CELL CYCLE

From radiation to Rad17

Eukaryotic genomes are subject to a barrage of potentially damaging agents such as ultraviolet (UV) light or ionizing radiation (IR). Cells fight back by activating checkpoints — control points that allow the cell cycle to be halted and any damage to be repaired. Many checkpoint pathways have already been characterized but, reporting in *Nature*, Robert Abraham, Xiao-Fan Wang and colleagues describe a new link in regulation of the damage-induced G2 checkpoint.

Studies in fission yeast have implicated members of the so-called Rad family — which includes Rad17, Rad1, Rad9 and Hus1 — in the activation of DNA damage and replication checkpoints. Rad17 is thought to load a complex of the other three proteins onto the damaged DNA during an early stage of checkpoint activation. But what regulates Rad17?

Abraham, Wang and colleagues tackled this question by asking what proteins Rad17 associates with. Immunoprecipitations revealed an interaction between human Rad17 (hRad17) and two related checkpoint kinases — 'ataxia-telangiectasia, mutated' (ATM) and ataxia-telangiectasia and Rad3-related kinase (ATR). These interactions were strengthened if cells were exposed to IR and also, in the case of ATR, to UV light.

So could hRad17 be a substrate for these kinases? The authors found that wild-type ATR can indeed phosphorylate hRad17, both *in vitro* and in intact cells treated with IR. Two serine residues (Ser635 and Ser645), which are conserved in Rad17 across several species, were crucial for the phosphorylation. Abraham, Wang and colleagues then overexpressed a catalytically inactive ATR mutant (ATR^{KI}), and showed that the phosphorylation of Ser635 and Ser645 in response to UV light was reduced considerably. Interestingly, though, phosphorylation of Ser645 in response to IR was not as sensitive to expression of ATR^{KI}. This is consistent with a model in which ATM mediates the phosphorylation of hRad17 in response to IR, whereas ATR controls the response to other forms of genotoxic stress.

To test the functional consequences of the interaction between hRad17 and ATM/ATR, the authors overexpressed a mutant hRad17 ($hRad17^{AA}$) in which the two crucial serine residues had been mutated to alanine. The cells failed to activate the G2/M checkpoint after exposure to IR, and many showed signs of apoptotic death. Further analysis showed that although $hRad17^{AA}$ was not phosphorylated, it could nonetheless still interact with ATR and ATM.

Finally, Abraham, Wang and colleagues used the $hRad17^{AA}$ mutant to see whether phosphorylation of hRad17 modulates its interaction with the hRad1/hRad9/hHus1 complex. Sure enough, whereas wild-type hRad17 co-precipitated with hRad1 in response to IR, this complex was not seen when $hRad17^{AA}$ was used.

The conclusion, then, is that ATM and ATR are upstream regulators of hRad17. This work not only reinforces the importance of these kinases in DNA-damage-induced checkpoint pathways, but also places hRad17 squarely in the middle of the responses to genotoxic stress.

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References and links

ORIGINAL RESEARCH PAPER Bao, S. *et al.* ATR/ATM-mediated phosphorylation of human Rad17 is required for genotoxic stress responses. *Nature* **411**, 969–974 (2001)

FURTHER READING Kastan, M. B. & Lim, D.-s. The many substrates and functions of ATM. *Nature Rev. Mol. Cell Biol.* **1**, 179–186 (2000)

IN BRIEF

CELL CYCLE

A DNA damage response pathway controlled by Tel1 and the Mre11 complex.

Usui, T., Ogawa, H. & Petrini J. H. J. *Mol. Cell* **7**, 1255–1266 (2001)

By examining checkpoint activation in *Saccharomyces cerevisiae*, the authors have defined a new DNA-damage response pathway controlled by the yeast ataxia-telangiectasia mutated (ATM) homologue Tel1, and the Mre11 complex. The Mre11 complex acts as the damage sensor, and its ability to repair double-stranded DNA breaks is enhanced by the pathway. According to the authors, these results indicate "that the diverse functions of the Mre11 complex in the cellular DNA damage response are conserved in mammals and yeast".

TECHNIQUE

Short tandem repeat profiling provides an international reference standard for human cell lines.

Masters, J. R. *et al. Proc. Natl Acad. Sci. USA* **98**, 8012–8017 (2001)

Although there are many ways to detect cross-contamination between cell lines, none so far has been suitable for use as an international reference standard. Masters and colleagues now report the study, on 253 human cell lines, of a technique that they believe could fill this void — short tandem repeat profiling. The profile is a simple numerical code that is reproducible between laboratories and inexpensive to generate.

TRANSCRIPTION

Structure of the histone deacetylase SIRT2.

Finnin, M. S., Donigian, J. R. & Pavletich, N. P. *Nature Struct. Biol.* **8**, 621–625 (2001)

SIRT2 is the human homologue of yeast Sir2, an NAD-dependent histone deacetylase that mediates transcriptional silencing at mating-type loci and telomeres. This report of the SIRT2 crystal structure — at a resolution of 1.7 Å — reveals an NAD-binding domain as well as a smaller domain composed of a helical module and a zinc-binding module. The helical module forms a pocket lined with hydrophobic residues that are conserved within the five classes of Sir2 proteins.

CELL ADHESION

Requirement for C3G-dependent Rap1 activation for cell adhesion and embryogenesis.

Ohba, Y. *et al. EMBO J.* **20**, 3333–3341 (2001)

C3G is a guanine-nucleotide exchange factor for Rap1 that is now shown to be needed for adhesion and spreading. Embryonic fibroblast cells prepared from conditional *C3G*-deficient mice — generated to overcome embryonic lethality of *C3G* knockouts — show decreased adhesion and spreading, and also migrate faster. The authors showed that C3G is required for cell-attachment-mediated Rap1 activation, but that Rap1 activation alone isn't sufficient for spreading — specific extracellular integrin matrices are needed.