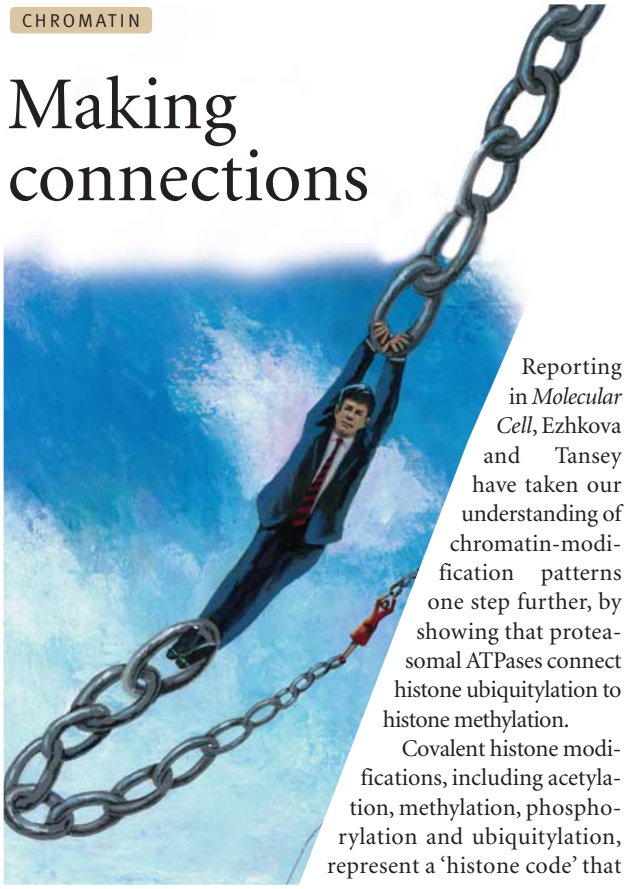
CHROMATIN

Making connections



Reporting in *Molecular Cell*, Ezhkova and Tansey have taken our understanding of chromatin-modification patterns one step further, by showing that proteasomal ATPases connect histone ubiquitylation to histone methylation.

Covalent histone modifications, including acetylation, methylation, phosphorylation and ubiquitylation, represent a 'histone code' that controls the transcriptional status of genes. In *Saccharomyces cerevisiae*, the chromatin of transcriptionally active genes is methylated on histone H3 at lysine residues K4 (H3-K4) and K79 (H3-K79), which functions as a short-term 'memory' of recent transcription. H3 methylation, which, intriguingly, is also required for transcriptional silencing, depends on the ubiquitylation of histone H2B at K123 (H2B-K123).

Using chromatin immunoprecipitation (ChIP) analysis and real-time PCR, Ezhkova and Tansey investigated the interactions of the *S. cerevisiae* 19S proteasome subunits Rpt4 and Rpt6 with chromatin. By comparing constitutively active and inducible genes, they showed that Rpt6 associates with both active and uninduced promoters, but that its binding is enriched during active transcription. Furthermore, tagged Rpt4 was detected along the length of genes during active transcription, but was only found at the promoter and the 5′ end of an inducible gene under non-inducing conditions. This indicates that proteasomal ATPases associate with promoter sequences before gene activation occurs and then spread along the transcribed sequences during activation.

Temperature-sensitive mutations in the ATPase domain of Rpt6 (*SUG1-25* and *SUG1-3*) and Rpt4 (*SUG2-1*) produced considerably lower levels of methylated H3-K4 and H3-K79 throughout the genome, although methylation of another lysine — H3-K36 — was not affected. A loss of gene silencing was also seen in these mutants. However, yeast that were mutant in different proteasome components — and therefore have compromised proteasome function — had relatively normal H3 methylation and showed no effect on gene silencing. So, Rpt4 and Rpt6 facilitate H3 methylation and gene silencing but these functions are independent of proteolysis.

