CELL BIOLOGY

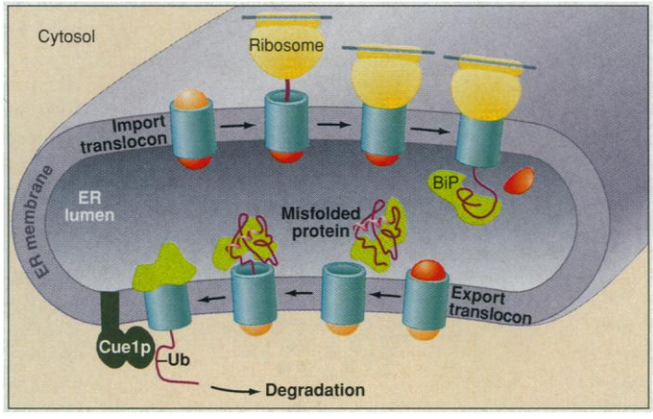
The Ins and Outs of Protein Translocation

Howard Riezman

Most cells manufacture proteins destined for export, which will be used by the organism either as structural elements or information-carrying signaling molecules. In preparation for their secretion, these proteins are first translocated as a long chain of amino acids through a pore, also made of protein, into the endoplasmic reticulum (ER), a complex network of membrane tubes inside the cell. Once inside the ER, these proteins fold into their mature state, assemble with other proteins, and move toward their final destination outside the cell.

But errors can happen, so eukaryotic cells carefully monitor the accuracy of protein folding and assembly in the ER. When unfolded proteins accumulate—or proteins are misfolded, unassembled, or denatured as a result of various stresses—the cell sends a signal to the nucleus to activate a program to destroy these mistakes. For many years it was thought that misfolded or unassembled proteins were destroyed in the lumen of the ER. But surprising evidence has shown recently that misfolded proteins are actually exported out of the ER to the cytosol in order to be tagged with ubiquitin, a highly conserved 76-residue polypeptide that marks them for destruction by an organelle specialized for protein degradation, the proteasome. On page 1806 of this issue, Biederer *et al.* (*1*) describe a new component of this machinery, Cue1p, a protein that docks crucial parts of the ubiquitination apparatus at the ER membrane. Without Cue1p, cells cannot efficiently ubiquitinate and degrade misfolded proteins. Indeed, in the absence of Cue1p, misfolded proteins remain in the ER lumen, suggesting that their export is usually coupled to their ubiquitination.

When proteins are imported into the ER during their translation from messenger RNA, they pass through an aqueous channel, found in a protein structure called the translocon (*2*) (see the figure). During this cotranslational import, the cytoplasmic face of the aqueous pore is sealed by docking of the ribosome, thus maintaining the permeability barrier of the ER membrane and ensuring that the nascent polypeptide is directed into the ER lumen. In the lumen, the nascent precursor is greeted by enzymes, including chaperones, oligosaccharyltransferase, and signal peptidase, which modify, fold, and assemble these unfolded proteins into native structures. The chaperone BiP is required for import into the ER, but cleavage of the signal peptide or glycosylation is not. Apparently, only unfolded proteins are translocated through the aqueous pore, so proper folding of the imported proteins ensure their unidirectional transport.



Quality control. Before protein import (top) into the ER, the import translocon (blue) is opened on the cytoplasmic side to receive the ribosome, which binds to the translocon and creates a seal. The lumenal gate can then be opened, and the polypeptide is translocated to the ER lumen where interaction with BiP and protein folding ensure unidirectional translocation. For export of misfolded proteins (bottom), BiP binds to a misfolded protein, and the lumenal gate opens to allow delivery of the misfolded protein to the export translocon (blue). It is not clear whether or how the exported protein is unfolded and delivered to the export translocon. The Cue1p complex ensures ubiquitination of the exported protein and its unidirectional export. For simplicity, the import and export translocons are drawn the same, but there may be components that are specific to one of the two. Both translocons are closed by a cytoplasmic gate (orange) and a lumenal gate (red). These may or may not be independent of the translocons.

