Protein translocation: **Rehearsing the ABCs**

Ann E. Cleves and Regis B. Kelly

Recent evidence that vacuolar enzymes in yeast can be delivered directly from the cytosol, rather than *via* the secretory pathway, alerts us to the increasing evidence for 'non-classical' forms of protein translocation that may involve ABC transporters.

Address: Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143-0534, USA.

Current Biology 1996, Vol 6 No 3:276–278

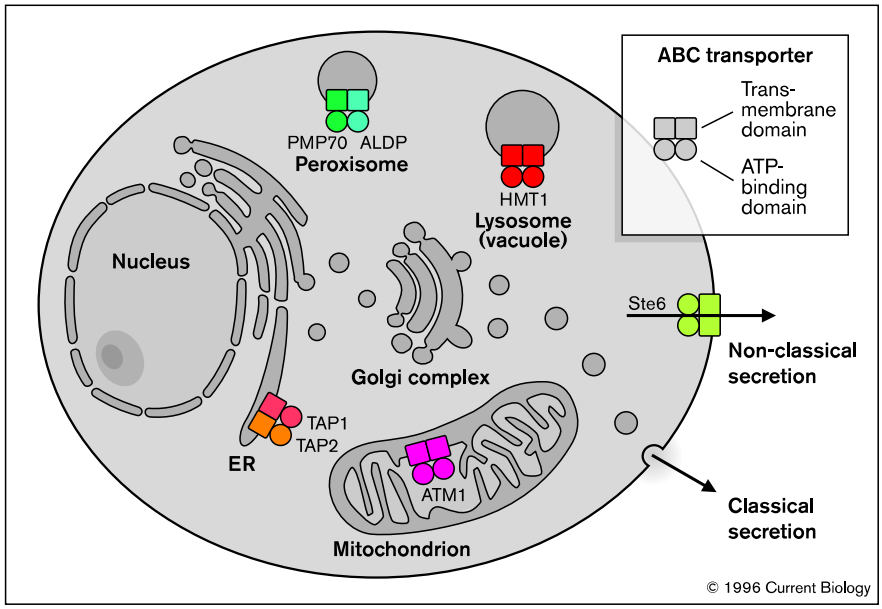
Signal-mediated translocation of proteins occurs across the membranes of several organelles, including the endoplasmic reticulum (ER), peroxisomes, mitochondria and lysosomes. In some cases, signal-mediated translocation involves a well-characterized molecular machine — or translocon — such as the Sec61 complex in the ER. In parallel with these systems, several membranes also have members of the ATP-binding-cassette (ABC) protein family (Fig. 1), transporters that are capable of translocating a variety of substrates across membranes, including peptides and proteins [1]. The recent discovery that some enzymes are translocated directly from the cytoplasm into the vacuole in yeast [2,3] prompts a re-examination of these alternative routes of transport that do not use the conventional signal-peptide-mediated mechanisms.

Transport across organelle membranes

The molecular machinery responsible for signal-mediated protein translocation across the ER membrane is well known [4]. In addition to this 'classical' translocation machinery, the ER also contains the TAP1–TAP2 (transporter associated with antigen presentation) heterodimer that transports peptides from the cytosol into the ER lumen, in an ATP-dependent manner. There, the peptides assemble with class I major histocompatibility complex (MHC) molecules for presentation to cells of the immune system [5]. TAP1 and TAP2 are members of the ABC family and are each a 'half transporter', consisting of a membrane-spanning domain (with six transmembrane regions) and an ATP-binding domain. Expression of TAP1 and TAP2 in insect cells showed that the TAP1–TAP2 complex requires no additional cofactors to function as a peptide transporter [6]. The TAP complex acts as a selective peptide pump, because human, rat and mouse TAP1–TAP2 complexes have been shown to display some specificity for peptide substrates [7].

In the case of the peroxisome, several pathways for the import of proteins are known. Targeting signals have been identified in proteins destined for peroxisomes, and receptors for the PTS1 and PTS2 signals have been identified. The corresponding translocon, however, remains unknown. Peroxisomal membranes contain two members of the ABC family, PMP70 [8] and ALDP [9], which, like

Figure 1



Examples of ABC transporters located in the organelle membranes of eukaryotic cells. A typical ABC transporter consists of two transmembrane domains that each span the bilayer six times and two ATP-binding domains. The transporters can be assembled from two half-transporter polypeptides or may be synthesized as a single polypeptide chain. See text for further details.

TAP1 and TAP2, are half transporters. PMP70 and ALDP may form both homodimers and heterodimers.

Mutations in PMP70 are found in a subset a patients with Zellweger syndrome, a human disease in which mature peroxisomes are absent and the activities of all peroxisomal enzymes are deficient [10]. Mutations in ALDP cause adrenoleukodystrophy (ALD), a severe peroxisomal disorder associated with the accumulation of very long-chain fatty acids (VLCFA), possibly due to a defect in the import of VLCFA acyl-CoA synthetase into peroxisomes [11]. The functions of PMP70 and ALDP are not known, although it has been suggested that they may function as protein transporters in the peroxisomal membrane. An ALDP homolog in the yeast *Saccharomyces cerevisiae*, Pxa1p, is also localized to peroxisomes.

