APOPTOSIS

A death or death situation

Take one molecule — tumour-necrosis factor (TNF)-α — that binds two receptors. The first of these receptors (TNF-RI) contains an intracellular death domain and induces apoptosis. The second, TNF-RII, contains no such death domain, and associates with anti-apoptotic molecules such as the TNF-R-associated factors (TRAFs) and 'inhibitor of apoptosis' (IAP) proteins. But signalling by the TNF-RII pathway potentiates the pro-apoptotic effects of TNF-RI-mediated signalling. How can this be? A report by Jonathan Ashwell and colleagues in *Nature* now shows how components of the TNF-RII pathway might interact to promote cell death.

Previous work had shown that signalling through TNF-RII results in decreased levels of the anti-apoptotic molecule TRAF2. The authors explored the mechanism behind this decrease by comparing levels of TRAF2 in response to TNF-α signalling in Jurkat T cells (which contain TNF-RI but very little TNF-RII) with those in 4E3 cells (Jurkat T cells transfected with TNF-RII). They observed decreased levels of TRAF2 in the 4E3 cells, but not in the Jurkat T cells.

Ashwell and co-workers repeated this experiment in the presence of a proteasome inhibitor, and this time saw no decrease in TRAF2 in the 4E3 cells. As proteins are targeted for proteasome-mediated digestion by the addition of polyubiquitin chains, the authors then looked for the presence of ubiquitylated TRAF2. Using anti-TRAF2 antibodies, they were able to immunoprecipitate a large amount of polyubiquitylated material in response to TNF-α.

So, what might target TRAF2 for degradation? Enter the IAPs, which belong to TNF-RII signalling complexes and have E3 ubiquitin ligase activity. To test their involvement, beads that were coated with glutathione *S*-transferase (GST)–IAP fusion proteins were incubated with *in vitro* translated TRAF2. The TRAF2 was pulled down by GST–c-IAP1 and GST–c-IAP2, but not by the other IAPs tested. However, only c-IAP1 caused ubiquitylation of TRAF2, as tested *in vitro* and also by co-transfection experiments. So c-IAP1 seems to cause the proteasome-mediated degradation of TRAF2.

Finally, Ashwell and colleagues used a mutant form of c-IAP1, which has no E3 activity, to study the functional importance of the c-IAP1-mediated ubiquitylation of TRAF2. As expected, they found that TNF-α-induced degradation of TRAF2 was prevented by overexpression of the c-IAP1 mutant — an event that also substantially reduced apoptosis. These findings are therefore very satisfying — not only do they explain how TNF-RII-mediated signalling can potentiate the pro-apoptotic TNF-RI pathway, but they also identify a physiological function for c-IAP1.

Alison Mitchell

References and links

ORIGINAL RESEARCH PAPER Li, X., Yang, Y. & Ashwell, J. D. TNF-RII and c-IAP1 mediate ubiquitination and degradation of TRAF2. *Nature* **416**, 345–349 (2002)

WEB SITE

Jonathan Ashwell's laboratory: http://www3.cancer.gov/intra/LICB/LICBPAGE.htm

ENDOCYTOSIS

A transient tail

The virus is nature's squatter — it breaks into a cell, and then makes itself at home. Different viruses use various means of entering cells, and a report by Ari Helenius and colleagues in *Science* now uncovers how the simian virus 40 (SV40) gets in.

SV40 is endocytosed through caveolae — indentations in the plasma membrane — that are pinched off as vesicles that contain caveolin-1 and a single virus particle. But can SV40 actively induce its own endocytosis? As a first step in studying this, the authors tried to block the process, and found that latrunculin A (which sequesters actin monomers), and general- and tyrosine-kinase inhibitors, prevented virus uptake and infection.

Helenius and co-workers next found that, after virus entry, the number of actin stress fibres was reduced. Instead, small actin patches and tails appeared. The site for actin-tail formation corresponded to the sites of caveolin-1 expression. Moreover, dynamin II — which is involved in internalization — was also recruited to the virus-containing caveolae.

These results indicate that the virus uses actin polymerization to enhance its own internalization, and the authors showed that SV40-induced tyrosine phosphorylation in the caveolae leads to the observed transient changes. The next step, then, will be to identify the kinase(s) that are responsible.

Alison Mitchell

References and links

ORIGINAL RESEARCH PAPER Pelkmans, K., Püntener, D. & Helenius, A. Local actin polymerization and dynamin recruitment in SV40-induced internalization of caveolae. *Science* **296**, 535–539 (2002)

IN THE NEWS

Cesar Milstein (1927–2002)

Cesar Milstein, one of the key figures in the development of monoclonal antibodies, died on 24 March 2002 at the age of 74.

Together with Georges Köhler, Milstein reported a hybridoma technique for the production of monoclonal antibodies in *Nature* in 1975. This discovery led to an enormous expansion in the use of antibodies in science and medicine, and they were awarded the Nobel Prize for Physiology or Medicine in 1984 (with Niels Jerne) for their pioneering work in this area.

Milstein was a key figure at the prestigious Laboratory of Molecular Biology in Cambridge. He joined the Division of Protein Chemistry in 1963 and, following the advice of Fred Sanger, Milstein changed his field of study from enzymes to antibodies.

Although monoclonal antibodies have not fully lived up to the initial 'magic bullet' expectations, Milstein lived to see the fruits of his studies develop into a new generation of treatments that specifically target cells of interest, such as the breast cancer treatment Herceptin and the rheumatoid arthritis treatment Remicade.

'The chief executive of the Medical Research Council, Professor Sir George Radda, led the tributes to Dr Milstein. He said: "No other MRC scientist has made such an outstanding contribution to Britain's science, health and wealth creation. The discovery of monoclonal antibodies revolutionized biomedical research and sparked an international billion-pound biotechnology industry. He was an inspiration to many young scientists and will be sorely missed by friends and colleagues at the MRC Laboratory of Molecular Biology and throughout the scientific world".' (BBC News Online, 26 March 2002).

Simon Frantz