

APOPTOSIS

A dysfunctional harlequin



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 pro-apoptotic molecule — the apoptosis-inducing 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

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 *Hq* 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_2O_2 . But when mutant granule cells were infected with retrovirus containing wild-type *Aif* sequences, the susceptibility to H_2O_2 was rescued. Moreover, overexpression of AIF in wild-type neurons resulted in a decreased sensitivity to H_2O_2 .

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

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 di-methylated 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-expression-profiling 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

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 tri-methylated.

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.

Arianne Heinrichs

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

Finally, abnormalities in the cell cycle have been observed in degenerating neurons, so the authors examined cell-cycle control in the *Hq* mice. A series of experiments showed that granule and retinal cells from *Hq* mice re-enter the cell cycle aberrantly, before they die by apoptosis, supporting the idea of a link between cell-cycle re-entry and oxidative stress.

How do these results square with AIF's known function as a pro-apoptotic molecule? The authors propose that AIF normally acts, either indirectly or directly, as a free-radical scavenger in the mitochondrial membrane — it prevents oxidative stress by mopping up H_2O_2 in particular. Under conditions that induce apoptosis, however, AIF translocates to the cytoplasm and nucleus, where it promotes chromatin condensation and other features of apoptosis. As well as shedding light on the functions of AIF, then, this study has pinpointed the first *in vivo* model for studying how oxidative stress might affect cell-cycle re-entry and apoptosis.

Alison Mitchell

References and links

ORIGINAL RESEARCH PAPER Klein, J. A. *et al.* The harlequin mouse mutation downregulates apoptosis-inducing factor. *Nature* **419**, 367–374 (2002)

DNA REPLICATION

Ku calls time

Not all genes are created equal when it comes to replication — whereas some genes are replicated early during S phase, others are not replicated until much later. The factors that control this difference are not well understood, but a report by Donaldson and colleagues in *Genes & Development* now describes one way of controlling replication timing at telomeric regions. Interestingly, though, the protein involved is probably more familiar to those who study DNA repair.

Replication timing is known to be influenced by several factors, including a gene's position on the chromosome or in the nucleus — those genes that are nearest to the nuclear periphery (and in yeast cells to the telomeres, which localize to the nuclear periphery) tend to replicate later.

Armed with this knowledge, Donaldson and colleagues wondered whether Ku — which acts at eukaryotic telomeres — might be involved in replication timing. The Ku heterodimer consists of two subunits (known as *yku70* and *yku80* in yeast), and it is important in the repair of double-stranded DNA breaks. In yeast, these proteins are required for the localization of telomeres to the nuclear periphery, and they are also involved in the recruitment of silencing (Sir) proteins to telomeres.

Donaldson and colleagues used the dense isotope transfer method to monitor replication in yeast cells going through S phase. As their model for telomere replication, they monitored the yeast chromosome V right region, which contains two active replication origins — *ARS501* and the so-called Y' ARS, both of which replicate late as might be expected from their telomeric positions. The authors also studied the replication time of internally located origins at some distance from the telomeres.

Replication was then compared at the various origins in wild-type and *Ku* deletion yeast strains. In a *yku70* strain, replication at the internally located origins was similar to that in wild-type strains. However, *ARS501* and Y' ARS replicated much earlier in the



mutant than in the wild-type strain. The authors also observed some 'escape replication' (whereby origins replicate before they are released from the block that is used for synchronization) of *ARS501* and Y' ARS — a phenomenon that has previously been reported for very early-replicating sequences. The authors found similar results at other telomeres, as well as in a *yku70yku80* deletion strain.

As Ku is involved in recruiting the Sir3 protein to telomeres, one possible explanation is that, when Ku is deleted, the localization — and hence function — of Sir3 is disrupted. So the authors tested the effects of deleting *sir3* in their system. The Y' ARS was replicated earlier in the *sir3* deletion strain than in wild-type yeast. However, *ARS501* was barely affected, indicating that the effect of deleting Ku is not simply to disrupt the recruitment of Sir3 to telomeres.

The conclusion, then, is that Ku has a specific function in determining the activation time of telomere-proximal replication origins. How it does this remains a mystery. There is no evidence that Ku is needed for the assembly of pre-replication complexes or that it associates with replication origins *in vivo*. So the authors favour a model in which the Ku complex is involved, through its telomere-localization function, in setting up a specialized chromatin context that can act over large distances to regulate the time at which telomere-proximal origins fire. And time will tell if they're right.

Alison Mitchell

References and links

ORIGINAL RESEARCH PAPER Cosgrove, A. J. *et al.* Ku complex controls the replication time of DNA in telomere regions. *Genes Dev.* **16**, 2485–2490 (2002)

WEB SITE

Anne Donaldson's laboratory:
<http://www.dundee.ac.uk/biocentre/SLSBDIV3add.htm>

