APOPTOSIS

Follow the fly path

It all started two years ago, say the authors, with the discovery of p53 in *Drosophila melanogaster*. They reasoned that if *Drosophila* has p53, then maybe there are parallels between the way that flies and mammals induce p53-dependent cell death. And it seems that they were right, as they now report in *Genes and Development*.

The authors in question — Arnold Levine and colleagues — started with a p53-dependent pathway that had been observed in flies but not in mammals. *Drosophila* p53 activates the transcription of a gene called *Reaper*, the product of which interacts with the inhibitor of apoptosis (IAP) protein DIAP and targets it for ubiquitin-mediated proteolysis. As DIAP's role is to inhibit caspases, its destruction frees up caspases to proceed with an apoptotic response.

So could this happen in mammals? To test this, Levine and co-workers first looked for proteins that might interact with CIAP1 — the human homologue of DIAP1. They generated stable clonal HeLa cell lines that expressed a haemagglutinin-Flag-tagged CIAP1 protein, and then co-purified CIAP1 and its partners from HeLa cell lysates using M2-agarose beads, which recognize the Flag tag. In response to etoposide-induced p53-dependent apoptosis, the authors pulled down two low-molecular-weight proteins. But these weren't CIAP1's binding partners — they were fragments of CIAP1 itself.

Levine and colleagues concluded that CIAP1 is cleaved during p53-dependent apoptosis. When they induced apoptosis in a p53-independent manner (using anti-Fas antibodies), the cleavage was no longer seen, suggesting that it requires the p53 pathway. As one characteristic of this pathway is the transcription of p53 target genes, the authors then asked whether *de novo* protein synthesis is needed for the cleavage. Treatment with cycloheximide blocked the cleavage, consistent with this idea.

Cleavage of CIAP1 was not blocked by caspase inhibitors, which means that the cleavage is independent of caspases and is a cause rather than an effect of the cell-death pathway. So might another type of protease be involved in the cleavage? Serine proteases have previously been implicated in apoptosis, so Levine and co-workers treated HeLa cells with etoposide and a general serine protease inhibitor, and

CHROMATIN

Dynamic repression

Heterochromatin protein 1 (HP1) is generally considered as the 'keeper' of heterochromatin — transcriptionally silenced, condensed DNA that is also typically associated with the centromeric regions. It is thought to do this by crosslinking chromatin, thereby creating a dense chromatin environment that is impermeable to transcriptional activators. Two papers, published in *Science*, from teams led by Richard Festenstein and Tom Misteli, now challenge this view.

Both groups studied the dynamics of HP1 — a major component of heterochromatin — using fluorescence recovery after bleaching (FRAP) on cells that express green fluorescent protein (GFP)–HP1 isoform α, β or γ fusion proteins. Misteli and colleagues stably transfected immortalized Chinese hamster ovary (CHO) cells, whereas the Festenstein group generated transgenic mice that expressed GFP–HP1β isoform protein specifically in T cells.

In CHO cells, fluorescence recovery was rapid for all HP1 isoforms; complete recovery was reached within 5 s in less dense euchromatin and within ~60 s in heterochromatin. In resting T cells taken from the transgenic mice, recovery was much slower and incomplete; ~70% was recovered in 150–200 s in heterochromatin, and ~90% in 90–100 s in euchromatin. HP1 mobility in heterochromatin was reduced compared with euchromatin, which possibly reflects the higher density of HP1-binding sites in heterochromatin. Incomplete recovery in resting T cells indicates the presence of an immobile fraction of HP1β molecules, which is larger in heterochromatin than in euchromatin.

To examine the effects of T-cell activation — which triggers gene activation and cell-cycle induction — on HP1 mobility, Festenstein and colleagues measured FRAP in T cells that were taken from mice and activated *ex vivo*. GFP–HP1β mobility was significantly increased, both in heterochromatin and euchromatin, compared with unstimulated cells. Moreover, the immobile HP1β fraction in heterochromatin was reduced to ~10%. The recovery time in heterochromatin in activated T cells (50–80 s) was similar to that in CHO cells (~60 s), and the fluorescence recovery was indeed almost complete — as in CHO cells.

So, it seems that HP1 binds transiently to heterochromatin and euchromatin, which leads the authors to conclude that heterochromatin is not inaccessible to other factors, and that the continuous exchange of HP1 allows transcriptional regulators to compete for binding, thereby determining the fate of the heterochromatin region. In addition, the increased mobility of HP1 in immortalized cells and activated T cells allows the heterochromatin to be restructured, which might facilitate cell-cycle entry and transcriptional activation.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPERS Festenstein, R. *et al.* Modulation of heterochromatin protein 1 dynamics in primary mammalian cells. *Science* **299**, 719–721 (2003) | Cheutin, T. *et al.* Maintenance of stable heterochromatin domains by dynamic HP1 binding. *Science* **299**, 721–725 (2003)

found that both CIAP1 cleavage and apoptosis were blocked. They obtained similar results using primary mouse thymocytes — thymocytes with no p53 genes failed to cleave CIAP and failed to undergo apoptosis.

