APOPTOSIS

Lifesaver

How the pro-apoptotic molecules Bak and Bax — which are potentially lethal — are maintained in an inactive, monomeric confirmation in viable cells is poorly understood. However, recent structural insights into the monomeric Bax molecule have provided a possible mechanism for its inactive status. And now, reporting in *Science*, Stanley Korsmeyer and colleagues have identified a protein — voltage-dependent anion channel 2 (Vdac2) — that keeps Bak in check.

Bak and Bax are required for mitochondrial apoptosis — 'BH3-only' members of the Bcl-2 family respond to death signals and subsequently trigger the activation of Bak and Bax, which leads to mitochondrial membrane permeabilization and the release of cytochrome *c*. This then initiates the caspase cascade.

To investigate whether Bak interacts with another mitochondrial protein that regulates its activity, Korsmeyer and colleagues used protein crosslinkers to identify a candidate protein (X) that complexes with Bak in purified mitochondria or whole cells. This Bak–X complex was lost when mitochondria were treated with the BH3-only protein tBid or when cells were treated with death stimuli. By testing various BH1- and BH3-domain mutants of both tBid and Bak, the authors concluded that X interacts with the Bak pocket that is formed by the BH1, BH2 and BH3 domains and can be displaced, directly or indirectly, by BH3-only molecules.

Protein X was identified as Vdac2, a low-abundance isoform of the Vdac outer-mitochondrial-membrane porin. Vdac2 was further implicated when the authors found that *Vdac2*-deficient embryonic stem cells lacked the Bak–X complex, which appeared when Vdac2 was re-expressed in these cells. Using haemagglutinin (HA)-tagged Vdac2, endogenous Bak — but not Bax — was coprecipitated. Similarly, HA-tagged Bak — but not Bax — coprecipitated endogenous Vdac2.

Bak in its active, oligomeric conformation is more susceptible to proteolysis than the inactive form, and the absence of Vdac2 increased its susceptibility. So, Vdac2 interacts with Bak, but not Bax, and regulates its conformation.

Next, Korsmeyer and co-workers set out to determine how Vdac2 modulates Bak-dependent apoptosis. Is Vdac2 an inhibitor of Bak-mediated apoptosis, or does it function as a proapoptotic factor itself when released from Bak? Vdac2 expression in $Bax^{-/-}$ cells inhibited apoptosis, but had no effect in $Bak^{-/-}$ cells, which argues against the latter possibility. Also,

GENE EXPRESSION

Repeat performance silences the crowd

A newly discovered role in epigenetic gene silencing for the retrotransposon long terminal repeats (LTRs) that are scattered throughout eukaryotic genomes indicates that they might be involved in gene regulation during development.

We have known for some time that repetitive DNA and gene silencing are linked, and the idea that repeats might be involved in gene regulation is an old one. However, direct evidence was lacking until now.

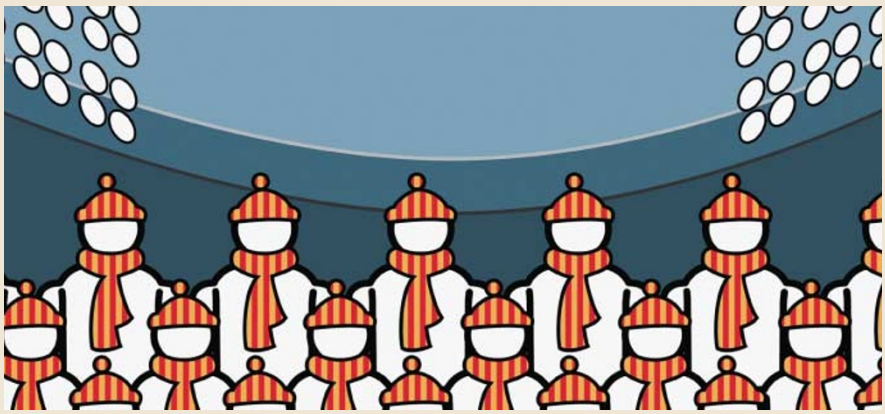
The initial aim of Vera Schramke and Robin Allshire's study was to determine whether RNA interference (RNAi) — through both post-transcriptional gene silencing (PTGS) and chromatin-based gene silencing (CBGS) — could silence non-centromeric genes in fission yeast in the same way that it had been shown to silence centromeric repeats.

