port to a model in which monomeric katanin subunits in the ADP-bound state exchange their bound ADP for ATP and oligomerize on a microtubule. Oligomerization allows binding of multiple katanin subunits to multiple adjacent tubulin subunits in the microtubule. Oligomerization of katanin subunits also causes ATP hydrolysis through katanin–katanin contacts. A conformational change in the oligomer creates a pushing or pulling force on the underlying tubulin subunits, resulting in dissociation of tubulin–tubulin interactions. The hydrolysis-dependent conformational change in katanin would at the same time promote disassembly of the katanin oligomer back to monomers.

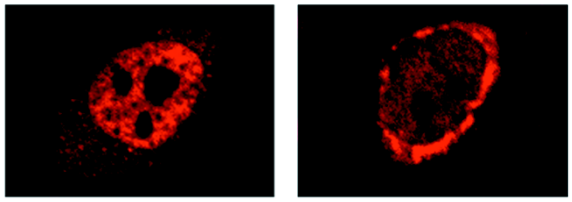
The X-ray crystal structure of the AAA protein NSF[8] indicates that the oligomerized form of all AAA proteins may be a hexameric ring with radial symmetry. Such a radial katanin hexamer would be consistent with the 14–16-nanometre rings seen in katanin preparations by rotary shadowing. Indeed, hydrodynamic analysis of the E334Q mutant katanin in the presence of ATP indicated that the oligomer stabilized by the mutation is a hexamer. However, given that microtubules act as a template for the assembly of the katanin oligomer, it is not inconceivable that the oligomer formed on the microtubule has a different arrangement to that formed in solution. A particularly perplexing problem is that a flat doughnut with 6-fold symmetry (the katanin ring) cannot be docked on the side of a microtubule in such a way as to create multiple identical interactions between katanin subunits and tubulin subunits.

Two possible solutions to this paradox can be tested in future experiments. A katanin hexamer could be docked inside the 15-nanometre lumen of the microtubule, where its 6-fold symmetry would match the 12-fold symmetry of a 12-protofilament microtubule. In this extremely unorthodox view, katanin monomers would enter the microtubule through dynamic lattice defects in the microtubule wall and assemble into the oligomer inside the microtubule. A conformational change in the katanin oligomer might push outwards on bound tubulin subunits or trap transient disruptions that occur during microtubule 'breathing'. A second, more mundane solution would be the assembly of higher-order oligomers of hexameric rings on the outside of the microtubule. Unlike a single hexameric ring, these could form multiple homologous contacts with tubulin subunits in the wall of the microtubule. A coordinated conformational change in this super-oligomer could push or pull on non-adjacent tubulin subunits to disrupt tubulin–tubulin interactions.

Hartman and Vale's results[1] have implications for the actions of all AAA proteins. VPS4 and NSF represent two variations on the AAA theme. VPS4 forms a stable homodimer in the ADP-bound state, probably through a predicted coiled-coil domain adjacent to the AAA domain. VPS4 has been trapped as an oligomer, probably a dodecamer or double ring, only as a mutant[7]. NSF, an AAA protein involved in vesicle fusion in the secretory pathway, consists of two AAA domains called D1 and D2. The D2 domain of NSF does not hydrolyse ATP and, as a result, NSF forms a stable hexameric ring that can be observed by hydrodynamics or electron microscopy[9]. The D1 domain of NSF, which does hydrolyse ATP, may switch between a splayed ring and a closed ring in an ATP- and protein-substrate-dependent manner (Fig. 2).

Because the protein ligands for both VPS4 and NSF are associated with membranes, protein-ligand-dependent oligomerization of VPS4 and the D1 domain of NSF cannot be analysed by hydrodynamics. A FRET assay like that used for katanin might be useful in

A dual function for RAD9



A provocative paper by Hong-Gang Wang and colleagues in this issue (*Nature Cell Biol.* **2,** 1–6; 2000) describes an unexpected pro-apoptotic (cell-death-promoting) function of the human RAD9 protein. This seems surprising given that the fission yeast (*Schizosaccharomyces pombe*) Rad9 protein does not appear to promote apoptosis in yeast, but in fact these results may not be as contradictory as one might think.

Wang and colleagues show that, when overexpressed in mammalian cells, human RAD9 interacts with the anti-apoptotic proteins BCL-2 and BCL-x_L and sensitizes cells to death induced by growth-factor withdrawal. Both effects are inhibited by deleting a short region of RAD9 that shares weak sequence homology with a BCL-2 homology domain known as BH3. The authors obtain similar results when they express *S. pombe* Rad9 in mammalian cells. Expression of an antisense *RAD9* DNA construct protects cells from the genotoxic effects of the DNA-damaging agent methyl methanesulphonate (MMS). MMS induces wild-type RAD9 to move to the nuclear envelope (right panel; the left panel shows RAD9 localization before treatment with MMS), where it localizes with BCL-2, but it does not induce the relocalization of a mutant RAD9 lacking the BH3-like domain. All of this suggests that RAD9 promotes cell death by antagonizing the anti-apoptotic activity of BCL-2-like proteins.

But a pro-apoptotic role for RAD9 is surprising in light of the previously described function of mammalian and *S. pombe* Rad9 in cell-cycle arrest in situations where DNA is damaged or incompletely replicated. In mammalian cells, Rad9 might induce either cell-cycle arrest or cell death, according to the extent of DNA damage. But yeast *rad9* mutants, which fail to arrest the cell cycle after irradiation-induced DNA damage, are hypersensitive to irradiation — which is at odds with Wang *et al.*'s data because one might think that, if *S. pombe* Rad9 were also pro-apoptotic, *rad9* mutations would render cells more, not less, resistant to irradiation.

But actually the fact that Rad9 does not seem to promote apoptosis in yeast may not be that surprising. Although an apoptotic morphology can be induced in yeast by overexpression of mammalian pro-apoptotic proteins such as Bax or by mutating genes such as *cdc48*, cell death may not be a normal part of the yeast life cycle. The yeast genome does not seem to encode any other components of the mammalian cell-death machinery. Yeasts also seem to use a different machinery to respond to DNA damage: they do not express any homologues of p53, a protein required in most mammalian cells as a critical DNA-damage-checkpoint protein. Finally, although overexpression of mammalian Bax or Bak induces apoptosis in yeast, so-called 'BH3-domain-only' proteins of the Bcl-2 family (which, like human RAD9, do not contain the BH1, BH2 or BH4 domains) are non-functional in yeast (Wang *et al.*'s unpublished data).

It seems that, during evolution, RAD9 has acquired another function: it is involved not only in a DNA-damage checkpoint, but also in the control of apoptosis. It will be interesting to see whether other members of the Rad9 checkpoint family also promote cell death, and whether the interaction of human BCL-2 with RAD9 is relevant to the reported effect of overexpression of mammalian Bcl-2 in delaying entry into S phase.

VALERIE DEPRAETERE