CELL DEATH

Fatal cut

Autophagic cell death is thought to be a fail-safe mechanism of suicide that the cell switches to under conditions in which apoptosis does not work. A new study from the group of Hans-Uwe Simon indicates that the reverse situation can also occur — the cell can switch the autophagic system towards apoptosis.

Simon and colleagues noticed that overexpression of the autophagy-related gene-5 (ATG5) protein increased the cell's susceptibility to undergo apoptosis in response to cell-death stimuli, including certain anti-cancer drugs. By contrast, ATG5-deficient cells showed a strongly reduced sensitivity towards anti-cancer agents.

When Simon and co-workers investigated a possible role for ATG5 in apoptosis by using a model of spontaneous neutrophil apoptosis, they detected a truncated ATG5 product in apoptotic, but not in non-apoptotic, neutrophils. They suggested that ATG5 might be a target of proteases that are activated during apoptosis, and identified calpain-1 and -2 as ATG5-specific proteases. Indeed, incubation of a calpain inhibitor with neutrophils prevented both apoptosis and ATG5 cleavage. Overexpression of an ATG5 mutant that lacked the calpain cleavage site in ATG5-deficient cells did not increase sensitivity to anti-cancer drugs. The authors therefore concluded that calpain-mediated cleavage of ATG5 is a crucial pro-apoptotic event. In addition, the authors found that autophagic activity is not required for the apoptotic effect of truncated ATG5.

> “…this finding has clearly important implications for anti-cancer therapies.”

Simon and colleagues showed that truncated ATG5 induced cytochrome *c* release and apoptosis in cells that were not protected by high levels of the anti-apoptotic protein BCL2. ATG5 colocalized with a mitochondrial marker, and the appearance of truncated ATG5, but not of full-length ATG5, in the mitochondrial fraction coincided with cytochrome *c* release and caspase-3 cleavage. The authors also showed that truncated ATG5-mediated apoptosis could be blocked by a caspase inhibitor.

But how does truncated ATG5 trigger cytochrome *c* release? The authors proposed that truncated ATG5 might bind to BCL2-family members. They found that full-length and truncated

CHROMATIN

A twist in the tail

The post-translational covalent modification of histone tails is crucial for eukaryotic gene regulation. Now, Tony Kouzarides and colleagues identify a novel non-covalent histone modification. They report in *Cell* that isomerization of histone-tail proline residues affects histone lysine methylation and, ultimately, gene expression.

Proline isomerization converts peptidyl-proline residues between the *cis* and *trans* conformations, which changes the secondary structure of polypeptides and functions as a regulatory switch in signalling pathways. As histone tails have conserved proline residues, Kouzarides and co-workers asked whether isomerization of histone-tail prolines might affect nucleosome structure and, therefore, gene expression.

The authors first showed that the proline isomerase Fpr4, a member of the FK506-binding protein family in *Saccharomyces cerevisiae*, binds to the N-terminal tail of histones H3 and H4 through its nucleolin-like domain. Chromatin immunoprecipitation analyses in *S. cerevisiae* confirmed that Fpr4 associates with chromatin at constitutively active and inducible genes, intergenic regions and at the silent-mating-type loci.

Using *in vitro* isomerization assays, the authors identified two substrates for Fpr4 — H3P30 and H3P38, proline residues that lie close to the Fpr4-binding site in the H3 tail. An adjacent lysine residue, K36, is methylated by the Set2 histone methyltransferase, and a series of observations indicated crosstalk between H3P38 isomerization and H3K36 methylation.

Methylation of H3K36 prevented the isomerization of H3P38 *in vitro* and, similarly, the *cis:trans* equilibrium of adjacent proline residues affected H3K36 methylation — when H3P38 is in the more common *trans* conformation, Set2 lysine methylation occurred rapidly. However, the addition of catalytically active Fpr4 increased the *cis* conformation of H3P38 and slowed the rate of H3P38 methylation. These results imply that structural changes in the H3 tail affect the action of chromatin-modifying enzymes.

A further set of *in vivo* experiments using *fpr4*-mutant yeast strains yielded some intriguing results. At inducible genes, isomerization of proline residues by Fpr4 inhibits H3K36 methylation, maintaining low amounts of trimethyl H3K36, which

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ATG5 did not bind to BAX; however, only truncated ATG5 binds to BCL-X_L in apoptotic, but not in non-apoptotic, cells. The authors speculated that truncated ATG5 promotes apoptosis by blocking the function of the anti-apoptotic 'survival' protein BCL-X_L, which leads to BAX and/or BAK activation. Although this hypothesis requires further testing, it is supported by the observation that ATG5-induced cell death was blocked by high levels of BCL2.

Although the details of this molecular link between autophagy and apoptosis need to be worked out further, this finding has clearly important implications for anti-cancer therapies.

Arianne Heinrichs

ORIGINAL RESEARCH PAPER Yousefi, S. *et al.* Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis. *Nature Cell Biol.* **8**, 1124–1132 (2006)
FURTHER READING Codogno, P. & Meijer, A. J. Atg5: more than an autophagy factor. *Nature Cell Biol.* **8**, 1045–1047 (2006)

is necessary for the rapid induction of gene expression. By contrast, constitutively active genes are trimethylated at H3K36, and the amount of lysine methylation at these loci is unaffected by the deletion of *fpr4*.

