whether other essential factors might also have been immunodepleted, and purified an SMN complex from HeLa cells. They found 16 proteins in this complex, including SMN, Gemin2, the Sm proteins and a number of additional proteins. Addition of this SMN complex to the immunodepleted extracts was able to restore assembly of the Sm core.

This is, say the authors, "the first direct evidence for an essential role for the SMN complex in the formation of the Sm core of U1 snRNP". And, as SMN has been shown to interact with components of small nucleolar ribonucleoproteins (snoRNPs), it is possible that SMN might act as a general assembly factor for different classes of RNPs. The authors now intend to use their cell-free system to dissect the assembly pathway of the other spliceosomal snRNPs, and to take a closer look at how the SMN complex might be involved in the biogenesis of snoRNPs.

Alison Mitchell

References and links

ORIGINAL RESEARCH PAPER Meister, G. *et al*. A multiprotein complex mediates the ATP-dependent assembly of spliceosomal U snRNPs. *Nature Cell Biol*. **3**, 945–949 (2001)

challenged with tBID, they remained viable for 24 hours, but by 48 hours were beginning to show signs of mitochondrial dysfunction and death. By contrast, double-knockout cells that lacked *Bax* and *Bak* proved completely resistant to apoptosis that was induced by all tested BH3-domain only molecules. It seems, then, that the definitive commitment to cell death occurs proximal to the mitochondria, at the step of BAX/BAK activation.

Taken together, these results paint a picture where the BH3-domain only molecules activate the multidomain molecules to trigger a mitochondrial pathway that activates both caspase-dependent death and caspase-independent mitochondrial dysfunction.

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References and links

ORIGINAL RESEARCH PAPER Cheng, E. H.-Y. A. *et al*. BCL-2, BCL-X_L sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. *Mol. Cell* **8**, 705–711 (2001)

CELLULAR MICROBIOLOGY

Bud in, bud out

HIV-1 assembles at the host cell's plasma membrane into enveloped particles that bud off to infect other cells. Garrus and colleagues now report in *Cell* that this process might use the cellular machinery that buds vesicles into the lumen of multivesicular bodies.

The p6 domain of the viral protein Gag contains a short motif (PTAP) known to be the docking site for cellular factors that assist viral budding. In this study, a two-hybrid screen identifies Tsg101 as the cellular factor recruited by the PTAP motif. Indeed, depleting Tsg101 by siRNAs blocked viral budding at a late stage.

The amino-terminal PTAP-binding domain of Tsg101 has homology with E2 ubiquitin-conjugating enzymes, and, although it is not enzymatically active, it does bind ubiquitin, as it had higher affinity to p6–ubiquitin than to p6 alone. So ubiquitylation probably regulates the recruitment of the budding machinery.

Ubiquitylation was recently shown to participate in the formation of intralumenal vesicles in multivesicular bodies. Moreover, Tsg101 is the mammalian homologue of Vps23, which functions in the vacuolar protein sorting (vps) pathway in budding yeast. Consistent with a role of the vps machinery in viral budding, dominant-negative mutants of Vps4, which acts in the same pathway as Tsg101, also inhibited the budding of HIV-1 particles from the cell surface.

Although it may seem surprising at first, it makes sense for HIV-1 to use the machinery of the vps pathway for budding. Indeed, the formation of intralumenal vesicles in multivesicular bodies is the only process in the cell that is topologically equivalent to budding from the cell surface — in both cases, the membrane invaginates away from the cytoplasm.

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FURTHER READING VerPlank, L. *et al*. Tsg101, a homologue of ubiquitin-conjugating (E2) enzymes, binds the L domain in HIV type 1 $Pr55^{Gag}$. *Proc. Natl Acad. Sci. USA* **98**, 7724–7729 (2001)

STRUCTURE WATCH

DNA repair unwinds…

During DNA replication, most, if not all, replication forks encounter some damage — in the form of a base lesion or a single-stranded nick in the DNA — which would stall the machinery unless there are measures to carry out on-site repairs to restart the process. One protein, known as RecG, is involved in the processing of stalled replication forks and works by reversing the fork past the damage to create a four-way (Holliday) junction that allows the template to switch strands and bypass the lesion. To find out how RecG can achieve this, Singleton and colleagues determined the crystal structure of the *Thermatoga maritima* RecG complexed with ADP and a synthetic three-way DNA junction (which resembled a stalled replication fork). They found that the DNA was predominantly bound to a large amino-terminal domain of RecG, which had not been discovered in other DNA helicases. This not only clamps onto and splits open the junction, but also stabilizes the unwinding of the fork. The authors propose that RecG uses a new mechanism for DNA unwinding (pictured) in which the template DNA is bound across the interface between the amino- and carboxy-terminal domains.

REFERENCE Singleton, M. R., Scaife, S. & Wigley, D. B. Structural analysis of DNA replication fork reversal by RecG. *Cell* **107**, 79–89 (2001)

…and relaxes

But this is not the only mechanism an organism can use to protect against DNA damage that impedes the progress of a replication fork. Specialized low-fidelity DNA polymerases can also be recruited to damaged regions to temporarily substitute for the normal high-fidelity DNA polymerases — which are very sensitive to such regions — to allow synthesis to continue. One family of enzymes that can replicate damaged DNA is the Y-family of polymerases, which includes the *Sulfolobus solfataricus* DNA polymerase, Dpo4. Ling and colleagues determined the crystal structure of Dpo4 to uncover the mechanisms behind this family of enzymes. They found the structural configuration and conserved active site of Dpo4 is similar to all high-fidelity polymerases, but that it also makes limited and non-specific contacts with the replicating base pair. This relaxes base selection, and allows translocation of two template bases to the active site. These findings indicate a mechanism for error-prone and lesion-bypass DNA synthesis.

REFERENCE Ling, H. *et al.* Crystal structure of a Y-family DNA polymerase in action: a mechanism for error-prone and lesion-bypass replication. *Cell* **107**, 91–102 (2001)