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COMMENTARY

Surfing the Sec61 channel: bidirectional protein translocation across the ER membrane

Karin Römisch

University of Cambridge, Cambridge Institute for Medical Research, Wellcome Trust/MRC Building, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2XY, UK
(e-mail: kbr20@cam.ac.uk)

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SUMMARY

Misfolded secretory and transmembrane proteins are retained in the endoplasmic reticulum (ER) and subsequently degraded. Degradation is primarily mediated by cytosolic proteasomes and thus requires retrograde transport out of the ER back to the cytosol. The available evidence suggests that the protein-conducting channel formed by the Sec61 complex is responsible for both forward and retrograde transport of proteins across the ER membrane. For transmembrane proteins, retrograde transport can be viewed as a reversal of integration of membrane proteins into the ER membrane. Retrograde transport of soluble proteins through the Sec61 channel after signal-peptide cleavage, however, must be mechanistically distinct from signal-peptide-mediated import into the ER through the same channel.

Key words: Yeast, Translocation, ER, Sec61, Quality control

INTRODUCTION

Protein secretion is an essential process. In most cases, however, cells are better off secreting a reduced amount of a specific protein than secreting a defective form of this protein. Misfolded secreted and cell surface proteins could interfere with cell-cell communication; this would have disastrous consequences for both development and the immune system in multicellular organisms, and for cell wall integrity and mating in yeast. Even intracellularly, dysfunctional proteins are too dangerous to be let loose. Eukaryotes have therefore developed a stringent quality-control mechanism at the site of secretory protein synthesis and maturation, the ER (Hammond and Helenius, 1995). Proteins that fail to pass quality control cannot enter ER-to-Golgi transport vesicles and are retained in the ER (Hammond and Helenius, 1995). Most of these proteins are subsequently degraded (Klausner and Sitia, 1990; Hammond and Helenius, 1995). The notion that degradation takes place in the ER lumen has always been difficult to reconcile with the presence of secretory proteins in the process of folding – and thus susceptible to proteolysis – in the same compartment (Klausner and Sitia, 1990). The proteases responsible for 'ER degradation', however, turned out to be cytosolic proteasomes, which suggests that folding and degradation are compartmentalized: like many other incompatible processes in eukaryotic cells they are separated by a membrane (Ward et al., 1995; Jensen et al., 1995; McCracken and Brodsky, 1996; Wiertz et al., 1996a; Biederer et al., 1996; Hiller et al., 1996; Werner et al., 1996). Cytosolic degradation of misfolded secretory proteins requires that these proteins be transported across the ER membrane back to cytosol.

After more than two decades of research on the topic, we have a detailed understanding of how proteins cross the ER membrane and enter the ER lumen (Matlack et al., 1998); export of misfolded proteins across the same membrane to the cytosol has only been studied for a few years, but we now have enough pieces of the puzzle to develop experimentally testable theories that might help to fill the gaps.

THE PROTEIN TRANSLOCATION CHANNEL IN THE ER MEMBRANE

The protein-conducting channels in the ER membranes of yeast and mammalian cells are formed by the heterotrimeric Sec61 complex, which comprises Sec61p (Sec61α in mammals), Sbh1p (Sec61β in mammals) and Sss1p (Sec61γ in mammals) (Fig. 1). Sec61p is an essential polytopic protein that has 10 transmembrane domains and lines the protein-conducting channel in the ER membrane (Wilkinson et al., 1996). Sbh1p is a single-spanning transmembrane protein most of whose bulk resides on the cytosolic face of the membrane (Panzner et al., 1995; Toikkanen et al., 1996); in contrast to the two other subunits of the channel it is stable on its own (Panzner et al., 1995; Esnault et al., 1994). The *SBH1* gene is not essential,

