GENOME STABILITY

Assembling the repair kit

A painstaking new analysis fills in some important gaps in our knowledge of how the human genome repairs the double strand breaks (DSBs) in DNA that are induced by ionizing radiation (IR).

Ataxia telangiectasia (AT) is a hereditary disorder with a complex molecular aetiology. AT cells show cell-cycle checkpoint defects; they are also highly sensitive to IR-induced DSBs, indicating that ATM — the protein that is mutated in AT — is involved in the response to DSBs. Because the link between these two phenotypes is unknown, the mechanism that underlies this sensitivity is a mystery, as is the role of the phosphorylated form of a histone H2A variant (γ-H2AX) and other proteins that gather at an IR-induced DSB site.

Paradoxically, AT cells undergo V(D)J recombination — a process that involves DSBs and a recently identified nuclease, Artemis. Artemis-deficient cells, although they are also radiation-sensitive, efficiently repair DSBs.

Careful comparison between DSB formation and repair in ATM- and Artemis-defective cells indicated to Riballo *et al.* that both ATM and Artemis are required to repair a subset of DSBs that are rejoined slowly after a phase of rapid loss of the γ-H2AX foci. This and other experiments indicated that ATM and Artemis operate in a common 'slow-rejoining' DSB pathway.

So it seems that although most DSBs rejoin rapidly and require the core non-homologous end-joining proteins, a distinct fraction are rejoined more slowly and require ATM and Artemis.

To assess whether the ATM-dependent pathway might be implicated in end-processing, the authors induced high-complexity DSBs, which are likely to require end-processing before rejoining to low-complexity DSBs. A clear correlation between DSB complexity and the magnitude of ATM-dependent DSB rejoining strongly indicated that the ATM-dependent pathway is involved in end-processing.

But what about the relationship between the different components of the DSB-response pathway? The authors showed that radiation-induced hyperphosphorylation did

EPIGENETICS

Breaking the silence

Silencing of whole regions of chromosomes by packaging them into heterochromatin provides an effective way of shutting off gene expression. But what keeps heterochromatin from invading surrounding regions and turning off genes that need to be expressed? A recent study by Ania Ebert and colleagues provides some important insights into the mechanisms that are involved.

Drosophila melanogaster genetics has provided important tools for probing the details of heterochromatin formation. One of these is the phenomenon of position-effect variegation (PEV), in which expression of a gene is reduced or abolished if it is moved to a region of heterochromatin. Screens for mutations that counteract this effect have identified a class of suppressor of position-effect variegation (Su(var)) genes that include important components of heterochromatin. The product of one of these, *Su(var)3-9*, methylates the lysine 9 residue of histone H3 (H3-K9), a key step in heterochromatin formation, and it was a screen for suppression of Su(var)3-9-mediated silencing that provided Ebert and colleagues with a clue as to how the spread of silencing is regulated.

The authors screened for mutants that reversed silencing of the *white* gene that had been translocated to a heterochromatic region, an effect that requires Su(var)3-9 function. The allele that gave the strongest effect — the previously identified *Su(var)*3-1 — mapped to the *JIL-1* gene and caused the opposite phenotype to null mutations in this gene, indicating a gain-of-function effect. *JIL-1* encodes a histone kinase, but the *Su(var)*3-1 mutation had no effect on JIL-1 kinase activity, indicating a second function for this protein.

To pinpoint how JIL-1$^{Su(var)3-1}$ counteracts silencing, Ebert and colleagues first checked whether this mutation alters patterns of histone methylation, which are crucial for determining the activation state of chromatin. When this turned out not to be the case, the authors proposed that JIL-1$^{Su(var)3-1}$-mutant proteins might instead affect the expansion of heterochromatic regions along the chromosome.

To test this, they used a PEV rearrangement in which a large chromosomal region that includes the genes *Notch* and *white* is moved to a region that lies next to heterochromatin. When two additional copies of *Su(var)3-9* are expressed, the region is visibly compacted into a heterochromatic structure, with high levels of H3-K9 dimethylation and expression of *Notch* and *white* mutant phenotypes. In a JIL-1$^{Su(var)3-1}$ background, however, all of these features are reversed, with the translocated region restored to its normal state. Similar results for seven other PEV rearrangements indicate that JIL-1$^{Su(var)3-1}$ prevents or reverses Su(var)3-9-mediated silencing in a range of situations.

