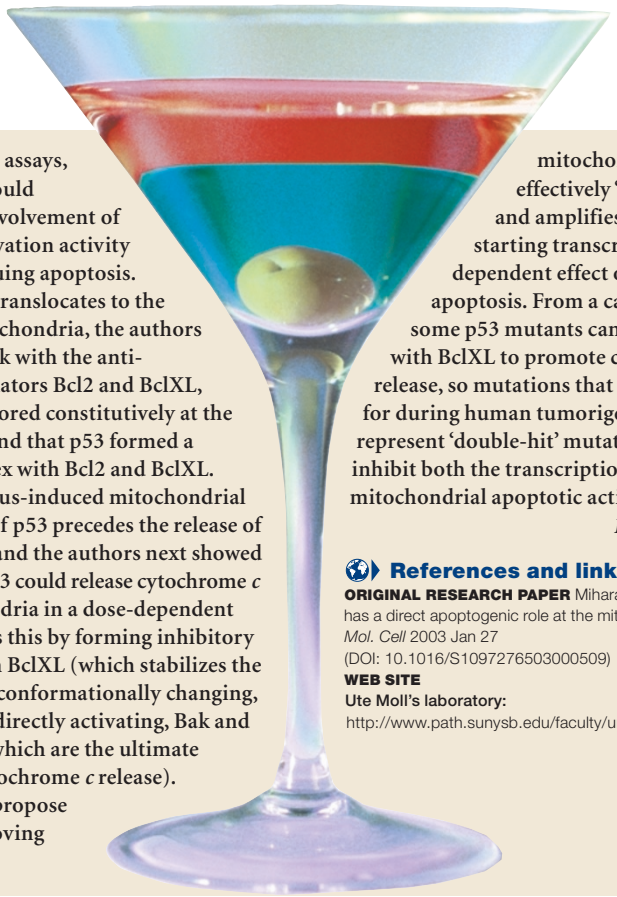

APOPTOSIS

Double trouble

p53 can kill cells by transcription-dependent means, and it can enhance this effect by inducing death by a transcription-independent mitochondrial pathway. Reporting in *Molecular Cell*, Ute Moll's group show that p53 does this by translocating to mitochondria, and inducing permeabilization of the outer mitochondrial membrane (OMM) and the release of cytochrome *c*.

Previously, the authors saw that a fraction of p53 translocates rapidly to mitochondria after apoptotic stimuli (DNA damage and hypoxia) in malignant and immortal cells. So they γ-irradiated mouse thymocytes to see if this also occurs in primary cells. Within 1 h of treatment, p53 moved to the mitochondria.

By targeting p53 directly to mitochondria of p53-null cells and using several sensitive transcriptional assays, Moll's group could discount the involvement of p53's transactivation activity during the ensuing apoptosis.

Because p53 translocates to the surface of mitochondria, the authors looked for a link with the anti-apoptotic mediators Bcl2 and BclXL, which are anchored constitutively at the OMM, and found that p53 formed a specific complex with Bcl2 and BclXL.

Death-stimulus-induced mitochondrial translocation of p53 precedes the release of cytochrome *c*, and the authors next showed that purified p53 could release cytochrome *c* from mitochondria in a dose-dependent manner. It does this by forming inhibitory complexes with BclXL (which stabilizes the OMM) and by conformationally changing, and thereby indirectly activating, Bak and Bax proteins (which are the ultimate effectors of cytochrome *c* release).

The authors propose that p53, by moving rapidly to the mitochondria, effectively 'jump-starts' and amplifies its slower-starting transcription-dependent effect on apoptosis. From a cancer aspect, some p53 mutants can't interact with BclXL to promote cytochrome *c* release, so mutations that are selected for during human tumorigenesis might represent 'double-hit' mutations that inhibit both the transcriptional and mitochondrial apoptotic activites of p53.

Katrin Bussell

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WEB SITE

Ute Moll's laboratory: http://www.path.sunysb.edu/faculty/umoll/default.htm

LIPID RAFTS

A deadly gang

When attacking prey, animals often work together as a deadly gang and, on a smaller scale, it's teamwork that is crucial for a successful attack by the three subunits of the *Bacillus anthracis* anthrax toxin. The first subunit, the 83-kDa form of protective antigen (PA83), binds to the host cell's anthrax toxin receptor (ATR), which induces a cleavage reaction that produces PA63. PA63 then oligomerizes to form $(PA63)_7$, binds to the other two subunits lethal factor (LF) and oedema factor (EF) and facilitates their entry to the cytoplasm, where these factors exert their toxic effects. The details of this initial entry process have been unclear, but now, in *The Journal of Cell Biology*, van der Goot and colleagues provide new insights.

The authors first showed that, whereas PA83 associates with detergent-soluble domains of the plasma membrane, PA63 associates with detergent-resistant membranes (DRMs or lipid rafts) or, more specifically, with "noncaveolar cholesterol and sphingolipid-rich domains of the plasma membrane". They then showed that free ATR is not raft associated, so it seems that PA63 forces ATR into rafts. Furthermore, they found that LF, which binds $(PA63)_7$ and not PA63, associates with DRMs in PA63-treated cells, which indicates that $(PA63)_7$ is predominant in rafts.

So, is it ATR clustering, which is a result of PA63 heptamerization, that causes the raft association? It seems so, because when van der Goot and co-workers used an alternative method to cluster ATR — that is, they labelled ATR with PA83 (which cannot heptamerize) and then added monoclonal or polyclonal antibodies against PA83 — they found that clustered PA83 was mainly present in DRMs, in contrast to non-clustered PA83. In addition, they showed that PA clustering was essential and sufficient to promote its internalization.

Having ruled out a caveolin-mediated entry pathway, van der Goot and colleagues showed that PA enters cells by clathrin-mediated endocytosis. They found that dominant-negative mutants of dynamin (which is involved in caveolin- and clathrin-mediated uptake) and Eps15 (which is required specifically for clathrin-mediated endocytosis) had inhibitory effects on PA internalization.

The physiological role of ATR is not clear, but this work has shown that "its trafficking properties, i.e., slow endocytosis as a monomer and rapid clathrin-mediated uptake on clustering, make it an ideal anthrax toxin receptor". In addition, this study has highlighted the potential importance of lipid rafts as therapeutic targets for drugs against anthrax.

Rachel Smallridge

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Gisou van der Goot's laboratory: http://www.medecine.unige.ch/recherche/groupes/f/fondamentale/02.html