IN THE NEWS

Alternative death route

Apoptotic cell death is well established, but other types of programmed cell death have remained elusive. Reporting in *Nature Chemical Biology*, researchers from Harvard Medical School have come a step closer to unravelling a distinct form of non-apoptotic cell death.

The treatment of cells with 'death-receptor ligands' triggers the canonical apoptosis pathway and induces cell death even in the presence of a general caspase inhibitor. The resulting necrosis-like, non-apoptotic cell-death phenotype — which the researchers termed 'necroptosis' — has been observed in many cell types, which implies that it might represent a common pathway.

"The one thing that has been lacking so far has been a way to figure out what proteins are involved in these other pathways", said Shai Shaham, Rockefeller University (*The Scientist*, 31 May 2005). But, by screening a library of chemical compounds, Junying Yuan and colleagues might have found a way — they identified necrostatin-1, which specifically inhibits all known examples of necroptosis.

"The next step…", according to Alan Faden of Georgetown University Medical Center, "…is to use necrostatin-1 to identify the components of the signal transduction cascade responsible for necroptosis." (*The Scientist*, 31 May 2005).

The inhibitor was also used to explore the *in vivo* role of necroptosis. Ischaemic brain injury, as seen in stroke, is associated with both apoptotic and non-apoptotic cell death. Injecting necrostatin-1 into the ventricles of mice with stroke-like injury significantly reduced the volume of dead brain tissue. "This protection suggests that necroptosis is involved in this form of pathologic cell death", the authors say (*The Scientist*, 31 May 2005).

Arianne Heinrichs

ENDOCYTOSIS

And…cut

The formation of clathrin-coated vesicles (CCVs) from clathrin-coated pits (CCPs) during endocytosis has now been visualized using live-cell imaging. It might not quite be the stuff of blockbuster movies, but this observation has enabled Merrifield, Perrais and Zenisek to show that CCP invagination and scission are tightly coupled, and that actin polymerization plays an essential part.

In their study, Merrifield *et al.* fused the extracellular domain of the transferrin receptor (an endocytic marker) to super-ecliptic phluorin, a pH-sensitive variant of green fluorescent protein (Tfnr–phl). The fluorescence of super-ecliptic phluorin is almost completely quenched when the pH changes from 7.4 to 5.5. They transfected Tfnr–phl into cells that contained fluorescently labelled clathrin, and then observed a high amount of colocalization between clathrin-coated 'structures' (CCSs) and Tfnr–phl. When the external pH was switched from 7.4 to 5.5, most — but not all — fluorescence was quenched. There were some acid-resistant Tfnr–phl spots, which appeared suddenly at CCSs — the first frame in the image series at which these were detected was designated the moment of scission. This protocol gave spectacular movies of CCVs appearing across the membrane of living cells.

Whether or not CCPs can support several rounds of CCV formation has been an unresolved issue, so Merrifield *et al.* studied CCPs that formed *de novo* during the imaging session as well as those that were already present from the beginning, and showed that CCVs developed from both newly-formed CCPs and

RNA

Shuttle discovery

The 'RNA era' has seen an expansion in our knowledge of tRNA biology, and recent mechanistic insights have revealed that the maturation of tRNA and its export from the nucleus are interlinked and complex processes. Now, a group of scientists in Japan shows that the subcellular movement of tRNA is more complicated than initially believed — Tohru Yoshihisa and co-workers found that, in yeast, mature cytoplasmic tRNAs are actively reimported into the nucleus, and they report their findings in *Science*.

The authors used fluorescence *in situ* hybridization to track the subcellular movement of tRNA in *Saccharomyces cerevisiae* and showed that mature cytoplasmic tRNAs re-entered the nucleus in an energy-dependent process.

Although most of the imported tRNAs were in the aminoacylated form, they comprised diverse tRNA species — tRNAs encoded by both intron-containing and intronless genes, full-length tRNAs and tRNA molecules with truncated 3′ ends formed part of this migratory pool. Surprisingly, the small GTPase Ran, an important component of many nuclear transport systems, is not required for tRNA import — although the nuclear import of proteins was inhibited in Ran•GAP-deficient mutants, tRNA influx was unimpaired.

As to why tRNAs shuttle between the cytoplasm and the nucleus, Yoshihisa and his team suggest several possibilities. It has been proposed that, prior to nuclear export, tRNAs must pass a 'quality control test' that is linked to tRNA aminoacylation — defective tRNA molecules that cannot accept an amino acid are degraded in the nucleus. As tRNAs are long-lived molecules, they are at risk of adverse modifications, and the authors contend that this intranuclear 'quality control check' might be required to eliminate inactive tRNAs, ensuring the removal of aberrant tRNAs from the cytoplasm.

Importantly, the active transport of mature tRNAs into the nucleus raises a provocative, but exciting, possibility — perhaps these aminoacyl-tRNAs are imported to facilitate intranuclear protein synthesis. Nuclear translation is a controversial topic about which there is at present no consensus; however, these findings will certainly do much to revive this fascinating debate.

Shannon Amoils

References and links

ORIGINAL RESEARCH PAPER Takano, A., Endo, T. & Yoshihisa, T. tRNA actively shuttles between the nucleus and cytosol in yeast. *Science* 19 May 2005 (doi:10.1126/science.1113346)

FURTHER READING Hopper, A. K. & Phizicky, E. M. tRNA transfers to the limelight. *Genes Dev.* **17**, 162–180 (2003)