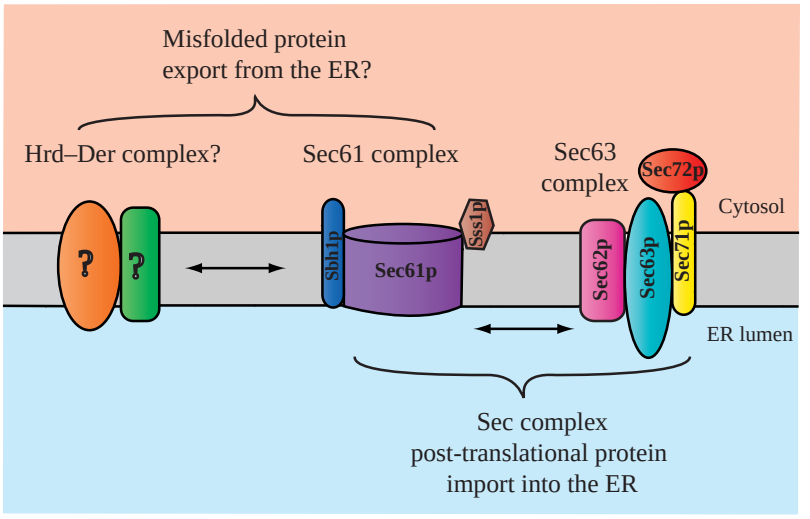

Fig. 1. Interaction of the Sec61 complex with other membrane protein complexes. Note that the Sec61 channel in the ER membrane is formed by multiple Sec61 complexes. The Sec61 channel on its own mediates cotranslational protein import into the ER in both yeast and mammalian cells. In yeast, the Sec61 complex can associate with the Sec63 complex to form the heptameric Sec complex responsible for post-translational protein import into the ER. Association of the Sec61 complex with Hrd or Der proteins in the membrane might allow retrograde transport of misfolded proteins through the Sec61 channel to the cytosol.

neither is a second gene encoding a closely related protein, Sbh2p; deletion of both genes, however, results in temperature-sensitive growth and protein translocation defects (Toikkanen et al., 1996; Finke et al., 1996). The role of Sbh1p in translocation is unclear, but in mammalian cells it mediates association of signal peptidase with the protein translocation channel (Kalies et al., 1998). Sss1p is an essential 9 kDa protein that binds to Sec61p (Esnault et al., 1994; Wilkinson et al., 1997). This association stabilizes both proteins and may also be responsible for stabilizing the structure of the Sec61 channel (Esnault et al., 1994; Wilkinson et al., 1997). A non-essential homologue of Sec61p, Ssh1p, forms a complex homologous to the Sec61 complex that contains Sbh2p and Sss1p (Finke et al., 1996); this complex might have a specialized role in cotranslational translocation into the yeast ER (Finke et al., 1996); the function of its mammalian equivalent remains unknown.

Hanein and colleagues first glimpsed the Sec61 channel structure: they observed channels in the ER membrane whose formation in the mammalian ER was dependent on the presence of a functional signal sequence; in *S. cerevisiae* their formation depended upon the presence of a second protein complex, the Sec63 complex (Hanein et al., 1996) (Fig. 1). The Sec63 complex consists of four proteins (Sec63p, Sec62p, Sec71p and Sec72p), which are required for post-translational import of proteins into the yeast ER (Deshaies et al., 1991; Panzner et al., 1995). The complex interacts with the Sec61 complex, forming the heptameric Sec complex, and is required for signal-sequence recognition (Lyman and Schekman, 1997; Plath et al., 1998). In addition, Sec63p itself contains an ER-lumenal DnaJ domain, which is responsible for anchoring the ER-lumenal Hsp70 BiP to the translocation channel (Corsi and Schekman, 1997). In yeast, BiP facilitates import of secretory proteins into the ER (Sanders et al., 1992). In mammalian cells, Johnson and colleagues have shown that BiP forms the ER-lumenal seal of the protein translocation channel (Hamman et al., 1998).

RETROGRADE PROTEIN TRANSLOCATION ACROSS THE ER MEMBRANE

ER degradation of misfolded proteins was poorly understood until a series of papers suggested that misfolded secretory proteins are not degraded by ER lumenal proteases, but primarily by proteasomes in the cytosol (Ward et al., 1995; Jensen et al., 1995; Wiertz et al., 1996a; Biederer et al., 1996; Hiller et al., 1996; Werner et al., 1996). At the time, the process was renamed ‘ER-associated degradation’ (ERAD) (McCracken and Brodsky, 1996), but because the proteasomes responsible for degradation are not necessarily ER associated, either term is used now to describe the process.

