CELL SPREADING

Come closer to the edge

They belong to the same subfamily, but the small GTPases Rap1 and K-Ras show sibling rivalry when it comes to cell adhesion. Oncogenic mutations in K-Ras cause cells to lose adhesion, whereas Rap1 has a positive influence on cell spreading and adhesion. William Arthur, Lawrence Quilliam and Jonathan Cooper used the fact that the Rap1 yeast orthologue activates a Rho protein by stimulating a guanine nucleotide-exchange factor (GEF) to explore the possibility that, in mammalian cells, Rap1 might also mediate cell spreading by activating Rho-family GTPases. Their findings are reported in *The Journal of Cell Biology*.

When suspended cells were re-plated onto a fibronectin surface, Rap1 and the Rho-family GTPase Rac1 were both activated. And cells expressing active forms of Rap1, Rac1 or Rac GEFs were all similarly spread. On further investigation of an interrelationship between Rap1 and Rho-family GTPases, the authors found that inhibiting Rac1, but not Cdc42 or RhoA, antagonized Rap1-induced cell spreading, which implies that Rap1 requires Rac1 for spreading. In support of this, Rap1 activation increased Rac1–GTP levels. And activating Rac1 when Rap1 function was compromised restored cell spreading.

Faced with several possibilities for how Rap1 might induce cell spreading through Rac1, the authors explored potential interactions of Rap1 with Rac GEFs. Rap1 could bind to the central Dbl-homology and pleckstrin homology (DH–PH) domains of VAV2 and Tiam1, but not to SWAP-70 or COOL-1. But although activated versions of these GEFs enhanced spreading in control cells, they couldn't do so in cells in which Rap1 activity was inhibited — unlike the case mentioned above, in which activated Rac1 could overcome impaired Rap1 activity.

Again, there were many possible reasons for the requirement of Rap1 activity for VAV2 and Tiam1 function. Active Rap1 didn't seem necessary to stimulate the catalytic activities of the GEFs, so, instead, the authors studied the potential influence of Rap1 on GEF localization. In studies using extracts from fractionated pseudopodia and by immunofluorescence, both GDP- and GTP-bound Rap1 were found in membrane protrusions that were associated with the substratum. So, too, was VAV2, but not

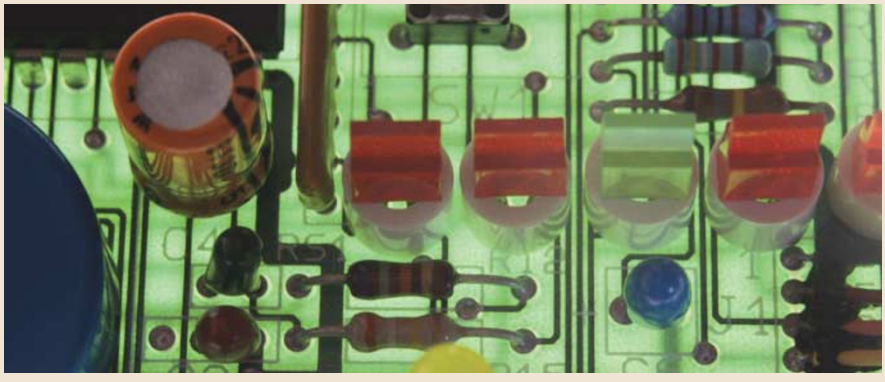
P53

Complex control

The regulation of the tumour suppressor and transcription factor p53 is highly complex and involves different types of post-translational modification, including phosphorylation and ubiquitylation. Recent findings reported in *Nature* now add to the complexity, as Danny Reinberg and colleagues have shown that p53 can be specifically methylated, and that this regulates its stability and the expression of target genes.

