APOPTOSIS

Cheating death

The ability of p53 to induce apoptosis is a key defence against cancer. That cancer can, sometimes, occur indicates that this defence is not impenetrable, although the blame cannot always be laid on p53. Regulators of p53 are equally culpable, which makes their identification and characterization essential. Previously, Xin Lu's group identified two ASPP proteins — ASPP1 and ASPP2 — that act as potent activators of p53. Now, Lu and colleagues report, in *Nature Genetics*, the identification of a third member of this p53-regulating protein family — but, in this case, it acts as an inhibitor.

The authors recognized the importance of the ASPP proteins in regulating p53, so they turned to the worm as a more tractable biological model. However, homology searches with ASPP1 and ASPP2 revealed only one ASPP gene in *Caenorhabditis elegans*. Lu and co-workers knocked down the gene function using RNA interference (RNAi) and saw an increase in apoptotic germ cells — a surprising observation, given the pro-apoptotic function of the previously known ASPPs! Similarly, the use of antisense RNA in human cell lines caused an increase in apoptosis. These findings indicated that the newly identified ASPP protein had an inhibitory effect on apoptosis, earning it the name iASPP.

The authors showed, using co-immunoprecipitation, that iASPP binds p53 and subsequently mapped the binding site to an SH3 domain. Given that this p53-binding domain is common to all three ASPP proteins, it seemed plausible that the proteins compete for p53. This turned out to be the case, as increased iASPP corresponded with reduced ASPP1 and ASPP2 co-immunoprecipitation with p53, and vice versa when ASPP1 and ASPP2 were increased. In addition, expression of iASPP *in vivo* abolished the pro-apoptotic function of ASPP1 and ASPP2.

The ability of iASPP to inhibit p53 implies that it has oncogenic properties, and the demonstration that its expression stimulated Ras-mediated transformation *in vitro* supports this claim. In addition, Lu and colleagues found that iASPP is overexpressed in several cancers containing wild-type p53 and normal levels of ASPP1 and ASPP2.

Binding of ASPP1 and ASPP2 enhances the capacity of p53 to turn on death-promoting genes. At the same time, binding to p53-regulated cell-cycle arrest genes is not enhanced. The authors observed the same specificity for the apoptosis programme with iASPP. Future studies will reveal whether iASPP has a dominant-negative effect on ASPP1 and ASPP2 or whether iASPP is a direct inhibitor of p53.

Regardless of how iASPP inhibits p53, it is now clear that the apoptotic function of p53 is stimulated by ASPP1 and ASPP2 and inhibited by iASPP — opposing roles for such close family members.

David Gresham,
Associate Editor, Nature Genetics

References and links

ORIGINAL RESEARCH PAPER Bergamaschi, D. *et al.* iASPP oncoprotein is a key inhibitor of p53 conserved from worm to human. *Nature Genet.* **33**, 162–167 (2003)

STRUCTURE WATCH

A major invasion

The food-borne pathogen *Listeria monocytogenes* enters human cells by interacting with the host-cell receptor E-cadherin using internalin (InIA), a major invasion protein. This results in the bacteria being phagocytosed by cells — such as intestinal epithelial cells — that are usually non-phagocytic. Now, in *Cell*, Heinz and colleagues provide new insights into this interaction. They present the crystal structures of the functional domain of InIA (InIA′) alone and in complex with the amino-terminal immunoglobulin-like domain from human E-cadherin (hEC1).

The authors found that InIA′ forms an extended, sickle-shaped structure, and that hEC1 specifically binds to and fills the central cleft that is formed by the curved leucine-rich repeats (LRRs) of InIA′. LRRs are usually quite rigid, but a hinge region in LRR6 of InIA′ provides the flexibility needed to form the InIA′–hEC1 complex.

The Pro16 residue of hEC1 is important for the recognition of human E-cadherin by InIA, and in mice, where Pro16 is replaced by glutamate, InIA does not bind epithelia. Consistent with this, the authors confirmed that Pro16 is essential for the InIA′–hEC1 interaction using structural and mutagenesis studies. Their work has therefore provided "...a detailed picture of the first steps leading to human infection by *L. monocytogenes*", insights into "...the structural basis for host tropism", and the possibility of new therapeutic approaches.

REFERENCE Schubert, W. D. *et al.* Structure of internalin, a major invasion protein of *Listeria monocytogenes*, in complex with its human receptor E-cadherin. *Cell* **111**, 825–836 (2002)

Dimension difficulties

Protein synthesis on a ribosome is terminated when a stop codon enters the decoding centre (DC) of the 30S ribosomal subunit and is recognized by a class-I release factor (RF). A conserved motif in RFs (SPF in RF2) is thought to interact directly with the stop codon in the DC, and the GGQ motif of RFs is then thought to interact with the peptidyl-transferase centre (PTC) of the 50S ribosomal subunit to stimulate peptide release. There is, however, a problem with this model — the distance between the DC and PTC is ~73 Å and, in the X-ray structure of RF2, the SPF and GGQ motifs are only 23 Å apart.

Two studies in *Nature* — from Frank and colleagues and van Heel and co-workers — now give us the solution to this dimension difficulty. Both groups used cryo-electron microscopy to study the structure of the bacterial RF2–ribosome complex at ~10–14-Å resolution. They found that RF2 has an open conformation when bound to ribosomes, which allows it to span the distance between the DC and the PTC. The authors therefore propose a model in which stop-codon recognition by the RF2 SPF motif causes a structural change that converts RF2 from a compact to an open conformation, which places its GGQ motif near the PTC. In addition, the authors showed that RF2 mimics transfer RNAs functionally, and not structurally as was previously thought.

REFERENCES Rawat, U. B. S. *et al.* A cryo-electron microscopic study of ribosome-bound termination factor RF2. *Nature* **421**, 87–90 (2003) | Klaholz, B. P. *et al.* Structure of the *Escherichia coli* ribosomal termination complex with release factor 2. *Nature* **421**, 90–94 (2003)