Wiertz and coworkers produced the first hint that the Sec61 channel is responsible for retrograde transport of these degradation substrates from the ER lumen to the cytosol: early in 1996 they had demonstrated the complete dislocation of a transmembrane protein from the ER membrane to the cytosol prior to degradation (Wiertz et al., 1996a); in a second paper they showed that this single-spanning transmembrane protein, MHC class I heavy chain, when destined for degradation, associates with Sec61β prior to export to the cytosol (Wiertz et al., 1996b). Wiertz et al. (1996b) observed this interaction both in virus-infected cells, in which one of the virus-encoded proteins physically guides the MHC class I heavy chain to the export channel and out to the cytosol, and in cells treated with DTT, which reduces the disulfide bonds of the heavy chain and thus makes it a substrate for export and degradation. They are so far the only investigators who have isolated an exported ER protein bound to the proteasome.

Pilon, Schekman and I first demonstrated that the Sec61 channel functions in export of misfolded proteins from the yeast ER (Pilon et al., 1997). Pilon had created two new mutants in *SEC61* that in contrast to the previously isolated temperature-sensitive alleles were cold-sensitive for growth and protein import into the ER; we found that these *sec61* mutants are defective for protein export at any temperature (Pilon et al., 1997). Thus we were able to investigate export defects under conditions that allow import of secretory precursors through the same channel. These new *sec61* alleles encode stable Sec61 proteins that have point mutations close to the lumenal side of transmembrane domains III and IV; this suggests that this region is specifically important for export (Pilon et al., 1997). Furthermore, in these *sec61* mutants the unfolded protein response is induced, which indicates that misfolded proteins accumulate in the ER of these cells (K.

Römisch, unpublished). In a large collection of cold-sensitive *sec61* mutants import, and export defects mostly correlate; there are, however, some exceptions, which suggest that it is possible to create export-specific mutants in *SEC61* (Pilon et al., 1998).

Temperature-sensitive *sec61* mutants isolated as defective in protein translocation into the ER are also defective in export of misfolded secretory proteins from the ER to the cytosol (Pilon et al., 1997; Plemper et al., 1997). The mutant Sec61p in these cells is unstable and the amount of Sec61p in the ER is reduced even at the permissive temperature. Thus both export and import defects in these mutants are likely to be attributable to the reduced amount of Sec61p in these cells (Biederer et al., 1996; Plemper et al., 1997; Pilon et al., 1998). Because Sec61p is required for the insertion of most transmembrane proteins into the ER membrane, and the import of ER-resident lumenal proteins, defects in export from the ER in these temperature-sensitive *sec61* mutants may also be caused indirectly by reduced amounts of Sec61-dependent, ER-resident proteins.

Export of misfolded secretory proteins from the ER to the cytosol may simply be the reversal of posttranslational protein import into the ER. If this is true, all seven Sec complex subunits should be required for both import and export. Deletion of *SBH1* or *SBH2*, however, has no effect on export of a soluble misfolded form of carboxypeptidase Y, CPY*, from the yeast ER (Plemper et al., 1997). This could be due to redundancy in function of Sbh1p and Sbh2p; the *Δsbh1 Δsbh2* double knockout, however, has not yet been investigated. The role of Sss1p in export is not yet known, but deletion of the gene encoding the Sec61p homologue, *SSH1*, has no effect on export of misfolded proteins from the ER (Plemper et al., 1997; K. Römisch, unpublished). Analysis of a single temperature-sensitive allele defective in protein import into the ER suggests that Sec62p is not required for export (Pilon et al., 1997; Plemper et al., 1997). Deletion of *SEC71* also has no effect on export. Sec72p is unstable in *Δsec71* cells; thus Sec72p is probably also dispensable for export (Feldheim and Schekman, 1994; Plemper et al., 1997). The requirement for Sec63p itself is somewhat controversial: my lab and Plemper and colleagues found similar increases in the half-lives of mutant alpha-factor precursor and CPY* in *sec63-1* ER. Compared with the defects in some *sec61* mutants, this effect is marginal and probably is a consequence of the slow import of proteins into the ER rather than direct defect in export; Plemper and colleagues, however, suggested that Sec63p is required for export, and felt that the fact that mutations in BiP, which is anchored to the Sec complex via Sec63p, also interfere with export confirm this idea (Plemper et al., 1997). The analysis of more *sec63* mutant alleles might resolve this issue. So far, however, only the central component of the Sec complex, Sec61p itself, has proven to be essential for protein export from the ER.

