruption of the gene *EHMT1* (euchromatic histone-lysine *N*-methyltransferase 1) [3]. EHMT1 (also known as G9a-like protein (GLP)) together with its homolog EHMT2 (G9a) is part of a histone-methyltransferase complex that specifically dimethylates histone 3 on lysine 9 (H3K9me2), a post-translational histone modification generally associated with negative regulation of transcription (gene silencing). Although this modification can also be found in heterochromatin, EHMT1 and EHMT2 are responsible for selective H3K9 dimethylation in euchromatin [4].

In a comprehensive study published recently in *PLoS Biology*, Annette Schenck and colleagues (Kramer *et al.* [5]) identify the *Drosophila melanogaster* gene *G9a* as the sole *Drosophila* homolog of mammalian *EHMT1* (*GLA*) and *EHMT2* (*G9a*). Because of this, the authors have renamed *Drosophila G9a* as *EHMT*. Organism-wide deletion of this gene in *Drosophila* (by imprecise P-element excision) is not lethal, and so the authors were able to analyze the cellular and behavioral characteristics of mutant larvae and adults and to correlate these with changes in genomic histone methylation.

## Epigenetics and nervous system plasticity

The plasticity of the nervous system - the capacity to modify the neuronal network in response to neuronal signaling - is essential to nervous system function. One essential determinant of neuronal plasticity is the complexity of organization of the dendritic tree - the set of branched processes through which a neuron mainly receives signals from other neurons. Kramer *et al.* [5] found that *EHMT*-mutant *Drosophila* larvae display sparser dendrite branching than wild-type larvae, as assessed in type-4 multidendrite neurons in the peripheral nervous system. These neurons are involved in larval locomotory behavior and, accordingly, the mutant larvae displayed an altered crawling pattern. The dendritic-branching phenotype could be rescued by expression of wild-type *EHMT* in the type-4 neurons. However, wild-type *EHMT* expression did not reinstate normal larval locomotory behavior, suggesting that the deregulation of this behavior in the *EHMT* mutant is independent of the change in dendritic branching. The involvement of *EHMT* in dendritic plasticity is in line with the recent identification of a role for *Ehmt2* in cocaine-induced dendritic plasticity in the mouse nucleus accumbens, which is a brain structure of the reward system and thereby implicated in addiction and emotional learning [6].

Kramer *et al.* [5] also studied the role of *EHMT* in learning and memory in adult *Drosophila*. They measured habituation learning using a light-off jump reflex habituation assay, and found that in *EHMT* mutants this form of non-associative memory function was impaired. Short-term and long-term courtship memory was also compromised in *EHMT* mutants, suggesting a role for *EHMT* in the consolidation or retrieval of new memories. Impairment of courtship memory could be rescued by pan-neuronal expression of wild-type *EHMT* under the

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control of the *elav* promoter, which restricts expression to neurons. More importantly, *7B-Gal4*-driven expression of *EHMT* in the mushroom body only (a region of the *Drosophila* brain associated with learning and memory [7]) was sufficient to reverse the mutant phenotype. As these promoters are already active during development of the nervous system, this type of approach does not rule out developmental effects of reintroduced *EHMT* expression. For that reason, the authors confirmed the reversibility of the observed memory impairments by inducing wild-type *EHMT* expression on only three consecutive days during adulthood, thus ruling out a developmental effect. In line with this, it has previously been reported that adult-forebrain-specific ablation of *Ehmt2* and *Ehmt1* in the mouse causes memory impairment without obvious alterations in dendritic organization and brain development [8].

## Genome-wide changes in histone methylation in *EHMT* mutants

As EHMT is a H3K9 histone-methyltransferase, one would expect its loss to lead to the simultaneous deregulation of numerous genes. In mice, chromatin immunoprecipitation followed by DNA sequencing (ChIP-seq) has been used to detect epigenetic alterations in brain-region-specific gene-expression profiles that define genetic programs important for memory formation [9]. Using a similar approach, Kramer *et al*. identified genomic regions that show reduced H3K9me2 in *EHMT*-mutant larvae. Downregulation of H3K9me2 was most prominent in the promoter regions (defined as the region ≤1 kb upstream of the transcription start site) and downstream of the poly(A) sites, which mark, respectively, the 5' and the 3' ends of genes. The cross-correlation of these data with microarray analysis of expressed mRNAs allowed the authors to identify genes that are usually repressed by EHMT-mediated H3K9 dimethylation. Pathway analysis of these genes revealed a striking enrichment of genes implicated in nervous-system development and in other pathways associated with learning and memory. The finding of depletion of H3K9me2 at the 3' ends of upregulated genes in addition to depletion at the promoter region underscores the significance of changes in chromatin modifications to all levels of transcriptional regulation. This is also revealed by other recent data on the genome-wide distribution of several histone modifications, which showed functional diversification in different genomic regions. In addition to affecting transcriptional initiation, several different histone modifications have been found to be involved in the regulation of transcriptional elongation, exon-intron definition and the activity of transcriptional characters, for example [9,10].

In mice, in contrast to *Drosophila*, the EHMT1 and EHMT2 methyltransferases have been found to control the repression of non-neuronal genes after neural differentiation, and therefore aid memory consolidation and retrieval in maintenance mode, rather than in relation to developmental plasticity [7]. This difference might reflect the different developmental stages analyzed (larvae versus adult mice); Kramer *et al*. emphasize that a significant proportion of the genes found in the mouse study are conserved in *Drosophila* and show decreased levels of H3K9me2 in the *EHMT* mutants, which means that the set of EHMT-target genes in the two species can be assumed to overlap, at least in part. From a different point of view, the apparently conflicting results might also reflect the fact that epigenetic regulators control transcriptional programs or networks, rather than specific target genes, and that these networks can differ to some extent even in closely related species.

As mental-retardation syndromes are usually diagnosed only after birth, the ability to reverse symptoms in the individual will be crucial in the eventual translation of experimental results into clinical applications. The findings of Kramer *et al*. [5], showing that some effects of EHMT loss are reversible, could point to a general feature of genetic diseases caused by disruption of the epigenetic machinery. As this machinery regulates gene-environment interaction, it may be a suitable and easily accessible interface for clinical intervention. Further investigation of the network of H3K9 dimethylation to identify other specific methyltransferases and/or antagonizing demethylases, as well as key target genes and alternative players in epigenetic gene regulation, could result in additional therapeutic possibilities, and will also give important insight into the general mechanisms underlying the more common 'sporadic' diseases of the nervous system - those that have no readily discernable genetic basis.

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