antigen processing.

Who needs peptide transporters?

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The discovery of genes in the major histocompatibility complex (MHC) that code for subunits of proteasomes and putative ATP-driven transmembrane transporters has led to a great deal of speculation about their involvement in antigen processing and peptide binding to MHC class I molecules (the developments were discussed at the time in News and Views1,2). The most favoured hypothesis is that proteasomes digest cytoplasmic proteins into fragments which are then, in an ATP-dependent step, translocated across the membrane of the endoplasmic reticulum (ER) by the transporters. Writing in Cell, Lévy et al.3 challenge this view. They report that ATP is required for the binding of peptide to MHC class I molecules rather than for peptide transport into the lumen of the ER.

MHC class I molecules are composed of two subunits, a membrane-spanning heavy chain and β2-microglobulin, a small exoplasmic protein. Heavy chains are polymorphic and encoded by genes in the MHC. Shortly after their synthesis, heavy chains assemble non-covalently with β2-microglobulin in the ER. Binding of peptides, nine or ten amino acids long, stabilizes the association between heavy chain and β2-microglobulin and makes the complex competent for transport to the cell surface, where MHC class I molecules activate cytotoxic T-cells by presenting peptides to them.4

Several mutant cell lines have been characterized in which MHC class I molecules are synthesized but not expressed on the cell surface. Only after the addition of peptides are these molecules found on the cell surface.5,6 In one cell line the defect could be repaired by transfection with a gene encoding a member of the superfamily of ATP-driven transporter proteins.7 It was therefore proposed that peptide transport from the cytosol into the lumen of the ER is an active, ATP-dependent process. The experiments now described by Lévy et al.3 aim at a direct demonstration of the participation of ATP-driven transporters in supplying class I molecules with peptide.

An in vitro system was used in which class I heavy chains were synthesized and cotranslationally inserted into membranes derived from the rough ER of lymphoblastoid cells. The membranes, also called rough microsomes, form spherical vesicles that are impermeable to proteases added from the outside. Microsomes have been widely used to study the translocation of nascent secretory proteins. This process requires the presence of an ER signal sequence on the nascent polypeptide, GTP, possibly also ATP, and several cytosolic and membrane factors.7 Most secretory proteins can be translocated only cotranslationally, but some small ones with relative molecular masses of about 6,000–8,000 (M, 6–8K) are also translocated post-translationally.10 Microsomal membranes were generally found to be impermeable to proteins lacking a signal sequence, but small peptides, such as those that are substrates for an ER oligosaccharyl transferase,8 can pass through the membrane. When added to cells or microsomes, peptides with 3–5 amino-acid residues are found inside the lumen of the ER or of microsomes, respectively, and become glycosylated. The efficiency of membrane permeation strongly depends on the size and physical properties of the peptides. How these peptides cross the ER membrane is unclear.

To test whether ATP-binding proteins are involved in peptide transport across the ER membrane, Lévy et al.3 translated messenger RNA coding for class I heavy chains in a reticulocyte lysate supplemented with rough microsomes from the lymphoblastoma cell line Raji. The microsomes contain endogenous β2-microglobulin. After translation, ATP was depleted and a peptide from the nucleoprotein infilus A (NP, 384–394) in its biotinylated form was added.9 In the presence of ATP, the peptide stabilized the oligomeric assembly of heavy chains with β2-microglobulin and bound to this complex. Depletion of ATP prevented the assembly of heavy chains with β2-microglobulin and prevented the binding of peptide. In the absence of ATP, peptide was bound by a chaperone (BiP, the human immunoglobulin-binding protein) and released from it in the presence of ATP; BiP is a soluble heat-shock protein analogue of the ER lumen10, and has been shown to interact with partially folded and misfolded proteins in an ATP-sensitive fashion. The two experiments convincingly demonstrate that binding of peptide to MHC class I molecules occurs only in the presence of ATP, and that at least some peptides can enter the lumen of microsomal vesicles in the absence of ATP.

To see whether cytosolic domains of transmembrane proteins are involved in peptide transport, Lévy et al. incub...
Cost of the charge
Sequestration of a charged side-chain in the low-dielectric-constant heart of a globular protein has always been surmised to exact a ruinous thermodynamic cost. Sometimes paired charges have proved so repulsive that they have driven the protein core apart, as in the case of fibronectin (1). Now S. Dao-pin et al. [Biochemistry 30, 11521–11526 (1991)] have made two mutants of T4 phage lysozyme, one with lysine for methionine on the inward-facing surface of an α-helix, the other with glutamate for a leucine. The conformations are less stable, but both chains fold, giving products with 35% per cent and 4 per cent activity. The first mutant has been crystallized and the isolated helix is appreciably wobbly. The pK of the intruding lysine is 6.5 and the structure survives down to pH 3. Such mutations, then, are evidently not as calamitous as has been supposed.