ER PROTEINS INVOLVED IN PROTEIN EXPORT TO THE CYTOSOL

The regulation of bidirectional protein trafficking through the Sec61 channel remains a mystery. The channel is a passive conduit, and directionality of transport is almost certainly determined by accessory proteins. Matlack et al. (1999) have shown recently that post-translational protein import into the ER is driven by binding of multiple BiP molecules to the lumenal end of the translocation substrate. BiP binding interferes with passive backward movement of the substrate through the Sec61 channel, but not with forward movement, and thus drives import into the ER lumen. The Hsp70 homologue BiP also recognizes unfolded proteins in the ER and chaperones their folding (Hammond and Helenius, 1995). In mammalian cells, BiP forms the lumenal seal of the Sec61 channel (Hamman et al., 1998). A number of BiP mutant alleles interfere with export of several soluble proteins from the yeast ER, but the role that BiP plays in export remains unclear (Plemper et al., 1997; Brodsky et al., 1999). BiP might keep soluble export substrates from aggregating, or it might actively target these proteins to the export channel. Alternatively, the gating function of BiP might be required for channel opening from the ER lumen.

Gruss et al. (1999) have shown recently that Sec61β in mammalian ER can be phosphorylated and that phosphorylation has a moderate stimulatory effect on protein import into the ER. Given that Sec61β can mediate the association of the Sec61 channel with other protein complexes in the ER membrane, its phosphorylation might regulate interactions between the channel and accessory molecules in the cytosol or the ER membrane that facilitate transport of misfolded proteins to the cytosol (Kalies et al., 1998). Several investigators have isolated mutants in ER-resident transmembrane proteins essential for degradation of CPY* and HMG-CoA reductase (*DER* genes: Knop et al., 1996a; Bordallo et al., 1998; *HRD* genes: Hampton et al., 1996). The Der and Hrd proteins are candidates for modulators of transport through the Sec61 channel; whereas Hrd1p/Der3p and Hrd3p are required for ER degradation of several misfolded proteins, Der1p seems to be substrate-specific (Hampton et al., 1996; Bordallo et al., 1998; Plemper et al., 1998, 1999b). A recent review by Brodsky and McCracken (1999) provides a comprehensive summary of the proteins required for the degradation of individual ERAD substrates in yeast.

CYTOSOLIC FACTORS INVOLVED IN EXPORT

Several investigators have suggested that export of misfolded proteins from the ER is driven by a molecular ratchet mechanism: in a similar manner to BiP during protein import, cytosolic chaperones could bind to the cytosolically exposed end of an export intermediate and, ultimately, cause its export across the ER membrane (Biederer et al., 1997; Brodsky and McCracken, 1997; Riezman, 1997; Mayer et al., 1998; Bordallo et al., 1998; Matlack et al., 1999). Brodsky et al. (1999) have shown that the major cytosolic Hsp70s in yeast are not required for export. A potential candidate for a chaperone ratchet is cytosolic Hsp90, which associates with mutant insulin receptors destined for degradation (Imamura et al., 1998). Imamura et al. blocked Hsp90 binding by microinjecting anti-Hsp90 antibodies and found that the mutant receptors became stable in the ER membrane; this suggests that Hsp90 binding is necessary for degradation of these substrates by proteasomes. By contrast, inhibition of interaction between Hsp90 and one of its folding substrates, cystic fibrosis transmembrane conductance regulator (CFTR),

results in misfolding of CFTR and its accelerated proteasomal degradation (Loo et al., 1998). The features that Hsp90 recognizes, and what determines whether it promotes folding or degradation of any given substrate, remain to be investigated.

Cytosolic ubiquitination of misfolded proteins concomitant with export through the Sec61 channel may also drive export to the cytosol (Riezman, 1997; Biederer et al., 1997; deVirgilio et al., 1998; Bordallo et al., 1998): Biederer et al. (1997) identified a yeast ER-membrane protein, Cue1p, which is required for the membrane localization of the ubiquitin-conjugating enzymes Ubc6p and Ubc7p. In the absence of Cue1p, the soluble degradation substrate CPY* remains in the ER lumen, which indicates that ubiquitination is required for export of CPY* (Biederer et al., 1997; Bordallo et al., 1998). In mammalian cells, a soluble truncated form of ribophorin I, RI_{332}, is rapidly exported from the ER and degraded in a ubiquitin- and proteasome-dependent fashion. Inhibition of proteolysis results in cytosolic accumulation of RI_{332}, but expression in cells with a temperature-sensitive mutation in the ubiquitin-activating enzyme E1 leads to accumulation of RI_{332} in the membrane fraction at the restrictive temperature, which suggests that ubiquitination is indeed required for export of this protein to the cytosol (deVirgilio et al., 1998). The majority of ER degradation substrates investigated so far are ubiquitinated prior to degradation, but at least two proteins, mutant alpha-factor precursor in yeast and cytochrome P-450 in mammalian cells, are degraded in a ubiquitin-independent fashion (Werner et al., 1996; Roberts, 1997); 20S proteasomes are sufficient for their degradation, and the 19S ATPase cap, which makes the 26S proteasome specific for ubiquitinated substrates, is not required (Roberts, 1997; K. Römisch, unpublished).