The authors conclude that JIL-1 is a general antagonist of Su(var)3-9 function, although the molecular details have yet to be examined. These results reveal a dynamic process that regulates the balance between silent heterochromatin and active euchromatin, and once again show the importance of *Drosophila* in epigenetics research.

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References and links

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not occur in cells expressing tagged Artemis cDNA that were treated with an ATM inhibitor — strong evidence for a role of ATM in this process.

The authors formulated an intriguing model for biphasic rejoining of DSBs in which ATM hyperphosphorylates Artemis, activating its nuclease and end-processing abilities. Other proteins — such as 53BP1, H2AX, Nbs1 and Mre11 — might provide a scaffold that keeps Artemis at the DSB site and/or activate ATM.

Regardless of how accurate this model proves to be, the identification of Artemis as a downstream component of the ATM signalling pathway has gone a long way towards explaining AT radiosensitivity.

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European Journal of Human Genetics

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CANCER GENETICS

Tumour suppressor super models

The tumour suppressor p53 binds DNA and activates transcription to control the cell cycle and apoptosis, and is mutated in over 50% of human cancers. Mutations in *TP53* also cause Li–Fraumeni syndrome, which predisposes patients to a broad spectrum of malignancies, particularly sarcomas and carcinomas. However, the range of tumours seen in Li–Fraumeni syndrome and spontaneous cancers cannot be explained simply by a loss of wild-type p53; for example, mice that lack p53 develop lymphomas and sarcomas but not carcinomas, and these tumours tend not to metastasize. Furthermore, p53 is an unusual tumour suppressor because it is commonly altered through missense mutation rather than deletion. Now, two research groups have generated mouse models that closely resemble Li–Fraumeni syndrome and have used these models to investigate why the *TP53* mutations seen in human cancers are so oncogenic.

Kenneth Olive and co-workers produced mice with missense point mutations in two of the most commonly mutated p53 codons in human cancer: $Trp53^{R172H}$ affects the overall structure of the p53 DNA-binding domain, and $Trp53^{R270H}$ affects a residue that makes direct contact with DNA. Although $Trp53^{R270H/-}$ and $Trp53^{R172H/-}$ mice developed distinct tumour spectra, both developed different tumour phenotypes compared with $Trp53^{-/-}$ mice, indicating that missense *Trp53* mutants have pro-tumorigenic or oncogenic functions that cannot be explained simply by the loss of wild-type p53. In particular, strains carrying these two mutant alleles developed metastatic carcinomas and are therefore more accurate models of Li–Fraumeni syndrome.

The possibility that mice carrying *Trp53* missense mutations could be used as models of Li–Fraumeni syndrome was further supported by work carried out by Gene Lang and colleagues, who also generated mice that possessed the $Trp53^{R172H}$ structural mutation (which they refer to as $Trp53^{515A}$). However, the results from the two laboratories show that the same *Trp53* mutation causes different tumour spectra in different mouse strains; whereas Olive and co-workers found that $Trp53^{R172H/+}$ mice developed more carcinomas than $Trp53^{+/-}$ mice, Lang *et al.* show that $Trp53^{R172H/+}$ mice developed metastatic tumours.

Lang and colleagues also found that $Trp53^{R172H/R172H}$ and $Trp53^{R172H/+}$ mouse embryonic fibroblasts grow faster, have more DNA synthesis and have greater transformation potential than $Trp53^{+/+}$, $Trp53^{+/-}$ or $Trp53^{-/-}$ cells, supporting the idea that p53 mutant proteins function differently to wild-type p53. So, how do missense mutant p53 proteins exert their oncogenic effects?

p53 interacts with its family members p63 and p73, which themselves activate several p53 target genes in response to DNA damage. Both groups found evidence that $p53^{R172H}$ interacts with and inhibits endogenous p63 and p73 in cell lines that are derived from mouse tumours expressing this protein. Lang and colleagues also found that the disruption of p63 and p73 causes increased transformation of $Trp53^{-/-}$ cells and augments DNA synthesis to levels seen in $Trp53^{R172H/R172H}$ cells. The researchers conclude that the ability of mutant p53 to bind and inhibit p63 and p73 could explain why mutant p53 is more detrimental than the lack of p53, and why *TP53* missense mutations — rather than deletions of *TP53* — are so commonly found in human tumours.

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