MITOCHONDRIA

Mitoskeleton, you said?

Cells do not like simplicity. So instead of being spherical, mitochondria form tubular networks with complex inner membranes. How do cells build these complicated structures? To tackle this question, a few years ago, Rob Jensen's laboratory screened for *Saccharomyces cerevisiae* mutants defective in mitochondrial shape, and isolated one mutant, *mmm1*, in which mitochondria were large and spherical.

The Jensen group have now analysed the localization of an Mmm1–GFP chimera in living yeast cells. They found Mmm1 in spots on the surface of mitochondria, adjacent to a subset of mitochondrial DNA (mtDNA) nucleoids. In *mmm1* mutants, mtDNA collapses into one large structure, and cannot be properly segregated during mitochondrial division, which leads to its loss.

But Mmm1 is an outer membrane protein, so how can it bind to mtDNA inside mitochondria? The authors suggest that Mmm1 functions in the formation of contact sites between the outer and inner membranes, and this is supported by the fact that inner membranes collapse in *mmm1* mutants.

So the authors' model is that Mmm1 is part of a scaffold-like complex — the 'mitoskeleton' — that holds the outer and inner membranes together and is required for normal mitochondrial shape and mtDNA segregation.

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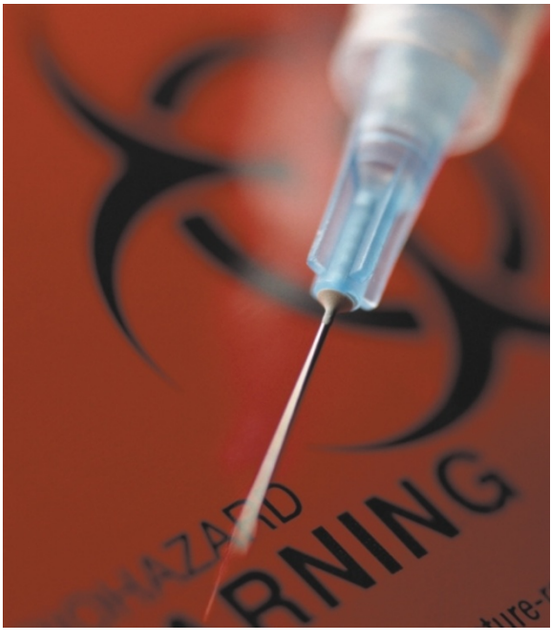
References and links

ORIGINAL RESEARCH PAPER Aiken Hobbs, A. E. *et al.* Mmm1p, a mitochondrial outer membrane protein, is connected to mitochondrial DNA (mtDNA) nucleoids and required for mtDNA stability. *J. Cell Biol.* **152**, 401–410 (2001)

CELLULAR MICROBIOLOGY

Lethal injection

Many pathogenic bacteria have the nasty habit of injecting proteins into the cytoplasm of their host to interfere with its signalling pathways. Gram-negative bacteria use syringes — type III secretion systems — but Gram-positive bacteria are not equipped with such sophisticated tools. So how do they get their deadly messengers across the host's plasma membrane? Madden and colleagues report in *Cell* that *Streptococcus pyogenes* might inject one of its virulence factors through pores formed by a bacterial cholesterol-dependent cytolysin, streptolysin O (SLO).



Infection of keratinocytes by *S. pyogenes* is accompanied by modulation of the host's pro-inflammatory response. Madden and colleagues noticed that a bacterial factor appeared in the host cytosol only when SLO was active. They identified the factor as SPN (*S. pyogenes* NAD-glycohydrolase), which can produce the second messenger cyclic ADP-ribose and could therefore be the effector that is responsible for modulating host cell signalling during infection.

The functions of both SPN and SLO, as well as bacterial adherence, are required for cytotoxicity. The emerging model is that bacteria adhere and secrete SLO to form pores through which SPN can access the host's cytoplasm to trigger cytotoxicity. Whether this occurs by diffusion or by an active process is not completely clear, but the authors present circumstantial evidence that transport occurs in a vectorial manner: co-infection of keratinocytes with strains that express either SPN or SLO alone does not trigger cytotoxicity, indicating that the action of the two proteins is tightly coordinated. Hence, transport of SPN into the host is probably not a random diffusion process.

The ability of SLO to form 30-nm pores that allow the passage of fully folded proteins has been known for a long time and is widely used by cell biologists for selective permeabilization of plasma membranes. This study provides the first indication that cytolysins, including SLO, might do more than just make the host cell leaky. But important questions remain, the most obvious being how vectorial transport of SPN through the pore occurs — if, indeed, vectorial transport it is.

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References and links

ORIGINAL RESEARCH PAPER Madden, J. C., Ruiz, M. & Caparon, M. *Cell* **104**, 143–152 (2001)

ENDOCYTOSIS

Tent pegs for clathrin

Every construction — no matter how temporary — needs sound foundations. So how are the clathrin coats that surround endocytic vesicles tethered to the plasma membrane? Two papers in the 9 February issue of *Science* reveal the importance of a phospholipid, phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2), in this process.

Several proteins involved in endocytosis, including epsin, clathrin assembly lymphoid-myeloid leukaemia protein (CALM) and its neuronal homologue AP180, contain an epsin amino-terminal homology (ENTH) domain. But what is its function? Itoh and colleagues couldn't find any proteins that bind epsin's ENTH domain, so they searched for phospholipid partners and discovered that PtdIns(4,5)P_2-containing vesicles bind it. NMR spectroscopy of epsin's ENTH domain bound to inositol-1,4,5-trisphosphate (Ins(1,4,5)P_3) revealed that Ins(1,4,5)P_3 binds to a positively charged pocket in an eight-helix bundle. Deletion of loop 1 or substitution of Arg63 or Lys76 for Ala in helix 3 almost abolished Ins(1,4,5)P_3 binding, and overexpression of a mutant epsin lacking the ENTH domain, or a Lys76Ala mutant, blocked endocytosis.

Ford and colleagues used X-ray crystallography to solve the structure of CALM's amino terminus bound to a series of inositol phosphates and phosphoinositides. CALM's amino terminus looks reassuringly similar to epsin's ENTH domain but, surprisingly, PtdIns(4,5)P_2 binds to a cluster of protruding lysines and a histidine not present in epsin's ENTH domain. Both CALM and AP180 were specifically sedimented by liposomes or tubules containing PtdIns(4,5)P_2, and mutation of the lysine cluster in either protein blocked sedimentation. Ford and colleagues could partially reconsitute clathrin-coat formation using PtdIns(4,5)P_2-containing phospholipid monolayers, clathrin, AP180 and the adaptor protein AP2. Substitution of PtdIns(4,5)P_2 for PtdIns, or omission of AP180, abolished the formation of clathrin lattices.

Why do two similar domains bind PtdIns(4,5)P_2 through different sites? Whatever the answer, the function of PtdIns(4,5)P_2 as a tent-peg *par excellance* is now undisputed.

Cath Brooksbank

References and links

ORIGINAL RESEARCH PAPERS Itoh, T. *et al.* Role of the ENTH domain in phosphatidylinositol-4,5-bisphosphate binding and endocytosis. *Science* **291**, 1047–1051 (2001) | Ford, M. G. J. *et al.* Simultaneous binding of PtdIns(4,5)P_2 and clathrin by AP180 in the nucleation of clathrin lattices on membranes. *Science* **291**, 1051–1055 (2001)