Proteins can be delivered to lysosomes — or their equivalent in yeast, the vacuole — *via* the secretory or endocytic pathways, and by autophagocytosis. In addition to these vesicular pathways, lysosomal proteins can also be imported directly from the cytosol. This uptake is mediated by the peptide motif KFERQ, which is recognized by the heat-shock protein (Hsp) 70 family member Prp73 [12]. Direct import of the vacuolar protein aminopeptidase I (API) is believed to be mediated by its amino-terminal 16 amino acids [3]. The machinery for direct vacuolar targeting is still unknown, but mutants defective in the import of API have been isolated [2]. The ABC transporter HMT1p is localized to the vacuole in the fission yeast *Schizosaccharomyces pombe*, and has been demonstrated to be an ATP-dependent transporter of phytochelatins, which are peptide–Cd^{2+} complexes involved in heavy metal tolerance [13].

Most proteins that are imported from the cytosol into the mitochondria contain an amino-terminal signal sequence, and the machinery responsible for the targeting and translocation of these proteins across the outer and inner mitochondrial membranes has been identified [14]. In addition to the classical, signal-mediated import machinery, an ABC-family member, Atm1p, is located in the mitochondrial inner membrane [15]. Although the *ATM1* gene is required for normal growth in yeast, the function of Atm1p remains unclear.

Eukaryotic and prokaryotic cell membranes

In addition to classical, vesicle-mediated protein secretion, across the plasma membrane, 'non-classical' secretion also occurs . Yeast Ste6p is an ABC protein that is transiently located in the plasma membrane and is known to be the transporter for the lipopeptide **a**-factor mating pheromone [16]. Ste6p is homologous to the mammalian multidrug resistance transporters (MDRs), one of which, MDR1, is found in the plasma membrane and is known to transport amphiphilic cytotoxic drugs out of cells [17]. There is evidence that MDRs can also function as peptide transporters: for example, mouse MDR3, when expressed in yeast, can transport **a**-factor at a low efficiency [18], and there is evidence that MDR1 can transport many hydrophobic peptides [19].

Bacteria possess many ABC transporters [1] in addition to having a classical protein secretion route that is mediated by SecA and shows similarities to ER transport in eukaryotes [20]. Bacterial ABC proteins transport a variety of substrates, including amino acids, peptides and sugars. Eukaryotic ABC transporters are known to transport peptides but, so far as we know, they do not transport proteins. Bacterial ABC transporters, by contrast, export a variety of proteins, mostly toxins and some as large as 177 kDa.

Non-classical protein export from mammalian cells

Mammalian cells produce a variety of proteins that lack a secretory signal sequence yet are exported from cells by non-classical mechanisms [21–24]. Examples of such proteins include interleukin-1β [23], basic fibroblast growth factor [25] and thioredoxin [23]. Pharmacological agents, such as monensin or brefeldin A, that perturb the function of the Golgi complex do not block secretion of these proteins, and export is enhanced by a variety of drugs that increase synthesis of heat-shock proteins.

Given the many examples of direct transport of peptides or proteins across organellar membranes, it is not surprising that some proteins in mammalian cells are directly exported from the cytosol across the plasma membrane. Non-classical export is not limited to specialized or damaged cells since it occurs in normal as well as neoplastic cells. Furthermore, we have recently shown that yeast also possesses the machinery for the non-classical secretion of proteins in addition to the a-factor peptide transporter, Ste6p (unpublished data). Since the mechanism of non-classical protein export in mammalian cells remains unknown, our yeast system may provide the first mechanistic insight into non-classical protein secretion in eukaryotic cells.

The normal housekeeping function of non-classical protein export from mammalian cells may be to remove toxic proteins, or simply to control the levels of normal cytosolic proteins. Through the course of evolution, these exported cytosolic enzymes might have acquired extracellular roles. This possibility is illustrated by the non-classical export of thioredoxin, an intracellular disulfide-reducing enzyme [23], in that adult T-cell-leukemia-derived factor, the secreted form of thioredoxin, upregulates the expression of the interleukin-2 receptor in an autocrine fashion [26]. Non-classical export of cytosolic proteins might even explain in part why some intracellular proteins are implicated in autoimmune disease: epitopes of certain cytosolic proteins, such as ribosomal P-proteins,

presented on the cell surface are the targets of autoantibodies, and cell lysis or damage seems an unsatisfactory explanation for the appearance of these cytosolic proteins at the cell surface [27,28].

