APOPTOSIS

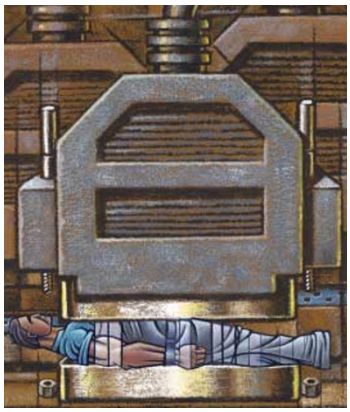
Death by an unusual adaptor

Apoptotic pathways seem to be full of adaptor proteins — a recently identified example is the apoptosis-associated speck-like protein (ASC). But not only does ASC have both a pyrin domain and a carboxy-terminal caspase-recruitment domain (CARD), which is quite unusual, it's now reported to associate with the pro-apoptotic protein Bax and to regulate a p53–Bax-intrinsic mitochondrial pathway of apoptosis. This work, by Ohtuka and co-workers, is presented in *Nature Cell Biology*.

On comparing gene expression plus or minus the tumour suppressor p53, the authors saw an induction of *ASC* mRNA and protein expression in the presence of p53. The *ASC* promoter contains a potential p53 recognition sequence, and further assays confirmed this specific protein–DNA interaction. Several p53 target genes can affect cell-cycle arrest or death, so the authors overexpressed ASC. This caused significant apoptosis. The effect, however, was markedly reduced in cells lacking Bax. ASC also exacerbated cell death when overexpressed in the presence of genotoxic agents.

The authors then used small interfering RNA (siRNA) to assess a direct role for ASC induction in p53- or genotoxic-stress-mediated apoptosis. A reduction in ASC expression inhibited apoptosis in both cases, which indicates that ASC is a downstream effector of p53 in DNA-damage-mediated apoptosis. As the absence of Bax inhibited ASC-induced apoptosis, and as Bax causes mitochondrial dysfunction followed by release of apoptotic stimulators, Ohtuka and co-workers investigated the effect of ASC overexpression on mitochondrial membrane potential and cytochrome *c* release. $Bax^{+/+}$, but not $Bax^{-/-}$, cells that expressed ASC had a significantly reduced membrane potential and released cytochrome *c* into the cytoplasm.

Most of the ASC protein localized to the mitochondrial fraction, and the authors found that the amino-terminal pyrin region was necessary for this



localization. The presence of a CARD prompted Ohtuka and co-workers to assess ASC's effect on caspases. Procaspase-2, -3 and -9 were cleaved to produce their active forms, but only when Bax was present. So ASC, like Bax, seems to localize to mitochondria and induce apoptosis. As both ASC and Bax have several properties in common, such as their regulation by p53, their mitochondrial and cytoplasmic localizations and their pro-apoptotic function, the authors investigated the possibility of a physical link between the two proteins, which was required in the p53–Bax-intrinsic mitochondrial apoptotic pathway. Endogenous Bax, but not other Bcl2-family proteins, interacted with endogenous ASC, again through ASC's pyrin domain, and both proteins colocalized in the cytoplasm and at mitochondria.

But the all-important question was whether ASC affected Bax localization. Overexpressing ASC increased the amount of Bax in the mitochondrial fraction coincident with a decrease in Bax levels in the cytoplasm. Further investigation showed that ASC expression induced a conformational change in Bax and resulted in its translocation to mitochondria. Finally, siRNA treatment against ASC inhibited the Bax conformational change and reduced the translocation of Bax to mitochondria after genotoxic stress.

So ASC functions downstream of p53 in DNA-damage-mediated apoptosis to regulate the Bax-intrinsic mitochondrial pathway. There is also emerging evidence that ASC might mediate caspase activation independently of Bax, so further investigation of this unusual adaptor is required.

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References and links

ORIGINAL RESEARCH PAPER Ohtsuka, T. *et al.* ASC is a Bax adaptor and regulates the p53–Bax mitochondrial apoptosis pathway. *Nature Cell Biol.* **6**, 121–128 (2004)

STRUCTURE WATCH

Size selection

In RNA interference (RNAi), short interfering (si)RNAs silence gene expression in a sequence-specific manner, and plants use RNAi to defend against RNA viruses. However, many of these viruses have a counter-defence mechanism. For example, the 19-kDa protein (p19) of tombusviruses can suppress RNA silencing, and has been shown to bind specifically to siRNAs. But how does p19 recognize siRNAs? Two papers now provide insights. Patel and co-workers (reporting in *Nature*) and Tanaka Hall and colleagues (reporting in *Cell*) both used X-ray crystallography to study a complex between p19 and a 21-nucleotide (19-base-pair duplex) siRNA.

These studies showed that a p19 homodimer binds to siRNA, and a concave β-sheet of this homodimer spans all 19 base pairs along one face of the siRNA duplex. This interaction is sequence independent (all of the interactions are directed towards the sugar–phosphate backbone in p19–siRNA), but is dependent on the size of the siRNA duplex. Each p19 monomer contains a pair of tryptophan residues that are positioned such that, in the homodimer, they stack over the terminal base pairs, and so measure and 'cap' the ends of the siRNA duplex. Using biochemical and *in vivo* assays, Tanaka Hall and colleagues verified the importance of these tryptophan residues in suppressing RNA silencing and, in addition, defined the siRNA characteristics that are important for recognition by p19. Together, the work by these groups might help us to further understand — and manipulate — RNA-silencing mechanisms in heterologous systems.

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Helical bundle conversion

Vinculin is a component and regulator of cell–cell (cadherin-mediated) and cell–matrix (integrin–talin-mediated focal adhesions) junctions. In its resting state, its head (Vh) and tail (Vt) domains interact to form a closed confirmation, but Vt can bind to the actin cytoskeleton when Vh binds to α-actinin in cadherin junctions and to talin in focal adhesions. In *Nature*, Bois and colleagues have now defined the mechanism of vinculin activation by determining the crystal structures of human vinculin in its inactive and talin-activated forms.

In its inactive form, Vh is composed of seven α-helices that are arranged as two four-helix bundles (α4 is long and spans both bundles), and Vh interacts with Vt to form the expected closed structure. On binding talin, marked changes occur in the amino-terminal bundle of Vh — the four-helix bundle completely rearranges to form a five-helix bundle that incorporates a helix from talin. No structural changes occur in the carboxy-terminal helical bundle of Vh, which functions as a scaffold. As both α-actinin and talin can bind Vh and displace Vt, it seems that, by inducing structural changes in Vh, these proteins can activate vinculin in cadherin junctions and focal adhesions, respectively. This work has also established "…helical bundle conversion as a signalling mechanism by which proteins direct cellular responses".

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