Old story
CONVENTIONAL thinking has it that clonally reproducing organisms don't last for long in evolutionary terms, at least compared with the sexually reproducing stock from which they derived. After all, asexually reproducing cells should not be harmed by the mutations that arise spontaneously. J. M. Quattro and colleagues in their estimation of the time over which a unicellular, all-female lineage of fish has persisted (Proceedings of the National Academy of Sciences 89, 3485–3502, 1992). Quattro et al. looked at a 'postformation' (that is, post-hybridization) mutations in the mitochondrial DNA and allozymes of the three-spined stickleback (P. gobio). The lineage manifests a high level of genetic diversity and is, the authors estimate, at least 100,000 generations old.

Steps in time
L. R. Bano, together with Thungas, has carried out a further appraisal of how the fossil tetrapod tracksway in the Coconino sandstone of northern Arizona came to be formed (Geology 19, 1201–1204, 1991). At issue is whether the sandstone, which is of Permian age, was deposited subaerially, as sand dunes, or under water. Brand and Tang produced diagrams of the orientation of the limb prints and direction of the fossil tracks, and compared them with those made by kneels on sand in a tank of flowing water 4 cm deep. In many of the fossil tracks, limb impression orientation and direction are different angles, a feature also evident in the impressions left by the living newts as they were drifted by the current. That, say the authors, points towards underwater origin for at least some of the sandstone.

bated microsomes with protease. This treatment, the authors argue, should have cleaved the cytoplasmically exposed ATP-binding domain of the transported transplongers. Because no effect on peptide binding to MHC class I molecules was evident, they concluded that transporbengers are not involved in this process. But because the protease sensitivity of the putative transporbengers is not known, the results are suggestive rather than conclusive.

The idea that ATP-driven transporters are involved in the transport of peptides across the ER membrane arose from analysis of mutant cells defective in surface expression of class I molecules. Mutant T2 cells derived from the B lymphoblastoid cell line T2 have a deletion in the MHC, and they express class I molecules intracellularly but not on the cell surface. Cerundolo et al.2 found that these cells had lost the ability to present intracellular antigen efficiently, whereas they could present extracellular antigen of eight and twelve amino-acid residues efficiently. One possible explanation for the defect in these cells is that peptides derived from cytosolic proteins do not reach the lumen of the ER. To test this possibility, Lévy et al. investigated the ability of microsomes from T1 and T2 cells to take up peptides and assemble them with class I molecules. They found that peptides were taken up into microsomes from T1 and T2 cells with similar efficiencies. But peptide-stimulated assembly of class I molecules was only seen with microsomes of T1 cells, not with those of mutant T2 cells. Lévy et al. propose that the defect is in luminal factors needed for peptide binding to the class I molecule.

The authors consider several explanations for the defect in the lumen of microsomes from T2 cells and the ATP requirement for peptide binding to class I molecules. Peptides may become concentrated by binding to BiP and the ATP is released from the peptide-carrying BiP (see figure). I consider this to be unlikely, because peptide would re-enter the free-peptide pool after release from BiP. Peptides may require trimming in the lumen of the ER before binding to class I molecules. This possibility remains open, however, and needs further experiments with peptides of different size.

Another possibility is that the oligomeric structure of MHC class I molecules needs a catalyst for proper folding. This is the most likely explanation for an ATP-dependent step in the formation of MHC I molecules, and the results of this study support the idea that ATP binding to the MHC I molecules is crucial for their proper folding and disulfide-bond formation in the protein. In the ER, the authors demonstrate that folding of a viral glycoprotein in the ER is an energy-dependent process. In the absence of ATP, the protein is not properly folded and does not aggregate.

Is there any requirement for peptide transporters? Indeed, for peptides up to about ten amino acids in length there may be no requirement for active transport across microsomal membranes. But in the case of peptides the ER membranes in intact cells cannot be released. Yet it may be interesting to see whether peptides are applied to the cytosol of cells by either micro-injection or after permeabilization of the plasma membrane can cross the ER membrane in an ATP-independent manner.

The results of Lévy et al. raise another important question. What size are the peptides present in the cytosol? In particular, are they the right size to interact with MHC class I molecules? Little is known about the peptide intermediates in the degradation of cytosolic proteins. It is conceivable that proteasomes sequentially process cytosolic proteins to fragments which can be actively transported across the ER membrane. In order to draw a more definite conclusion about the requirement for peptide transporters, intact protein substrates, not just the peptides, should be investigated for their requirements for proteolytic processing and membrane translocation. With the in vitro system used by Lévy et al. and a possible to analyse the entire pathway of proteolytic processing of cytosolic proteins, transport of peptides across the ER membrane and binding to the MHC I class I molecules. It is likely that several energy-requiring steps and factors mediating charging of MHC I class I molecules with peptides still await discovery.

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REFERENCES