TELOMERES

Extreme protection

The extremes of linear chromosomes are dangerous locations. Their structural resemblance to double-stranded DNA breaks can trigger unwanted DNA-damage-response pathways with catastrophic results for the cell. Telomeric DNA is therefore enveloped by a 'cap' of protective proteins that conceal these troublesome ends. Now, researchers have shed new light on the function of one of these telomere-binding proteins — TRF2.

TRF2 is a telomeric double-stranded-DNA-binding protein that has a role in the protection of chromosome ends, but its mechanism of action is unknown. A dominant-negative mutation of TRF2 that displaces the protein from its binding site leads to telomeric association of the ATM kinase — a critical mediator of the DNA-damage-response pathway. These findings indicate that TRF2 might oppose the action or the recruitment of ATM.

To investigate this further, Titia de Lange and co-workers overexpressed TRF2 in human primary fibroblasts, exposed these cells to ionizing radiation (IR) and then analysed the ATM-mediated response to DNA damage. Microscopy analysis revealed that an increased percentage of these cells had entered mitosis — indicating a failure of ATM-mediated cell-cycle arrest. Quantitative immunoblots showed decreased levels of the p53 protein and its downstream targets, and ATM-dependent phosphorylation of NBS1 — a DNA-double-strand-break-repair protein — was also impaired in these cells.

DNA damage that is induced by low levels of IR is usually associated with activation of ATM through autophosphorylation. However, when the authors co-expressed TRF2 and ATM, the level of phosphorylated ATM after IR exposure was reduced compared with controls. Similarly, endogenous ATM phosphorylation was reduced in cells that overexpressed TRF2.

So, does TRF2 directly interfere with ATM activation and function? Anti-ATM antibody co-precipitated

EPIGENETICS

Silent transmission

In mammals, DNA and histone methylation together provide an effective, long-term mechanism for silencing gene expression, but how specific methylation patterns are 'remembered' during cell division is unclear. In a recent paper, Sarraf and Stancheva showed that this depends on the coupling of the two types of methylation during DNA replication.

At sites of constitutive heterochromatin and transcriptionally silenced promoters, silencing is mediated by methylation of DNA at CpG dinucleotides and of histone H3 at lysine 9 (H3-K9). During DNA replication, the methyltransferase DNMT1 interacts with the replication machinery to ensure that DNA methylation patterns are faithfully copied. By contrast, little is known about how histone methylation is reproduced. One model proposes that this is somehow coordinated with DNA methylation, but evidence has so far been lacking.

By co-immunoprecipitation, Sarraf and Stancheva showed that MBD1 — a protein that specifically binds methyl-CpG groups — associates with a complex that contains an H3-K9-specific methyltransferase activity, providing a possible link between DNA and histone methylation. The other components of the complex were identified as the H3-K9-specific methyltransferase SETDB1 and CAF1, a protein involved in chromatin assembly. So, MBD1 bound to methylated DNA could recruit SETDB1 and, through its interaction with CAF1, promote H3-K9 methylation at specific sites during chromatin assembly.

Consistent with this, the three proteins were shown to form a complex *in vivo* specifically during DNA replication. The authors also showed how DNA replication is coupled to the activation of the CAF1–MBD1–SETDB1 complex. CAF1 is only transiently associated with MBD1 and SETDB1 during S-phase and this depends on MBD1 being displaced from DNA. Specific inhibition of replication elongation showed that this displacement depends on the progression of the replication complex. This seems to knock MBD1 off the DNA strand, allowing it to bind CAF1 and promote H3-K9 methylation on newly formed chromatin.

How does this relate to the silencing of specific genes? Sarraf and Stancheva identified several genomic MBD1-binding sites, including a CpG island in the *p53BP2* promoter region. In HeLa cells, *p53BP2* is usually transcriptionally silent, and the authors showed that this depends on both DNA methylation and methylation at H3-K9. Treatment with an inhibitor of DNA methylation or with small interfering RNAs against either MBD1 or SETDB1 led to loss of methylated H3-K9 at the *p53BP2* promoter and induced the expression of the gene. Similarly, reducing levels of DNA methylation or MBD1 expression resulted in loss of methylated H3-K9 at 23 other genomic MBD1-binding sites.

So, interactions between the DNA and histone methylation machinery seem to be important for maintaining patterns of epigenetic modification on a widespread basis. This provides one way of ensuring that transcriptional silencing is transmitted accurately through ongoing rounds of cell division, an essential requirement for normal mammalian development.

Louisa Flintoft
Assistant Editor, Nature Reviews

References and links

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endogenous and exogenous TRF2 in primary human fibroblasts, which indicates that these proteins interact *in vivo*. Pulldown experiments using fusion proteins that comprised different fragments of ATM showed that TRF2 bound to a specific domain of ATM, close to serine 1981 — the main site of autophosphorylation. Finally, immunofluorescence of IR-treated primary fibroblasts that overexpressed TRF2 detected the protein at telomeric foci, but not at chromosomal sites of DNA damage.

Based on these results, the authors propose a model in which TRF2 binds to ATM and inhibits its activation by preventing phosphorylation at S1981. Importantly, the subnuclear location of TRF2 indicates that this inhibition is restricted to telomeres, which would allow ATM to mediate vital DNA repair elsewhere in the nucleus.

