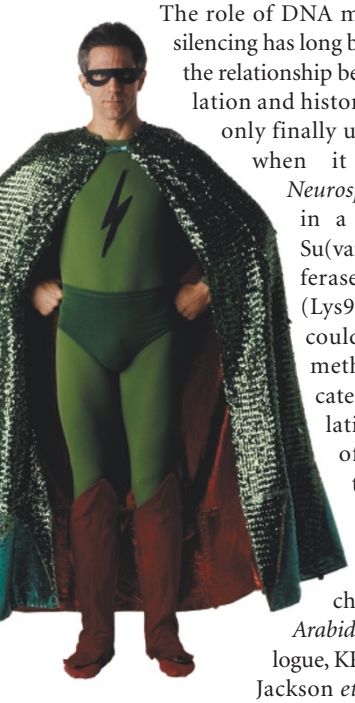
DNA METHYLATION

The silent superhero



The role of DNA methylation in gene silencing has long been established, but the relationship between DNA methylation and histone methylation was only finally unravelled last year, when it was shown in *Neurospora* that mutations in a widely conserved Su(var)-like methyl transferase and in lysine 9 (Lys9) of histone H3 could abolish all DNA methylation. This indicated that DNA methylation is downstream of histone methylation. But is this just a fungus-specific phenomenon? By identifying and characterizing the *Arabidopsis* Su(var) homologue, KRYPTONITE (KYP), Jackson *et al.* now extend the previous findings by showing that this relationship is also conserved in plants.

The study started with a genetic screen for suppressors of a hypermethylated allele of *SUPERMAN* (*SUP*), which causes disrupted flower morphology. As well as identifying mutations in *CHROMOMETHYLASE3* (*CMT3*), a known DNA methyltransferase, the screen yielded alleles of another gene, which the authors called *KYP*. *KYP* contains a SET domain that is characteristic of Su(var)9-3 proteins, which are known to be involved in histone H3 methylation and, as expected, it specifically methylates Lys9 of histone H3. Sequence analysis showed that the activity of the SET domain is either reduced or eliminated in *kyp* mutants, indicating that this domain is necessary for its function.

Given the dependence of DNA methylation on histone methylation in *Neurospora*, the authors decided to investigate the effects of loss-of-function mutations in KYP on DNA methylation. They used methylation-sensitive restriction enzymes and bisulphite sequencing to compare the methylation status of several loci, including *SUP*, which is predominantly methylated at CpNpG sites, and *FLOWERING LOCUS WA*, which is mostly methylated at CpG sites. The authors found that, as in the *cmt3* mutants, in *kyp* mutants DNA methylation was affected specifically at CpNpG sites in all of the sequences tested.

So how is the histone methylation that is brought about by KYP 'translated' into DNA methylation? The fact that CMT3 has a chromodomain made the authors wonder whether CMT3 could bind directly to Lys9 of histone H3, as does HP1, which binds to methylated, heterochromatic regions. Their *in vitro* studies and binding assays of tagged CMT3 and histone H3 in *Escherichia coli* gave negative results — instead, CMT3 turned out to bind directly to the *Arabidopsis* HP1.

On the basis of their findings, the authors propose the following model. HP1 is attracted to Lys9 of histone H3, once it has been methylated by KYP. HP1 then binds to CMT3, thus bringing it to the site of histone methylation. It is then up to CMT3 to methylate the DNA. Although this model awaits *in vivo* validation, it suggests the existence of a eukaryote-wide mechanism for attracting DNA methyltransferases to the chromatin, in which HP1 has a central role.

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References and links

ORIGINAL RESEARCH PAPER Jackson, J. P. *et al.* Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* **416**, 556–560 (2002)

WEB SITE

Steve Jacobsen's laboratory: http://www.mcdb.ucla.edu/Research/Jacobsen/index.html

APOPTOSIS

Cancer cells come to a sticky end

Warning — matrix adhesion can seriously damage your health. Instead of providing support and protection, a close relationship between cells and the underlying extracellular matrix (ECM) can increase their susceptibility to death following DNA damage.

Loss of adhesion from the ECM is sufficient to trigger apoptosis in some normal cells, but what about in cancer cells, in which detachment is an essential step on the path to malignancy? Jean Lewis *et al.*, reporting in *Proceedings of the National Academy of Sciences*, showed that ligation to integrins — receptors that mediate the attachment of cells to the ECM — increased the level of apoptosis in fibrosarcoma cells that had been treated with the DNA-damaging drug araC. Although not universal among transformed cells, several other cell types — including human M21L melanoma cells and rhabdomyosarcoma cells, as well as mouse embryo fibroblasts (MEFs) — exhibited this differential behaviour in response to DNA damage, depending on whether their integrins were engaged.

To confirm that integrins were responsible for apoptotic susceptibility, the ability of agonistic anti-integrin antibodies to restore the apoptotic response in the detached fibrosarcoma cells was examined. Treatment of detached cells with antibodies that bind either the β1 or αVβ3 integrins was, indeed, sufficient to restore araC-induced cell death, using caspase activity as an assay of apoptosis.

So how do integrins mediate these effects? The checkpoint protein CHK1 was activated in both adherent and suspended cells, so the defect must occur further downstream. p53 is a crucial mediator of apoptosis, and is degraded by MDM2, which, in turn, can be sequestered by ARF, so could one of these components be responsible for the apoptotic resistance that occurs in suspended cells? Following detachment of MEFs from the ECM, ARF protein levels rapidly declined, whereas MDM2 levels remained high. MDM2-mediated degradation of p53 therefore increased; p53 protein levels decreased with slower kinetics than those for ARF, and this correlates with the onset of apoptotic resistance.

But p53 has a broader role as 'guardian of the genome', so could treatment of detached cells with cytotoxic agents increase genomic instability? Following irradiation, MEFs that were grown in suspension displayed a significantly increased number of chromosomal rearrangements compared with those that retained their integrin-mediated ECM attachments.

So do we need to re-think our approach to cancer therapy, given these results? Not only do detached cells escape chemotherapy-induced apoptosis, but the treatment might also accelerate tumour progression by promoting genomic instability. Activating integrins before conventional treatment — in the subset of cancer types that exhibit this behaviour — might be one solution to this problem.

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References and links

ORIGINAL RESEARCH PAPER Lewis, J. M. *et al.* Integrins regulate the apoptotic response to DNA damage through modulation of p53. *Proc. Natl Acad. Sci. USA* **99**, 3627–3632 (2002)

WEB SITES

Encyclopedia of Life Sciences: http://www.els.net Apoptosis: molecular mechanisms | Integrins: signalling and disease

Martin Schwartz's laboratory: http://www.scripps.edu/vb/schwartz/