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)

longer-lived CCSs. Moreover, multiple scission events could be detected at several CCSs, with similar kinetics. Each scission, however, gave rise to a Tfnr–phl spot with only a fraction of the fluorescence of the total original Tfnr–phl patch. And some scission events were 'terminal' — that is, no further scission occurred — possibly because the entire CCP was internalized or because the hotspot was undergoing its last round of scission.

Another point of debate has been whether the nanometer-scale movements of CCPs away from the plasma membrane occurs before or after scission. Using a combination of illumination techniques and the cyclical changing of the pH of the buffer surrounding the cells, the authors measured scission and clathrin displacement from the membrane. Movement of CCPs away from the plasma membrane was seen to begin ~40 sec before membrane scission, with the average movement during invagination being ~40 nm.

Finally, to address another outstanding question — when actin polymerization occurs relative to scission — Merrifield *et al.* fluorescently labelled the actin-binding protein cortactin and visualized it, together with Tfnr–phl, at sites of scission. Cortactin recruitment to the prospective membrane scission site began well before scission occurred (coincidentally, ~40 sec before scission), but peak cortactin recruitment coincided with membrane scission. Preventing actin polymerization using latrunculin B inhibited CCS dynamics and markedly reduced scission events. So, the authors' studies point to a scenario in which CCP movement and scission are normally coordinated and actin polymerization helps to physically separate the budding part of the CCP from its original site of formation.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Merrifield, C. J., Perrais, D. & Zenisek, D. Coupling between clathrin-coated-pit invagination, cortactin recruitment, and membrane scission observed in live cells. *Cell* **121**, 593–606 (2005)

STRUCTURE WATCH

Probing prion patterns

Our understanding of prion-protein structure has been limited owing to the difficulty of studying amyloid fibres using traditional structural methods, but three independent groups now provide new insights in *Nature*.

To understand the self-perpetuating conformational changes of prions, Krishnan and Lindquist studied the amyloid fibres formed by the yeast prion Sup35. They focused on the 250-residue NM (N-terminal and middle) region of Sup35, which encodes prion function. Their aim was to understand the arrangement of NM molecules in amyloid fibres, the mechanisms of fibre nucleation, and the structural basis of different prion strains.

The authors took advantage of the lack of cysteine residues in NM. They created a large number of variants that contained a cysteine at different locations and modified these cysteines with fluorophores or crosslinkers. Their data indicate that a large portion of N — the 'Central Core' — is sequestered by intramolecular contacts, and that the 'Head' and 'Tail' of N form intermolecular contacts. M is mostly flexible and solvent exposed. Contacts between monomers in fibres occur in a Head–Head and Tail–Tail manner. Head–Head contacts form first and these interactions nucleate fibre assembly, which can then occur bidirectionally. Importantly, Krishnan and Lindquist also showed that strain distinctions are the result of differences in both the length of the amyloid core and the nature of the intermolecular contacts.

In the second study, Eisenberg and colleagues zoomed in on a peptide from the N terminus of the N region of Sup35 — a seven-residue peptide that forms amyloid fibres. This peptide also forms closely related elongated microcrystals, which were used for X-ray diffraction studies. The core of amyloid fibres consists of a continuous array of β-sheets that are orientated perpendicular to the fibre axis — a 'cross-β' spine. The structure determined here confirmed the known overall features of the spine: a double β-sheet, with each sheet formed by β-strands that are stacked in parallel. However, it also provided an important new insight. The interface between the two β-sheets is 'dry' — it contains no water molecules. The sheets are zipped together by the shapes of the amino-acid side chains, which complement each other and interdigitate, forming van der Waals interactions. These interactions are non-specific, so a given sequence might form more than one self-complementing 'steric zipper'.

In the final study, Riek and co-workers investigated the C-terminal region of the prion HET-s from the fungus *Podospora anserina* to understand the relationship between structural features and prion infectivity. Using fluorescence and NMR techniques, they showed that this region contains four β-strands, which are arranged in two β-strand–turn–β-strand motifs. Their model for the arrangement of these strands into the fibre core is similar to the arrangement proposed by Eisenberg and colleagues for the Sup35 peptide and, using structure-based mutagenesis, Riek and co-workers showed that the β-sheet structure is the infectious prion conformation of HET-s.

REFERENCES Krishnan, R. & Lindquist, S. L. Structural insights into a yeast prion illuminate nucleation and strain diversity. *Nature* 9 June 2005 (doi:10.1038/nature03679) | Nelson, R. *et al.* Structure of the cross-β spine of amyloid-like fibrils. *Nature* 9 June 2005 (doi:10.1038/nature03680) | Ritter, C. *et al.* Correlation of structural elements and infectivity of the HET-s prion. *Nature* 9 June 2005 (doi:10.1038/nature03793)