COUPLING OF EXPORT AND DEGRADATION

Degradation and export are coupled in many cases: treatment of cells with the proteasome inhibitor lactacystin, or performing the experiments in yeast cells that lack proteasome activity, leads to retention of misfolded proteins in the ER (Yang et al., 1998; Xiong et al., 1999; Plemper et al., 1998; Mayer et al., 1998). The initial hypothesis that the proteasome is docked onto the cytoplasmic face of the Sec61 channel and pulls out the degradation substrates is almost certainly too simplistic: although proteasomes do bind to the ER, no interaction with the Sec61 channel has been demonstrated. Most of the proteasomes associated with the ER membrane of mammalian cells are 20S proteasomes, not the 26S proteasomes required for degradation of ubiquitinated substrates (Yang et al., 1995); in *S. cerevisiae*, however, 26S proteasomes are bound to the ER membrane, and these may well be responsible for the degradation of proteins exported from the ER (Enenkel et al., 1998).

MISFOLDED MEMBRANE PROTEINS

Export and proteasomal degradation are constitutively coupled for proteins with more than one transmembrane domain (Xiong et al., 1999; Plemper et al., 1998; Mayer et al., 1998). Some multispanning membrane proteins also seem to require an ER membrane-associated proteolytic activity different from the proteasome for the degradation (Moriyama et al., 1998; Xiong et al., 1999). The nature of this protease is unknown, but it might be identical to the signal peptide peptidase responsible for degradation of cleaved-off signal peptides (Lyko et al., 1995); large membrane proteins might need to be clipped in the membrane by this protease to facilitate their subsequent export from the membrane. Surprisingly, at least some single-spanning transmembrane proteins, such as MHC class I heavy chain and the α subunit of the T cell receptor, can be entirely dislocated from the membrane to the cytosol prior to degradation (Wiertz et al., 1996a,b; Huppa and Ploegh, 1997; Yang et al., 1998). Their transmembrane domains probably are masked after exit from the translocon, maybe by association with cytosolic chaperones. Currently, we do not understand what features in the substrate lead to 'cotranslocational' degradation; this mechanism might have evolved to prevent transmembrane proteins from forming potentially toxic aggregates in the cytosol.

Retrograde transport of misfolded transmembrane proteins is probably a reversal of the process that leads to their integration into the membrane in the first place, and involves lateral opening of the Sec61 channel towards the lipid bilayer (Do et al., 1996). After membrane integration, newly synthesized membrane proteins quickly dissociate from the channel (Do et al., 1996). The signals that promote reassociation of misfolded transmembrane proteins with the channel, and ultimately lateral opening and export, are unknown; the transmembrane domain responsible for channel opening during import is still present, however, and in conjunction with specific accessory molecules might be used to gate the channel laterally for export as well.

MISFOLDED SOLUBLE PROTEINS

Retrograde transport of soluble proteins through the protein-conducting channel is conceptually more difficult. During import into the ER the N-terminal signal peptide of secretory protein precursors serves a dual role: it targets these proteins to the ER membrane, and it is recognized by the Sec61 channel, which results in opening of the channel towards the lumenal side (Jungnickel and Rapoport, 1995; Plath et al., 1998). Both wild-type and misfolded soluble proteins complete translocation and dissociate from the Sec61 channel (Plemper et al., 1999a). In the ER lumen, the signal peptide is removed prior to re-export of misfolded proteins (Hiller et al., 1996; Werner et al., 1996). Opening of the channel from the lumenal side in the absence of a signal peptide must therefore be triggered by a mechanism that is fundamentally different from that used during protein entry. In the simplest scenario, terminally misfolded secretory proteins could be covalently modified in the ER lumen; like the presence of a signal peptide, this modification could target misfolded proteins to the Sec61 channel and trigger export to the cytosol (Fig. 2). The modification-acceptor sites would have to be relatively common in most protein sequences (occurring at a frequency similar to that of Ser/Thr phosphorylation sites or Lys acceptor sites for ubiquitin) and might be modified only if the substrate is associated with chaperones in the ER lumen for a prolonged period. Such a modification would probably be small, to