To identify the protease involved, Levine and colleagues went back to the literature. Previous reports had shown that mammalian IAPs can interact with a serine protease called HTRA2/OMI, so the authors did a northern blot analysis of *HTRA2* messenger RNA levels in HeLa cells during treatment with etoposide. They observed a seven-fold increase in *HTRA2* mRNA levels; a similar increase was also seen when HeLa cells were transfected with a p53 expression vector.

The authors therefore conclude that a parallel pathway to that first mapped out in flies indeed exists in mammals, with the subtle difference that CIAP1 is destroyed by protease-mediated cleavage rather than being targeted by ubiquitin for destruction.

Alison Mitchell

References and links

ORIGINAL RESEARCH PAPER Jin, S. *et al.* CIAP1 and serine protease HTRA2 are involved in a novel p53-dependent apoptosis pathway in mammals. *Genes Dev.* 17, 359–367 (2003)

MITOCHONDRIAL BIOGENESIS

NO energy

Mitochondria in brown adipocyte tissue (BAT) are larger and more numerous than in other cell types; their inner mitochondrial membrane contains uncoupling protein 1 (UCP1), which diverts energy from ATP synthesis to thermogenesis. Nitric oxide (NO) is known to regulate biological functions in mature brown adipocytes, but, until now, NO's role in mitochondrial biogenesis has not been studied.

Reporting in *Science*, Nisoli and colleagues looked at mitochondrial biogenesis in primary cultures of mouse brown adipocyte precursors. Treatment with an NO donor increased the mtDNA content above levels seen in untreated cells, which were due to spontaneous differentiation of the adipocyte precursors. This increase was abolished in the presence of the NO scavenger oxyhaemoglobin, indicating that it was mediated by NO generation.

The authors next showed that this effect occurred through activation of the peroxisome proliferation-activated receptor and co-activator 1α (PGC-1α) — a principal regulator of mitochondrial biogenesis in BAT, and cardiac and skeletal muscle. Using a cyclic GMP analogue and a guanylate-cyclase inhibitor, they also showed that the biogenesis depends on cGMP. And study of mouse white-fat 3T3-L1 and human monocytic U937 cell lines revealed that the biogenesis was not restricted to brown adipocytes and their differentiation processes.

To investigate the role of endogenous NO, the authors stably transfected HeLa cells with endothelial nitric oxide synthase (eNOS) — the only isoform that is expressed in brown adipocytes and 3T3-L1 cells under experimental conditions. Induction of eNOS increased mitochondrial biogenesis; an effect that was abolished by a NOS inhibitor.

Cold exposure triggers PGC-1α expression through activation of β_3-adrenergic receptors and increases in intracellular cAMP and Ca^{2+}, all of which stimulate NO production in brown adipocytes. So Nisoli *et al.* studied BAT functions in wild-type and *eNOS*$^{-/-}$ mice before and after cold exposure. At both temperatures, histological analysis indicated that *eNOS*$^{-/-}$ BAT was functionally inactive, and mitochondrial biogenesis was impaired.

When the authors looked at the control of biogenesis in the brain, liver and heart of the knockout mice, they found that deletion of *eNOS* was enough to reduce the number of mitochondria even in tissues that have a basal

level of neuronal, and possibly inducible, NOS expression.

In *eNOS*$^{-/-}$ mice, oxygen consumption rates — an indicator of metabolic rate — were decreased, indicating that BAT-dependent thermogenesis might be impaired. In genetic models of obesity, defective energy expenditure is involved in increased food intake and body-weight gain; eight-week-old *eNOS*$^{-/-}$ mice showed similar food consumption but weighed more than wild-type mice. So, the increased body weight of *eNOS*$^{-/-}$ mice could be accounted for by higher feed efficiency (weight gain/food intake) caused by defective energy expenditure.

So what does this mean? The features shown by *eNOS*$^{-/-}$ mice — reduced mitochondrial number and energy expenditure, weight gain, insulin resistance and hypertension — are all typical of the so-called metabolic syndrome. Millions of people are estimated to have metabolic syndrome, placing them at an increased risk of developing diabetes and cardiovascular disease. However, if the results reported by Nisoli and colleagues are applicable to humans, then we will have "…clues for the prevention or treatment of this condition".

Natalie Wilson

References and links

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WEB SITE

Center for Study and Research on Obesity:
http://www.unimi.it/ateneo/strutt/centric/centrob/centrobi.html