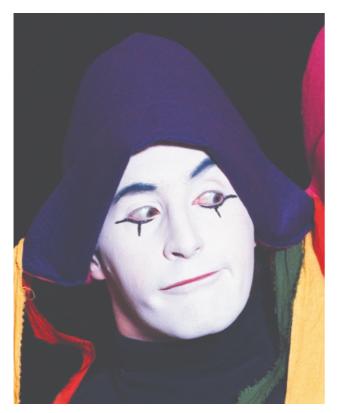
## HIGHLIGHTS

APOPTOSIS

# A dysfunctional harlequin



GENE EXPRESSION

# Numbers count

The pattern of histone modification in the chromatin surrounding a gene is important for its transcriptional activity. Methylation, acetylation and phosphorylation are among the different covalent modifications that contribute to the combinatorial potential of histone patterns. But a new study published in *Nature* by the Kouzarides group hints at the existence of yet another level of complexity that determines the activation state — the precise number of methylation events.

Tony Kouzarides and colleagues focused their attention on a group of proteins that contain so-called SET domains. SET domains catalyse methylation of specific lysine (K) residues in the amino-terminal tails of histones. The authors identified Set1 and Mitochondrial dysfunction has been implicated in several neurodegenerative disorders, including Alzheimer's and Parkinson's diseases. The changes in mitochondrial function can lead to increased energy production and oxidative stress, and subsequent apoptosis. Reporting in Nature, Susan Ackerman and co-workers now describe a genetic model for neurodegeneration that is mediated by oxidative stress. And, in so doing, they highlight a vital role for a key proapoptotic molecule - the apoptosisinducing factor (AIF) - in neuronal survival.

The authors were investigating a late-onset neurodegenerative mouse model called harlequin (Hq). The mutant mice showed a progressive loss of granule cells from the cerebellum, and TUNEL staining showed various characteristics of apoptosis in these cells, including nicked DNA, chromatin condensation and blebbing. There was also a progressive degeneration of retinal cells in the Hq mutant mice.

Ackerman and co-workers genetically mapped the *Hq* mutation, and showed that it is due to a proviral insertion in the *Aif* gene. This leads to an 80% reduction in *Aif* messenger

Set2 as the lysine methylases responsible for methylation of histone H3, and found that Set1 is specifically responsible for methylation of H3 at residue K4.

Given that lysine residues can be mono-, di- and even tri-methylated, Kouzarides and co-workers raised antibodies that can distinguish between di- and tri-methylated K4 of H3. Using these antibodies, they showed that Set1 is responsible for both di- and tri-methylation of K4 of H3.

Set1 is thought to be a transcriptional repressor. However, the detection of dimethylated K4 H3 at euchromatic loci which generally represent transcriptionally active loci — indicated that Set1 might also function as an activator. So, Kouzarides and colleagues carried out gene-expressionprofiling analysis, which revealed 480 genes whose activity was significantly reduced in the absence of Set1. Reduction of the top-20 genes varied between 53% and 38%, which indicates that Set1 is required, but not responsible, for gene activation.

Using chromatin immunoprecipitation assays, Kouzarides and colleagues showed that Set1-activated genes are methylated at RNA and protein relative to wild-type levels. Under normal physiological conditions, AIF is found in the mitochondrial intermembrane space. Here it acts as an oxidoreductase, a group of molecules that has been implicated in maintaining free-radical homeostasis. So the authors compared the levels of antioxidant enzymes (catalase and glutathione), lipid peroxidation and DNA oxidative damage between Hq and wild-type mice. They observed increases in the levels of all of these factors in the Ha mice, indicating that a loss of AIF function might lead to increased oxidative stress.

As the crystal structure of AIF is similar to that of glutathione reductase - an enzyme that is involved in the recycling of glutathione, and a potent scavenger of hydrogen peroxide  $(H_2O_2)$  — the authors examined the  $H_2O_2$ -sensitivity of Hq granule cells. They found that these cells were more sensitive than wild-type to both exogenously and endogenously generated H<sub>2</sub>O<sub>2</sub>. But when mutant granule cells were infected with retrovirus containing wild-type Aif sequences, the susceptibility to H2O2 was rescued. Moreover, overexpression of AIF in wild-type neurons resulted in a decreased sensitivity to H2O2.

K4 of H3, and that constitutively active genes contain both di- and tri-methylated K4 of H3. What came as a surprise was that the methionine-regulated *MET16* gene, as well as several other inducible genes, is both di- and tri-methylated at K4 of H3 — but only when the gene is active. In the repressed state, however, K4 of H3 is di- but not trimethylated.

The authors propose that "the role of dimethylated K4 H3 may be to determine a transcriptionally 'permissive' chromatin environment, whereas the trimethylated state may allow for an 'active' chromatin conformation". Finally, these data indicate that an — as yet unknown — mechanism must exist that prevents Set1 from adding a third methyl group.

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### References and links

ORIGINAL RESEARCH PAPER Santos-Rosa, H. et al. Active genes are tri-methylated at K4 of histone H3. Nature 419, 407–411 (2002)

FURTHER READING Marmorstein, R. Protein modules that manipulate histone tails for chromatin regulation. *Nature Rev. Mol. Cell Biol.* **2**, 422–432 (2002) WEB SITE

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http://www.welc.cam.ac.uk/groups/kouzarides\_group.shtml