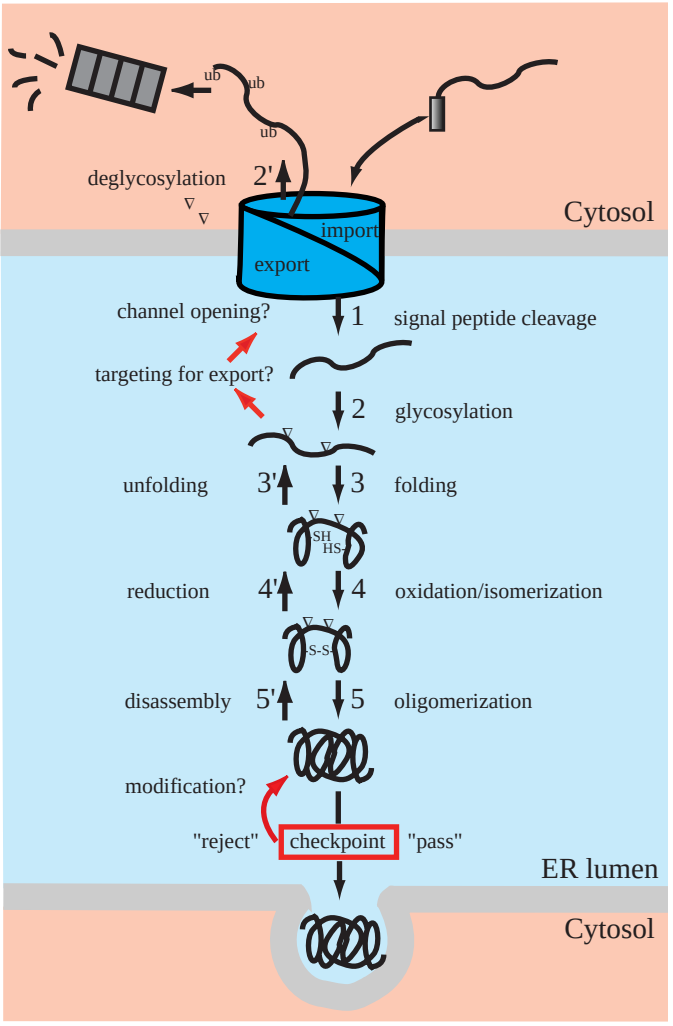

Fig. 2. Secretory protein translocation into and misfolded protein export from the ER. None of the steps shown in the figure is obligatory; individual steps in maturation are dependent on the nature of the specific secretory protein. The numbering does not represent an obligatory sequence of events but is introduced to illustrate that some steps are dependent on each other. Note that signal-peptide cleavage and glycosylation are not reversible in the ER lumen. Important steps required for export are highlighted in red and discussed in detail in the text.

prevent interference with export through the protein translocation channel, and either reversible in the cytosol or compatible with degradation by the proteasome. There is at present no evidence for any ER-lumenal modification specific for misfolded secretory proteins; there is, however, some evidence for competition between protein folding and N-glycosylation, and between N-glycosylation and O-glycosylation, in the ER lumen, which suggests that exposure of specific acceptor sites in slowly folding substrates leads to increased levels of modification (Holst et al., 1996; Holkeri et al., 1998). Inappropriate glycosylation often results in degradation of glycoproteins; this is most likely due to 'freezing' of the aberrantly glycosylated proteins in a non-native state rather than to N-glycosylation itself being an export signal.