The histone lysine methyltransferase Set9 is known to methylate Lys4 of histone H3 *in vitro*. While searching for other substrates for Set9, Reinberg and co-workers identified p53 as an *in vitro* substrate — other proteins that were tested did not serve as Set9 substrates, and alternative protein methyltransferases couldn't methylate p53. Importantly, however, other polypeptides that are present in HeLa-cell-derived extracts also served as substrates for Set9, and a recent report indicates that the transcription-initiation factor TAF10 can also be methylated by Set9. The p53 methylation site was mapped to a single Lys residue (Lys372) in the regulatory C-terminal region of p53, which is subjected to multiple post-translational modifications.

The affinity of Set9 for a p53 peptide was, in fact, higher than its affinity for an equivalent histone-H3 peptide. This led the authors to determine the structure of Set9 in complex with a monomethylated p53 peptide, and compare it with the previously solved complex of Set9 with a monomethylated histone-H3 peptide. Overall, the structures are very similar, and the Set9 residues that interact with the three amino-acid residues N-terminal to the methylated Lys residue are the same for the p53 peptide and the H3 peptide, despite the difference in peptide sequence. Reinberg and colleagues speculate that more distant residues in p53 might contribute to the substrate specificity.



To study the significance of Set9-methylation of p53 *in vivo*, the authors stably transfected cells that expressed endogenous p53 with wild-type Set9 or with a methylation-defective Set9 mutant. Using an antibody that is specific for Lys372-methylated p53, methylated p53 could be detected in extracts from the former cells but not the latter. When untransfected cells were treated with an anti-cancer drug that induces DNA damage and therefore a p53-responsive pathway, the amount of methylated p53 was increased compared with untreated cells. However, by introducing Set9 small interfering (si)RNA into these cells, the level of methylated p53 decreased after chemical treatment. So, Set9 methylates p53 *in vivo* and Set9-specific methylation of p53 occurs in response to DNA damage.

Next, the authors found that methylated p53 is exclusively nuclear, which drew their attention to the transcriptional activity of p53. Focusing on a well-characterized

when Rap1 activity was inhibited — indeed, this caused displacement of VAV2 from the cell edge.

It follows from these results, then, that artificially targeting Rap1-dependent Rac GEFs such as VAV2 and Tiam1 to the edge of the cell, where Rap1 is normally active, could compensate for a lack of Rap1 activity. By fusing the membrane-localization signals that are present in the Rap1 C-terminal 'CAAX' box and its adjacent N-terminal hypervariable domain to VAV2, the requirement for GTP-bound Rap1 in Rac1 activation could be bypassed.

The resulting Rac1-induced formation of productive membrane protrusions associated with the adjacent extracellular matrix thereby induces cell spreading. Such protrusive activity would, in turn, create new sites for Rap1 localization, and so the cycle should continue.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Arthur, W. T., Quilliam, L. A. & Cooper, J. A. Rap1 promotes cell spreading by localizing Rac guanine nucleotide exchange factors. *J. Cell Biol.* **167**, 111–122 (2004)

FURTHER READING Kinbara, K. *et al.* Ras GTPases: integrins' friends or foes? *Nature Rev. Mol. Cell Biol.* **4**, 767–776 (2003)

transcriptional target of p53 — p21 — they showed that the increased expression of p21 correlated with elevated levels of methylated p53. In addition, the induction of DNA damage further increased the level of p21 expression. By contrast, overexpression of catalytically inactive mutant Set9, or the siRNA-mediated depletion of Set9, impaired p21 expression and decreased the total level of p53.

This latter finding suggested to Reinberg and colleagues that methylation of p53 might affect its stability. Indeed, the stability of p53 increased in cells that expressed wild-type Set9, but not in those that expressed the methylation-defective mutant. Consistent with this 'hyper-stabilization' of p53, cells that overexpressed Set9 also showed higher apoptotic staining, which is indicative of a stronger p53-dependent apoptotic response.

The *in vivo* role of methylation in p53 regulation implies complex regulatory mechanisms, the coordination of which remains unclear. In addition, it raises the question of how the methylation of a single residue might interfere with the ubiquitylation and subsequent degradation of p53.