Using RNAi, the authors were able to silence the *ura4*$^+$ gene both at its native centromeric locus and when inserted at other non-centromeric locations, by expressing an inverted 280-bp section of the gene — short hairpin (*shuraSE*) — in the same strain. As expected, silencing was abolished in strains in which genes that encode crucial components of the PTGS pathway, such as *dicer* (*dcr1*), were mutated. Less predictably, *ura4*$^+$ silencing was also abolished in a strain that lacked the histone H3 lysine 9 methyltransferase Clr4, which was previously only known to be involved in the CBGS pathway.

By contrast, cells that lacked another CBGS component, the HP1 orthologue Swi6, retained silencing. So, it seems that Clr4 is not only involved in silencing through chromatin modification, but is also a component of the RNAi complex that generates the short interfering RNAs (siRNAs) that are the effectors of PTGS.

Schramke and Allshire, using chromatin immunoprecipitation, also assessed whether the *shuraSE*-silenced non-centromeric *ura4*$^+$ gene had the chromatin modifications — methylated histone H3 and bound Swi6 — that are characteristic of silenced centromeric repeats. Sure enough, not only did the relocated *ura4*$^+$ have an identical pattern of chromatin modification to centromeric repeats, but experiments in a strain that lacked Swi6 function indicated that, like these repeats, Swi6 was needed to spread silencing from the nucleation site and Rad21-cohesin was also recruited just like at centromeres.

To test whether such RNAi-mediated chromatin modifications are involved in endogenous gene regulation, the authors used RT-PCR to assess whether the silencing of meiotically-induced genes could be abolished in strains that lacked key components of the RNAi pathway, including Clr4 and Swi6. A notable pattern emerged: only genes with



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expression of Vdac2 inhibited tBid-induced apoptosis of *Bax*$^{-/-}$ cells, but not *Bak*$^{-/-}$ cells, which indicates that Vdac2 negatively regulates Bak-dependent apoptosis.

Cells deficient for Vdac2 were far more sensitive to death stimuli than *Vdac1*$^{-/-}$ and *Vdac3*$^{-/-}$ cells, both of which had similar sensitivities to wild-type cells. By re-expressing Vdac2, the susceptibility to apoptosis of *Vdac2*$^{-/-}$ cells reverted to normal. So, Vdac2 has a physiological role that is distinct from the other Vdac isoforms.

When analysing the apoptosis phenotype of *Vdac2*$^{-/-}$ cells, the authors noted an increased loss of mitochondrial transmembrane potential and the accelerated release of cytochrome *c*, compared with wild-type cells. After treatment with death stimuli, *Vdac2*$^{-/-}$ cells showed caspase activity and Bak oligomerization earlier than wild-type cells.

The authors concluded that Vdac2 is a specific inhibitor of Bak-dependent mitochondrial apoptosis, which, when absent, causes increased susceptibility to apoptotic death.

Arianne Heinrichs

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promoters close to an LTR were de-repressed (that is, unsilenced) in cells that lacked these RNAi-pathway components.

Further experiments showed that — at least for the two genes that were studied — RNAi-dependent modifications at LTRs mediate transcriptional repression, and removal of these LTRs leads to constitutive expression (de-repression) of the adjacent genes. Taken together, these results indicate that LTRs are needed to silence meiotically induced genes during vegetative growth. More generally, the authors conclude that LTRs can act as developmental effectors through the RNAi pathway, restricting the expression of a gene to a distinct differentiation pathway.

These fascinating results in fission yeast show that despite the recent flurry of RNAi research activity in a plethora of models, we still have a way to go before we fully understand this process. After Schramke and Allshire's study, the role of repetitive DNA elements in RNAi in other models is likely to be the focus of many new studies.