PROTEIN QUALITY CONTROL AND DEGRADATION

Components of the quality control machinery in the ER lumen can distinguish between folded and unfolded proteins; they are required for the initial recognition and subsequent retention of misfolded secretory proteins in the ER. Retention in the ER, however, does not always result in export to the cytosol and degradation (Young et al., 1993; Knop et al., 1996b; Jakob et al., 1998; Kowalski et al., 1998; Tortorella et al., 1998). The available data suggest that proteins have to have undergone a complete cycle of progressive maturation and quality control at each individual step, before reaching some kind of 'master checkpoint' at which the decision is made about their ultimate fate: packaging into ER-to-Golgi transport vesicles or retrograde transport to the cytosol for degradation (Fig. 1). In many cases, the individual steps in the pathway to the native conformation of a protein are dependent on each other; in the example shown in Fig. 1, the disulfide bond cannot be formed in step 4 unless the protein has acquired the appropriate fold in step 3. Proteins that fail at early steps in their maturation will neither be packaged into ER-to-Golgi vesicles nor exported for degradation, because they have never reached the decision-making checkpoint. An example of this class of misfolded protein is a form of CPY* that, in addition to the point mutation that causes export and degradation, lacks N-glycosylation sites; this unglycosylated form of CPY* is stable, because it is retained in the ER and not exported to the cytosol (Knop et al., 1996b). Similarly, when CPY* is expressed in yeast with mutations in oligosaccharyl side-chain-modifying enzymes of the ER, inappropriate N-linked oligosaccharyl structures are attached to it. This form of CPY* is also stable, which suggests that trimming of glycosyl side chains is an early step in CPY* maturation and that downstream folding events have to be completed, before the decision is made to export CPY* to the cytosol for degradation (Jakob et al., 1998).

The checkpoint in Fig. 2 might be an intellectual construct rather than a specific protein in the ER lumen. Passing this checkpoint might be equivalent to release from the network of ER chaperones that cooperates in folding newly synthesized proteins (Tatu and Helenius, 1997). Progressively more folded proteins should have a decreased affinity for chaperones and, ultimately, might be released from the network for packaging into vesicles. By the same token, slow folding will result in prolonged association with the chaperone network; this might prevent misfolded proteins from entering ER-to-Golgi transport vesicles and promote their covalent modification with an export-specific tag (Fig. 2).

Many steps in protein folding are equilibrium reactions and therefore reversible (Kowalski et al., 1998). Export of unfolded proteins to the cytosol removes these proteins from the equilibrium reactions and thus might be the driving force for progressive reversal of folding reactions that the degradation substrates have undergone prior to rejection at the checkpoint (Fig. 2, reactions 5′ to 3′). Proteins must be unfolded and disulfide bonds must be reduced prior to export through the Sec61 channel (Young et al., 1993; Tortorella et al., 1998). Proteins that cannot unfold completely, or aggregate, will remain in the ER and not be degraded (Valetti et al., 1991; Dafforn et al., 1999). Changes in redox potential or mutations in the active sites of protein disulfide isomerase that prevent disulfide reduction also interfere with export of misfolded

proteins from the ER (Young et al., 1993; Tortorella et al., 1998; K. Römisch, unpublished). N-glycosylation is not reversible in the ER lumen, but N-linked oligosaccharides per se do not interfere with export through the Sec61 channel (Wiertz et al., 1996a; Hiller et al., 1996); they are subsequently removed by a cytosolic N-glycanase (Fig. 1, step 2′; Wiertz et al., 1996a; Suzuki et al., 1998). As mentioned above, signal peptide cleavage is also irreversible; therefore an export-specific modification might be required for targeting to lumenal face of the Sec61 channel (Fig. 1). After unfolding is completed, such a modification could be recognized by an adaptor in the ER lumen that, if bound to an export substrate, triggers opening of the channel from the lumenal side. Channel opening may be achieved by interaction of the adaptor-substrate complex with BiP, which forms the lumenal seal of the Sec61 channel in mammalian cells (Hamman et al., 1998).

Once an export-specific modification has been identified, we will be able to determine whether any given misfolded protein retained in the ER is arrested on the anterograde branch of the folding pathway (steps 1-5, Fig. 1) or the retrograde branch (steps 5′-3′, Fig. 1); this will allow us to determine the order of the sequence of events in the ER lumen that lead to protein export. Isolation of export-specific mutants in *SEC61* and export-specific accessory molecules of the Sec61 channel would help us understand the molecular basis of channel opening from the lumenal side. The driving force for export could be identified by reconstitution of export from the ER in a cell-free system with purified cytosolic components.

CONCLUDING REMARKS

Only a few years ago, many investigators regarded transport of proteins from the ER lumen to the cytosol as an experimental artifact; thanks to the research summarized here, the cell biology community has revised its perception: we now know that retrograde transport from the ER to the cytosol is a highly regulated, physiological process. Given the current degree of interest, it might only be a few years before we fully understand the molecular mechanisms involved.

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