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References and links

ORIGINAL RESEARCH PAPER Chuikov, S. *et al.* Regulation of p53 activity through lysine methylation. *Nature* 18 Nov 2004 (doi:10.1038/nature03117)

EPIGENETICS

Let us join together in silence

It's so often the case — processes that were previously considered separate and distinct turn out to be intimately related. Whereas post-transcriptional silencing occurs through RNA interference (RNAi), transcriptional silencing is associated with 'silent' heterochromatin, which is mediated by histone methylation. The RNA-induced initiation of transcriptional gene silencing (RITS) complex is known to be required for heterochromatic gene silencing, but it also contains short interfering (si)RNAs that are generated by Dicer (Dcr), an enzyme that is involved in RNAi. In *Nature Genetics*, Noma *et al.* report the discovery of a self-enforcing loop mechanism through which RITS binding to heterochromatic loci enables the RNAi machinery to function in *cis* to destroy aberrant RNAs and generate siRNAs for heterochromatin maintenance.

Noma *et al.* began by showing, using chromatin immunoprecipitation (ChIP) analysis and immunofluorescence, that all three known subunits of the RITS complex — Argonaute, Chp1 and Tas3 — stably associate with all known heterochromatic domains in the *Schizosaccharomyces pombe* genome, such as centromeres, telomeres and the *mat* locus.

RITS is known to be essential for heterochromatin assembly at centromeres, so the authors investigated its possible role in heterochromatin assembly at the *mat* locus. They found that RITS cooperated with Atf1 and Pcr1, which belong to the ATF-CREB (cAMP-responsive-element-binding protein) family, to nucleate heterochromatin assembly at the *mat* locus. Mapping of RITS at the *mat* locus showed that, surprisingly, RITS could spread from the initial nucleation site, a centromere-homologous (*cenH*) repeat, to the neighbouring sequences. This presumably allows RITS to exert control over sequences that are incapable of initiating an RNAi response. For RITS to spread from *cenH* throughout the *mat* locus, though, Swi6 — a protein that mediates spreading of Lys9-methylated histone H3 — was required. Dcr-generated siRNAs, though, were not required for this spreading, but were needed for the initial targeting of RITS to *cenH*. The authors found that *cenH* was transcribed bidirectionally, and that the level of *cenH* transcript was regulated by both transcriptional and post-transcriptional silencing mechanisms: a lack of Swi6 (which is dispensable for RITS targeting at *cenH* but is required for transcriptional silencing) or a lack of Dcr (which isn't needed for heterochromatin maintenance at the *mat* region but does process dsRNAs into siRNAs) caused *cenH* transcripts to accumulate — substantially so in double mutants.

Noma *et al.* also investigated whether the stable association of RITS with heterochromatic loci was essential for the processing of rare repeat transcripts that escape transcriptional silencing. Indeed, they found that a point mutation in the H3-Lys9-binding domain of Chp1, or deletion of the Clr4 methyltransferase, not only prevented RITS from binding to chromatin but also abolished RITS-associated siRNAs. Moreover, loss of Clr4 caused centromeric-repeat transcripts to accumulate. So RITS needs to be associated with chromatin to process transcripts into siRNAs and to generate heterochromatin.

The model proposed by the authors establishes a mechanism through which RITS can function in *cis* to bring about effective gene silencing. Heterochromatic marks are initially established in a Dcr-dependent manner, and therefore presumably through the RNAi pathway. Such siRNAs somehow guide Clr4 and factors that mediate heterochromatin assembly to homologous target sequences. RITS then localizes at these specific loci and mediates the processing of nascent RNA transcripts, probably by recruiting Dcr or RNA-dependent RNA polymerase. This thereby generates more siRNAs — so the mechanism continues…

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References and links

ORIGINAL RESEARCH PAPER Noma, K. *et al.* RITS acts in *cis* to promote RNA interference-mediated transcriptional and post-transcriptional silencing. *Nature Genet.* **36**, 1174–1180 (2004)

WEB SITE

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