HIGHLIGHTS

IN BRIEF

EXOCYTOSIS

The β subunit of the Sec16p ER translocon interacts with the exocyst complex in *Saccharomyces cerevisiae*.

Toikkanen, J. H. *et al. J. Biol. Chem.* 2003 March 28 [epub ahead of print]

The exocyst affects protein synthesis by acting on the translocon machinery of the endoplasmic reticulum.

Lipschutz, J. H., Lingappa, V. R. & Mostov, K. E. *J. Biol. Chem.* 2003 March 28 [epub ahead of print]

Both of these groups — the first using yeast and the second using a mammalian cell line — report a new, unexpected link between the last step of the secretory pathway (exocytosis) and the first step (membrane and protein synthesis in the endoplasmic reticulum (ER)). Lipschutz *et al.* showed that Sec10, a component of the exocyst complex, interacted with the Sec61 β subunit of the Sec61 heterotrimer, a main component of the ER translocon. Overexpressing Sec10 in mammalian cells increased the synthesis and delivery of basolateral and secretory, but not apical, proteins. In yeast, the Sec61 heterotrimer interacted with two exocyst components. Overexpression of Sec61 β in yeast also increased secreted-protein production. The exact mechanism by which protein synthesis is increased is unclear, but decreased degradation is probably not the cause.

APOPTOSIS

Fas-associated death domain protein interacts with methyl-CpG binding protein 4: a potential link between genome surveillance and apoptosis.

Screaton, R. A. *et al. Proc. Natl Acad. Sci. USA* **100**, 5211–5216 (2003)

Fas-associated death domain protein (FADD) has been assumed to localize predominantly in the cytoplasm. The authors of this paper, though, report the primarily nuclear localization of FADD. Phosphorylation of FADD serine 194 was important for this localization and for interaction with a nucleocytoplasmic shuttling protein. FADD also interacted with methyl-CpG binding domain protein 4 (MBD4), a genome surveillance/DNA repair protein that affects apoptosis. So, FADD might have a new function in a new location.

CHROMOSOME DYNAMICS

Role of Polo-like kinase *CDC5* in programming meiosis I chromosome segregation.

Lee, B. H. & Amon, A. *Science* 2003 March 27 (DOI: 10.1126/science.1081846)

A distinct programme of chromosome segregation occurs during meiosis. Lee and Amon investigated the role of the yeast Polo-like kinase *CDC5* and found it to be a key regulator of meiosis I. It was required: at the onset of anaphase I to phosphorylate and remove cohesin from chromosome arms; during meiosis I to co-orientate sister kinetochores by phosphorylating Mam1; and to release the phosphatase Cdc14 from the nucleolus and for spindle assembly during anaphase I.

APOPTOSIS

What's your poison?

How does granzyme B (GrB) activate caspases and lead to the destruction of virus-infected or transformed cells? This is clearly an area of considerable interest at present — and some controversy — as, in addition to *The Journal of Cell Biology* paper described on page 345, work from the laboratories of Chris Bleackley and Joe Trapani has just been published on this subject in *Immunity*.

Cytotoxic lymphocytes mainly destroy target cells by exocytosis of the contents of secretory granules, which include serine proteases, such as GrB, and the pore-forming protein perforin. The enzymatic activity of granzyme B is thought to be central to its ability to induce cell death through the activation of caspases, but how it does this remains controversial. Previous work has shown that overexpression of BCL-2 — an anti-apoptotic protein, which acts by blocking death effectors that target mitochondria — protects cells against GrB-mediated destruction. This indicates that granzyme B is involved not only in the direct activation of caspases, including caspase-3, but also in the mitochondrial death pathway. Until now, the molecular mechanisms for this were not known.

To investigate this further, Bleackley and colleagues analysed the caspase-3 cleavage profile of BCL-2-overexpressing cells after treatment with GrB and adenovirus, which functions as a pore-forming protein to facilitate the release of GrB into the target cell. Caspase-3 activation occurs in two steps: an initial cleavage event, followed by autoactivation that is controlled by the self-catalytic activity of the caspase. In BCL-2-overexpressing cells, only partial cleavage of caspase-3 occurred, showing that high levels of BCL-2 prevented subsequent caspase autoactivation.

The authors reasoned that inhibitor of apoptosis proteins (IAPs) might be responsible for this block in caspase activation, and so they tested the effect of overexpressing XIAP on granzyme-induced cell death. Caspase-3 activation was blocked in cells overexpressing XIAP, and these cells were protected against the toxic effects of GrB. Expression of SMAC/DIABLO, a mitochondrial protein that is released from apoptotic cells and has been shown to displace IAPs from procaspases and promote caspase autoactivation, overcame this blockade in both the XIAP-overexpressing cells and BCL-2-overexpressing cells and rendered them susceptible to GrB-mediated destruction.

Data from Trapani and colleagues investigating the effects of GrB and perforin on Jurkat T cells also highlight the importance of the mitochondrial pathway in GrB-induced apoptosis. First, they showed that the pro-apoptotic BH3-interacting domain protein BID is cleaved by GrB and then translocates to the mitochondria. Next, they showed that exposure of Jurkat cells to GrB and perforin resulted in release of the pro-apoptotic factors cytochrome c, SMAC/DIABLO and HtrA2/OMI from mitochondria. BCL-2 overexpression blocked the release of these pro-apoptotic factors and blocked caspase autoactivation, which resulted in inhibition of GrB-mediated apoptosis of these cells. As above, overexpression of XIAP also resulted in the inhibition of caspase-3 autoactivation and protected these cells from the toxic effects of GrB.

Taken together, these results indicate the importance of the mitochondrial pathway in GrB-mediated cell death. Although GrB can directly cause the initial cleavage of caspase-3, for full caspase activation, pro-apoptotic mitochondrial proteins, including SMAC/DIABLO and HtrA2/OMI, are required to block the effects of IAPs, thereby allowing granzyme-mediated apoptosis to occur.

Jenny Buckland, Senior Editor, Nature Reviews Immunology

References and links

ORIGINAL RESEARCH PAPERS Sutton, V. R. *et al.* Caspase activation by granzyme B is indirect, and caspase autoprocessing requires the release of proapoptotic mitochondrial factors. *Immunity* **18**, 319–329 (2003) | Goping, I. S. *et al.* Granzyme B-induced apoptosis requires both direct caspase activation and relief of caspase inhibition. *Immunity* **18**, 355–365 (2003)