Nick Campbell, Associate Editor,
Nature Reviews Genetics

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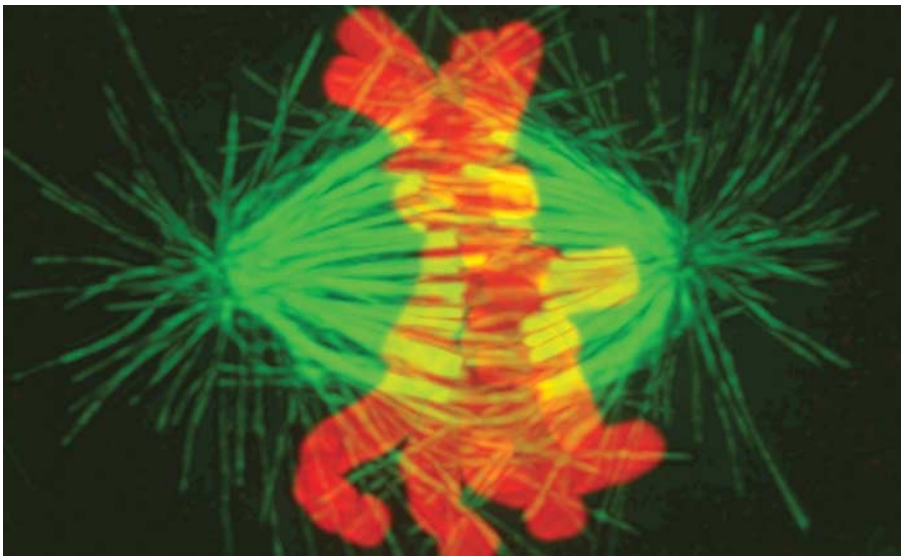
WEB SITE
Robin Allshire's laboratory:
http://www.wcb.ed.ac.uk/allshire.htm



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CYTOKINESIS

Right place, right time

The symmetrical arrangement of chromosomes and the mitotic spindle during metaphase creates an obvious point for initiating cell cleavage and ensures that the mother cell divides equally and symmetrically. It has previously been suggested that the spindle microtubules themselves play an integral part in choosing the cell-division plane and initiating cleavage-furrow formation.

In a recent report in *Nature*, Julie Canman and colleagues challenge the widely held view that the symmetrical bipolar spindle is required for cleavage-site formation. In an elegant set of experiments, they used a small-molecule inhibitor to block the kinesin Eg5, which is essential for establishing a bipolar spindle. As this inhibition activated the spindle checkpoint, they blocked this checkpoint as well.

What they found was that monopolar half-spindles formed, but that furrow formation and cytokinesis still occurred. The cell-division plane formed on the side of the cell facing the chromosomes and not at the poles. So, these results showed that two opposing microtubule arrays are not essential for cell division.

The authors then imaged fluorescently-tagged tubulin to monitor the dynamics of microtubules inside bipolar and monopolar cells undergoing cytokinesis. They noticed that a stable subpopulation of microtubules was associated with the cytokinetic furrows. These stable microtubules were associated with chromosomes, and only formed on the side of the asymmetric monopolar spindles that were associated with chromosomes, in the same place as the site of furrowing. Dynamic microtubules populated the cell poles, presumably acting to inhibit furrow formation outside of the cell equator.

The authors propose a model whereby, at least in some cultured mammalian cells, chromosomes form connections with the cell cortex through as-yet-undetermined microtubule stabilization factors. These stable microtubules then signal directly to the neighbouring cell cortex to form a furrow in their vicinity, and might act as tracks for motor proteins to deliver regulators and components of the cytokinetic furrow to the cell cortex.

In non-mammalian cells, it has also been unclear which part of the spindle apparatus is responsible for furrow positioning. Alsop and Zhang now report, in *The Journal of Cell Biology*, the systematic dissection of the role of each structural component.

Using micromanipulation, the authors removed asters and chromosomes from grasshopper spermatocytes in metaphase, leaving microtubules as the only structural constituent. This resulted in the disassembly of the spindle and, subsequently, the assembly of microtubule bundles. At first, the microtubules radiated towards the cell cortex; later, they underwent transient formations of bipolar and monopolar pseudospindles; and, ultimately, they formed disorganized arrays of bundled microtubules.

Induction of furrow initiation occurred at midzones of sustained bundles of microtubule arrays. Often, furrow induction occurred at multiple locations, but many furrows were transient and regressed. Furrow initiation and cytokinesis were delayed, however, probably because of microtubule reorganizations. So, Alsop and Zhang concluded that microtubules — regardless of their order or symmetry in the spindle — are sufficient to induce cell cleavage, reinforcing the idea that the microtubules themselves ensure that cells divide equally and symmetrically.

Mirella Bucci, Associate Editor, Nature

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