References

1. Higgins CF: ABC transporters: from microorganisms to man. *Annu Rev Cell Biol* 1992, 8:67–113.
2. Harding TM, Morano KA, Scott SV, Klionsky DJ: Isolation and characterization of yeast mutants in the cytoplasm to vacuole protein targeting pathway. *J Cell Biol* 1995, 131:591–602.
3. Segui'-Real B, Martinez M, Sandoval IV: Yeast aminopeptidase I is post-translationally sorted from the cytosol to the vacuole by a mechanism mediated by its bipartite N-terminal extension. *EMBO J* 1995, 14:5476–5484.
4. Walter P, Johnson AE: Signal sequence recognition and protein targeting to the endoplasmic reticulum membrane. *Ann Rev Cell Biol* 1994, 10:87–119.
5. Townsend A, Trowsdale J: The transporters associated with antigen presentation. *Sem Cell Biol* 1993, 4: 53–61.
6. Meyer TH, van Endert PM, Uebel S, Ehring B, Tampe' R: Functional expression and purification of the ABC transporter complex associated with antigen processing (TAP) in insect cells. *FEBS Lett* 1994, 351:443–447.
7. Momburg F, Roelse J, Howard JC, Butcher GW, Hammerling GJ, Neefjes JJ: Selectivity of MHC-encoded peptide transporters from human, mouse and rat. *Nature* 1994, 367:648–651.
8. Kamijo K, Taketani S, Yokota S, Osumi T, Hashimoto T: The 70-kDa peroxisomal membrane protein is a member of the Mdr (P-glycoprotein)-related ATP-binding protein superfamily. *J Biol Chem* 1990, 265:4534–4540.
9. Mosser J, Douar AM, Sarde CO, Kioschis P, Feil R, Moser H, Poustka AM, Mandel JL, Aubourg P: Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. *Nature* 1993, 361:726–730.
10. Gartner J, Valle D: The 70 kDa peroxisomal membrane protein: an ATP-binding cassette transporter protein involved in peroxisome biogenesis. *Sem Cell Biol* 1993, 4:45–52.
11. Mosser J, Lutz Y, Stoeckel ME, Sarde CO, Kretz C, Douar AM, Lopez J, Aubourg P, Mandel JL: The gene responsible for adrenoleukodystrophy encodes a peroxisomal membrane protein. *Human Mol Gen* 1994, 3:265–271.
12. Dice JF: Peptide sequences that target cytosolic proteins for lysosomal proteolysis. *Trends Biochem Sci* 1990, 15:305–309.
13. Ortiz DF, Ruscitti T, McCue KF, Ow DW: Transport of metal-binding peptides by HMT1, a fission yeast ABC-type vacuolar membrane protein. *J Biol Chem* 1995, 270:4721–4728.
14. Ryan KR, Jensen RE: Protein translocation across mitochondrial membranes: what a long, strange trip it is. *Cell* 1995, 83:517–519.
15. Leighton J, Schatz G: An ABC transporter in the mitochondrial inner membrane is required for normal growth of yeast. *EMBO* 1995, 14:188–195.
16. Michaelis S: STE6, the a-factor transporter. *Sem Cell Biol* 1993, 4:17–27.
17. Germann UA, Pastan I, Gottesman MM: P-glycoproteins: mediators of multidrug resistance. *Sem Cell Biol* 1993, 4:63–76.
18. Raymond M, Gros P, Whiteway M, Thomas DY: Functional complementation of yeast ste6 by a mammalian multidrug resistance mdr gene. *Science* 1992, 256:232–234.
19. Sharom FJ, DiDiodato G, Yu X, Ashbourne KJD: Interaction of the P-glycoprotein multidrug transporter with peptides and ionophores. *J Biol Chem* 1995, 270:10334–10341.
20. Economou A, Wickner W: SecA promotes preprotein translocation by undergoing ATP-driven cycles of membrane insertion and deinsertion. *Cell* 1994, 78:835–843.
21. Muesch A, Hartmann E, Rohde K, Rubartelli A, Sitia R, Rapoport TA: A novel pathway for secretory proteins? *Trends Biochem Sci* 1990, 15: 86–88.
22. Mignatti P, Rifkin DB: Release of basic fibroblast growth factor, an angiogenic factor devoid of secretory signal sequence: a trivial phenomenon or a novel secretion mechanism? *J Cell Biochem* 1991, 47:201–207.
23. Rubartelli A, Sitia R: Interleukin 1β and thioredoxin are secreted through a novel pathway of secretion. *Biochem Soc Trans* 1991, 19:255–259.
24. Kuchler K, Thorner J: Secretion of peptides and proteins lacking hydrophobic signal sequences: the role of adenosine triphosphate-driven membrane translocators. *Endocri Rev* 1992, 13:499–514.
25. Florkiewicz RZ, Majack RA, Buechler RD, Florkiewicz E: Quantitative export of FGF-2 occurs through an alternative, energy-dependent, non-ER/Golgi pathway. *J Cell Physiol* 1995, 162:388–399.
26. Tagaya Y, Maeda Y, Mitsui A, Kondo N, Matsui H, Hamuro J, Brown N, Arai K, Yokota T, Wakasugi H, Yodoi J: ATL-derived factor (ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin; possible involvement of dithiol-reduction in the IL-2 receptor induction. *EMBO J* 1989, 8:757–764.
27. Koren E, Reichlin MW, Koscec M, Fugate RD, Reichlin M: Autoantibodies to the ribosomal P proteins react with a plasma membrane-related target on human cells. *J Clin Invest* 1992, 89:1236–1241.
28. Naparstek Y, Plotz PH: The role of autoantibodies in autoimmune disease. *Annu Rev Immunol* 1993, 11:79–104.