The ubiquitin-conjugating enzyme Rad6, which ubiquitylates histone H2B, is required for methylation of H3-K4/K79, but not H3-K36. The authors found that

APOPTOSIS

Take a direct route

The tumour suppressor p53 induces apoptosis mainly by acting as a transcription factor and promoting the expression of several proteins that are involved in apoptosis. Reporting in *Science*, Doug Green and colleagues have now uncovered a more direct route for p53-mediated apoptosis — similarly to BH3-only proteins, cytosolic p53 can directly activate Bax and trigger apoptosis.

To test whether cytosolic p53 can induce the apoptotic programme, Green and co-workers pretreated p53-expressing cells with an inhibitor of nuclear import. The nuclear accumulation of p53 was blocked as evidenced by the lack of UV-induced p53-dependent gene expression. However, UV-induced apoptosis persisted, even in the absence of nuclear p53. Similarly treated *Bax*$^{-/-}$ cells were resistant to UV-induced apoptosis, which indicates that Bax is required for the induction of apoptosis in the absence of nuclear p53. The inhibition of nuclear import in cells that expressed a transcriptionally inactive form of p53 also resulted in its cytosolic accumulation and cell death — so the cytosolic localization of p53 is sufficient to induce apoptosis.

To clarify the cytoplasmic effects of p53, the authors microinjected purified p53 — wild-type p53, the transcription-deficient p53 mutant or a p53 mutant that lacked the proline-rich domain — into cells that expressed a cytochrome-*c*–green-fluorescent-protein fusion protein. Wild-type p53 and the transcription-deficient mutant each induced cytochrome-*c* release from mitochondria, whereas the mutant that lacked the proline-rich domain did not. Green and colleagues next showed that incubating purified p53 with isolated mitochondria induces cytochrome-*c* release, but only in the presence of Bax, and that the proline-rich domain of p53 is necessary for Bax activation.

Synthetic liposomes can be permeabilized in the presence of Bid — a BH3-only protein — and Bax. The authors observed similar effects when p53 and Bax were added to the lipid vesicles, the permeabilization of which mimics that of the mitochondrial outer membrane. Bid and another BH3-only protein, Bim, activate Bax by causing its oligomerization. Similarly, wild-type p53, but not the mutant that is defective in the proline-rich domain, induced the formation of Bax oligomers. So, the apoptotic function of cytosolic p53 seems to resemble that of BH3-only proteins.

Some BH3-only proteins, including Bid and Bim, bind to Bcl-2 and Bcl-xL. And the latter block apoptosis by sequestering pro-apoptotic Bcl-2-family proteins such as Bax. Other BH3-only proteins, such as Bad, instead promote apoptosis by releasing Bid and Bim from Bcl-2 and Bcl-xL. p53 also binds to Bcl-2 and Bcl-xL, and Green and co-workers showed that the binding of p53 to Bcl-xL released Bax and Bid from Bcl-xL. Again, this confirms the BH3-only-type function of cytosolic p53.

The authors note that this newly discovered, additional function of p53 in apoptosis provides yet another example of "…an emerging complexity that exists within components of the apoptotic machinery".

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPER Chipuk, J. E. *et al*. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science* **303**, 1010–1014 (2004)

WEB SITE

Douglas Green's laboratory:
http://www.liai.org/
http://www-biology.ucsd.edu/faculty/green.html

the deletion mutant *RAD6Δ* and *SUG1-25* yeast had similar defects in gene silencing, and, although the *SUG1-25* mutation has no effect on H2B ubiquitylation, *RAD6Δ* yeast are unable to recruit Rpt6 to active promoters. Furthermore, ChIP analysis showed that a point mutation in the gene encoding H2B, which prevents ubiquitylation of H2B, also inhibits Rpt4 recruitment to chromatin.