Shannon Amoils

References and links

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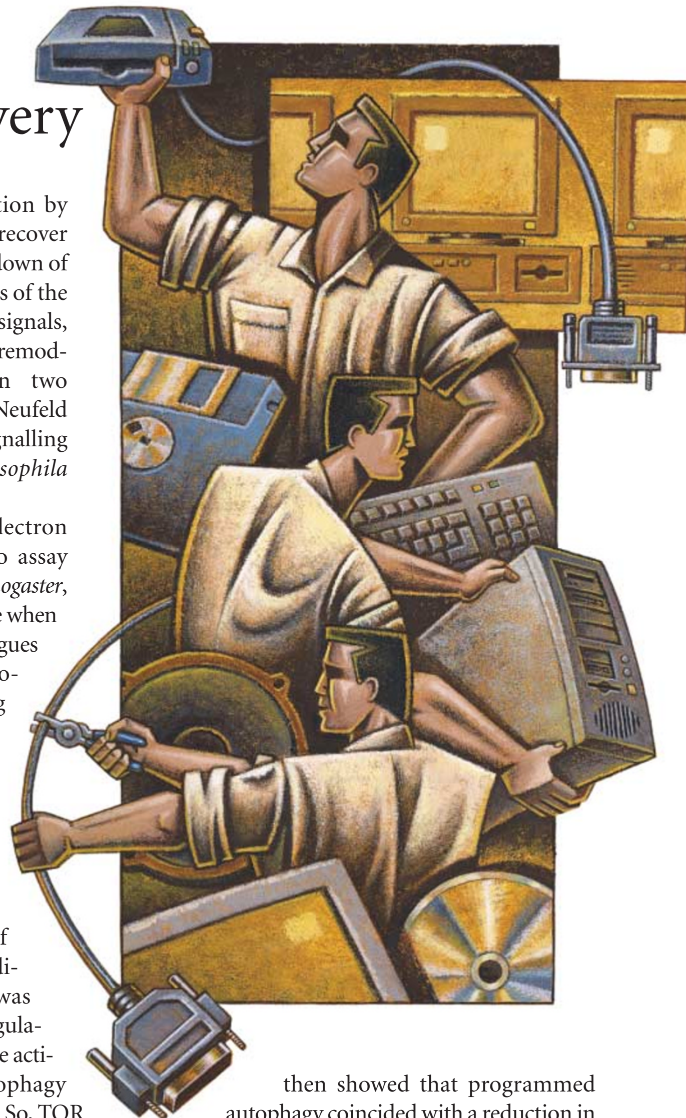
AUTOPHAGY

Breakdown recovery



Eukaryotic cells can respond to starvation by autophagy — a process whereby the cells recover essential nutrients by the lysosomal breakdown of organelles, proteins and other components of the cytoplasm. In response to developmental signals, 'programmed' autophagy promotes tissue remodelling and cell death. Reporting in two *Developmental Cell* papers, the groups of Neufeld and Stenmark have now uncovered the signalling pathways that control autophagy in *Drosophila melanogaster*.

Both groups used a combination of electron and fluorescent confocal microscopy to assay autophagy in the larval fat body of *D. melanogaster*, which showed a strong autophagic response when deprived of nutrients. Stenmark and colleagues also studied the various phases of programmed autophagy in the fat body during the final larval stage.

TOR (target of rapamycin) kinases, the central components of a conserved nutrient-sensing pathway, were suspected to function in the regulation of autophagy. To find out more, Neufeld and co-workers studied *TOR*-null flies and showed that the loss of TOR activity causes the induction of autophagy, regardless of the nutrient conditions. The same autophagic phenotype was observed when they expressed negative regulators of TOR in fat-body cells. Conversely, the activation of TOR signalling suppressed autophagy under conditions of nutrient deprivation. So, TOR signalling is both necessary and sufficient to suppress starvation-induced autophagy.

Components of the phosphatidylinositol 3-kinase (PI3K) signalling pathway, when overexpressed, also suppressed autophagy. However, overexpression of PI3K was unable to inhibit autophagy that was caused by the loss of TOR function, which indicates that PI3K signalling requires TOR activity to suppress autophagy.

Surprisingly, Neufeld and colleagues found that the inactivation of S6K, which is a central downstream effector of TOR, did not induce autophagy. In fact, the autophagy phenotype of starved *S6K*-mutant cells was significantly reduced compared with starved wild-type cells. This implies not only that the suppression of autophagy by the TOR pathway is independent of S6K, but that S6K is a positive regulator of autophagy.

The Stenmark group also studied the signalling pathways that are responsible for programmed autophagy, and found that the ecdysone hormone, which is important for larval development, triggered this event in the *D. melanogaster* fat body. Using a marker for PI3K activity, Stenmark and colleagues then showed that programmed autophagy coincided with a reduction in PI3K activity and could be blocked by the overexpression of components of the PI3K pathway. So, the authors propose that ecdysone signalling downregulates PI3K signalling, thereby inducing autophagy.

These new insights into the pathways that regulate autophagy raise new questions about its physiological role. For example, does autophagy contribute to the reduced cell growth that occurs when TOR activity is low? The answer seems to be no, as the inhibition of autophagy increased the severity of the phenotype that is caused by the loss of TOR signalling in *TOR*-mutant flies, which included reductions in cell size, growth rate and survival. Neufeld and colleagues concluded that, when TOR signalling is reduced, autophagy is required mainly for survival and normal cell metabolism. It will also be interesting to probe the role of TOR during programmed autophagy.

Arianne Heinrichs

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