The authors propose that the isomerization of H3P38 to its *cis* conformation by Fpr4 alters the secondary structure of the H3 tail so that H3K36 no longer fits into the active site of Set2. But, during active transcription, the passage of RNA polymerase through genes disrupts the nucleosome, exposing H3K36 to Set2 methylation. Trimethylated H3K36, in turn, inhibits Fpr4, over-riding Fpr4 regulation and locking the chromatin in an active state.

There are several nuclear proline isomerases in higher eukaryotes, and deciphering how isomerization interacts with the complex patterns of modified histones in mammalian cells will be the next challenge.

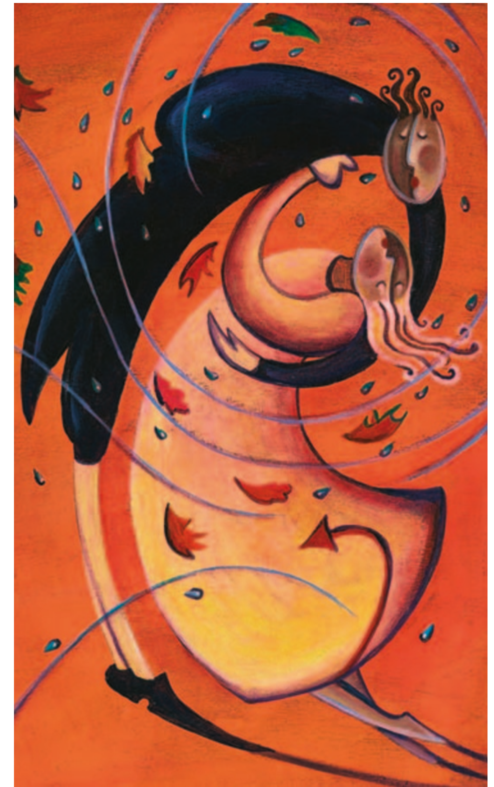
Shannon Amoils

ORIGINAL RESEARCH PAPER Nelson, C. J. *et al.* Proline isomerization of histone H3 regulates lysine methylation and gene expression. *Cell* **126**, 905–916 (2006)

DNA REPAIR

Deinococcus does the two-step

In 1956, *Deinococcus radiodurans* was isolated from canned ground meat that had been irradiated at a dose 250-times higher than that used to kill *Escherichia coli*. Radiation, heat and dehydration normally kill cells by causing double-stranded breaks (DSBs) in their DNA — one of the most difficult kinds of DNA damage to repair — but *D. radiodurans* can withstand 1.5 million rad, a thousand times more than any other organism. The ability of this extremophile to survive the virtual disintegration of its chromosome has attracted widespread interest. Now, reporting in *Nature*, Zahradka and colleagues describe evidence for a two-step DNA-repair mechanism that allows *D. radiodurans* to completely reassemble its radiation-shattered chromosome from hundreds of short fragments in just a few hours.



There are at least six different mechanisms — non-homologous end-joining; homologous recombination at the fragment ends; intra- and interchromosomal single-strand annealing; synthesis-dependent strand annealing (SDSA); break-induced replication; and copy choice — that can stitch together the fragments of partially overlapping DNA that are produced by DSBs. Until now, none of these mechanisms had been excluded for DNA repair in *D. radiodurans*, but this latest study excludes all of them and invokes a completely novel repair mechanism.

Zahradka *et al.* showed that following exposure to extreme radiation, massive DNA synthesis and assembly of DNA fragments occurs, which is dependent on DNA polymerase I. The DNA synthesis that was observed was faster than normal DNA replication, which was puzzling. Researchers recapitulated the classic Meselson–Stahl experiment, in which newly synthesized DNA is distinguished by labelling it with a heavy thymidine analogue (5-bromodeoxyuridine). Their results showed that, unlike normal semi-conservative DNA replication in *D. radiodurans*, DNA-polymerase-I-mediated synthesis and repair produces a patchwork of new and old DNA fragments that are stuck together in a 'distributive' mechanism of DNA repair.

By using a modified immunofluorescence-microscopy method to scrutinize DNA synthesis directly, Zahradka *et al.* showed that most, if not all, of the DNA synthesized by DNA polymerase I was single-stranded DNA that rapidly converted to double-stranded DNA. It seems likely that DNA polymerase I achieves fragment reassembly by extended synthesis-dependent strand annealing (ESDSA). How does ESDSA differ from SDSA? Crucially, it requires at least two genome copies that are broken at different positions. Once overlapping fragments have aligned, a single-round multiplex PCR-like step — a variant of PCR that simultaneously amplifies different target sequences by using multiple primer pairs — occurs to produce long single-stranded overhangs that anneal accurately to produce reassembled chromosomal segments. Last, RecA-mediated homologous recombination using long intermediates synthesized by DNA-polymerase-I produces full-length chromosomes.

Details of the mechanism still need to be refined, including the priming step for DNA-polymerase-I-mediated DNA synthesis and the identification of mechanisms that ensure the fidelity of DNA replication.

Susan Jones, Senior Editor,
Nature Reviews Microbiology

ORIGINAL RESEARCH PAPER Zahradka, K. *et al.* Reassembly of shattered chromosomes in *Deinococcus radiodurans*. *Nature* **443**, 569–573 (2006)
FURTHER READING Cox, M. M. & Battista, J. R. *Deinococcus radiodurans* — the consummate survivor. *Nature Rev. Microbiol.* **3**, 882–892 (2005)

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