So, Ezhkova and Tansey propose that H2B ubiquitylation by Rad6 recruits proteasomal ATPases to promoters. The proteasome components then move along the gene with RNA polymerase II, and use their ATP-dependent chaperone activity to reconfigure chromatin and allow access of histone methyltransferases to their target lysine residues — thereby coupling H2B ubiquitylation to transcription-dependent methylation of H3.

Lesley Cunliffe

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MEMBRANE TRAFFICKING

Adapting to a new role

In *Nature Cell Biology*, two papers now show that the Golgi-localized, γ-ear-containing, Arf-binding (GGA) family of adaptor proteins can interact directly with ubiquitin. Studies from Puertollano and Bonifacino, and the Piper and Boman laboratories, have found that these adaptors seem to have a new role in sorting ubiquitylated cargo at mammalian endosomes and the yeast *trans*-Golgi network (TGN), respectively.

GGAs are mainly associated with the TGN, where, in mammalian cells, they are known to sort mannose-6-phosphate receptors (MPRs) by binding to acidic-cluster–dileucine motifs in the MPR cytoplasmic tails. However, in the first study, Puertollano and Bonifacino showed that mammalian GGAs also colocalize with early endosomes. Using RNA interference, they showed that depleting GGA3 levels caused MPRs and internalized epidermal growth factor (EGF) to accumulate in enlarged early endosomes, which indicates that these proteins have entered a compartment they cannot leave.

These effects are similar to those seen after depleting proteins of the endosomal machinery that selects ubiquitylated cargo for delivery to the degradation pathway (ubiquitylated EGF receptors with bound EGF are sorted into this pathway). The authors therefore tested whether GGAs can bind ubiquitin and found that they can: GGA3 has the highest avidity of the mammalian GGAs and its VHS–GAT region is responsible for this binding.

In the final part of their study, Puertollano and Bonifacino showed that this region of GGA3 can also bind a subunit of the endosomal machinery mentioned above. They therefore propose that the effects of GGA3 depletion "…reflect a function of this protein in endosomes, which may be mediated by its interaction with the ubiquitin sorting machinery". However, they note that mammalian GGAs might also sort ubiquitylated cargo at the TGN.

The residues that mammalian GGAs use to bind acidic-cluster–dileucine motifs are not conserved in yeast Ggas. So, in the second study, Piper and co-workers used a yeast two-hybrid screen to identify sorting motifs that interact with Ggas. And, they found that Gga2 binds ubiquitin directly through its GAT domain.

Next, they investigated the role of Ggas in the trafficking of general amino-acid permease-1 (Gap1). Gap1 is expressed at the cell surface when the nitrogen source is poor. However, when the

nitrogen source improves, cell-surface Gap1 is ubiquitylated and sorted into the degradation pathway, and newly made Gap1 is ubiquitylated and sorted directly from the TGN to endosomes (and so bypasses the plasma membrane).

The authors found that, in the absence of Ggas, Gap1 is sorted from the TGN to the cell surface when it would usually be directed to endosomes. In addition, the endocytic delivery of cell-surface Gap1 to the degradation pathway seemed to be compromised. When they monitored Gap1 trafficking in the presence of Gga2 lacking its GAT domain, they again found that the TGN-to-endosome sorting of Gap1 is disrupted. However, in this case, the endocytic delivery of cell-surface Gap1 was mostly unaffected.

Piper and colleagues therefore propose that yeast Ggas bind ubiquitylated proteins at the TGN and divert them away from the secretory pathway to endosomes. And, together, these two studies have highlighted a new role for GGAs as ubiquitin-sorting receptors in membrane-trafficking events.

Rachel Smallridge

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FURTHER READING Bonifacino, J. S. The GGA proteins: adaptors on the move. *Nature Rev. Mol. Cell Biol.* **5**, 23–32 (2004)

WEB SITES

Juan Bonifacino's laboratory:
http://dir2.nichd.nih.gov/nichd/cbmb/Juan_Bonifacino.html

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http://www.physiology.uiowa.edu/faculty/faculty/piper.htm