Proteins that do not succeed in folding or assembling properly in the ER are shuttled back to the cytoplasm; some of the same machinery that is used for their import is also used for their export. For instance, yeast cells mutant for BiP (Kar2p) and two components of the yeast translocon, Sec61p and Sec63p, cannot export from the ER a fully translocated, mutant carboxypeptidase Y that does not fold properly (*3*). Proteins destined for export can be crosslinked to Sec61p, indicating that they are bound to this molecule, and in vitro experiments also implicate Sec61p in ER export (*4*). These findings raise several interesting issues.

Can a protein be recognized as hopelessly misfolded? How could such a misfolded protein be directed to the export channel? Although unfolded and misfolded proteins interact with the chaperone, BiP, it is not known whether this enzyme can differentiate between an unfolded protein that will eventually fold from one that will not. In addition, a misfolded protein may have too many folded domains to pass through the aqueous pore even though the pore is about 20 Å in diameter (*5*). Can BiP unfold these proteins? If so, BiP could also direct the unfolded protein to the export translocon, because BiP is known to bind to the DnaJ-like domain of Sec63p.

It is clear that some gene products are shared by the import and export translocons, but are they identical? Thus far, two components of the import translocon—Sec61p and Sec63p—have been shown to be required for protein export. Mutants in two genes encoding membrane proteins that are required for degradation of misfolded proteins, but not ER import, have been isolated (*6*). Functional characterization of these genes may help to clarify this issue.

During protein import the lumenal side of the channel is closed until the nascent peptide reaches about 70 amino acid residues in length. A putative gating protein for this side of the channel has been discussed (*2*), but the mechanism by which the lumenal side of the translocon is sealed is still unknown. How is the permeability barrier of the ER maintained during export of misfolded proteins? The export channel must be sealed on the lumenal side of the membrane while the exported protein is emerging on the cytoplasmic side. Therefore, export of misfolded proteins through an export translocon requires coordination of the events on the two sides of the ER membrane. How can this be accomplished?

How is unidirectional export ensured? Insight into this question comes from the new work by Biederer *et al.* Protein ubiquitination requires at least two enzymes: E1, which activates ubiquitin and transfers it to the second

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required protein, E2, a ubiquitin-conjugating enzyme (Ubc). In yeast, there are 13 Ubcs. Only one of these, Ubc6p, is a transmembrane protein, although it has some overlapping specificity with the soluble Ubc, Ubc7p. These two enzymes participate in the ubiquitination of both soluble and membrane-bound substrates. The new work reports that an ER protein, Cue1p, is required to recruit the soluble Ubc7p to the membrane. A deletion of Cue1p gives the same phenotype for ER degradation as a deletion of Ubc7p, suggesting that Ubc7p must be assembled with Cue1p to be active. In a yeast strain defective for ubiquitination of misfolded proteins, the latter are stabilized and remain in the lumen of the ER. This result suggests that attachment of ubiquitin may be required to drive unidirectional export of unfolded proteins from the ER.

If the presence of a ubiquitin branch on a protein can contribute to the unidirectional export of proteins from the ER, it may also be able to block import through the translocon. For ubiquitination to occur during cotranslational import into the ER, the seal between the ribosome and the membrane must be broken to allow access of the Ubcs to the protein. Evidence suggests that the ribosome seal can be broken during the translocation of transmembrane proteins without compromising the ER permeability barrier (*7*). This would allow for the ubiquitination and degradation of a misfolded transmembrane protein to begin before translation has terminated. Does this occur and could this serve to degrade improperly inserted membrane proteins? Given the rapid rate of recent progress in the fields of ER translocation and degradation, answers to these enigmas should come soon.

References

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QUANTUM PHYSICS

Artificial Atoms: New Boxes for Electrons

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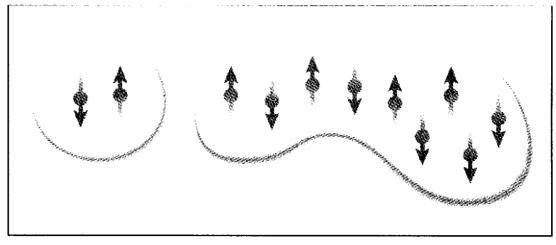
An atom can be thought of as a spherically symmetric box for electrons, where the walls of the box are set by the attraction of the electrons to the positively charged nucleus. The energy-level structure of electrons in these atomic boxes have been a central testing ground of quantum mechanics. For example, reproducing the measured hydrogenic spectrum of the one-electron atom was one of the first tests of the Schrödinger equation. Similarly, the excitation spectra of multielectron atoms have been key to the development of many of the more advanced ideas in multiparticle quantum mechanics. Now, similar experiments are being done on quantum dots (*1*).

These are small solid-state systems with discrete charge states and energy level structures that are reminiscent of atomic systems. Unlike in real atoms, however, a variety of geometries in these "artificial atoms" are possible, including one-dimensional (1D) rods (*2*), 2D pancakes (*3*, *4*), and 3D spheres (*1*). For example, on pages 1784 and 1788 of this issue, Kouwenhoven and colleagues at Delft Institute of MicroElectronics and Submicrotechnology and Nippon Telegraph and Telephone (*5*) and Stewart and co-workers at Stanford University (*6*) report on detailed studies of 2D quantum dots. In the former, a high-symmetry dot of a few electrons was studied, and in the latter, a low-symmetry dot containing many electrons was probed. These measurements demonstrate that many of the cherished ideas from atomic physics can be directly applied to artificial atoms. They also show, however, that artificial atoms offer new challenges to our understanding of interacting multiparticle quantum systems. In the related report on page 1792, Schedelbeck *et al.* (*7*) explain how two coupled dots were used to create artificial molecules.



Electrons in boxes. (**Left**) Schematic illustration of two electrons in a 2D circular quantum dot (quantum dot helium). In the ground state, the spins are oppositely oriented, but in the excited state, they can be in the same direction (triplet state) or in opposite directions (singlet state). (**Right**) Schematic of many electrons in an irregularly shaped 2D quantum dot. The states are expected to be complex, and the ground state may have a net spin polarization.

To probe the energy level spectra, the first two groups took electrical measurements on individual quantum dots using a technique developed in the early 1990s, where the dot is incorporated into an electronic device resembling a transistor (*4*). Current-voltage (*I-V*) measurements on these quantum-dot transistors directly probe the ground- and excited-state energies of electrons in the dots. Further, the charge state of the dot, that is, the atomic number of the artificial atom, can be controlled by a voltage applied to a nearby metallic gate. In this manner, the whole periodic table for a particular type of artificial atom can be studied in a single device.

In real atoms, the behavior of the ground and excited states is well known. For hydrogen, with one electron, the spectrum is given by the shell structure calculated by undergraduate physicists the world over. For the next simplest atom, helium, things get more difficult. The shell structure remains, but the coulomb interaction between the electrons complicates matters. For example, the coulomb repulsion plus the restrictions placed on the system as a result of the Pauli exclusion principle lead to the so-called exchange interaction, which favors a spin alignment of electrons in the atom. This ordering results, for example, in a splitting of the first excited state of He into a triplet state, where the electron spins are parallel, and a singlet state, where the spins are antiparallel. The exchange interaction is also the origin of Hund's rule in larger atoms with many electrons. This rule favors a spin-polarized, that is, magnetic, ground state for atoms with a partially filled shell.

In the experiment of Kouwenhoven *et al.* (*5*), the spectra of the first few electrons added to a circular quantum dot [resembling a pancake (see figure)] are studied. The dot is like a 2D atom and exhibits a shell structure whose states can be characterized by radial and angular momentum quantum numbers (*3*). The magnetic field dependence of the levels can be used to identify the angular momentum of the

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