REVIEW

Setting the Standards: Quality Control in the Secretory Pathway

Lars Ellgaard, Maurizio Molinari, Ari Helenius*

A variety of quality control mechanisms operate in the endoplasmic reticulum and in downstream compartments of the secretory pathway to ensure the fidelity and regulation of protein expression during cell life and differentiation. As a rule, only proteins that pass a stringent selection process are transported to their target organelles and compartments. If proper maturation fails, the aberrant products are degraded. Quality control improves folding efficiency by retaining proteins in the special folding environment of the endoplasmic reticulum, and it prevents harmful effects that could be caused by the deployment of incompletely folded or assembled proteins.

The term quality control (QC) was originally adapted to describe the process of conformation-dependent molecular sorting of newly synthesized proteins in the endoplasmic reticulum (ER) (*1*). The ER is the site of synthesis and maturation of proteins destined for secretion, for the plasma membrane, and for the secretory and endocytic organelles. Misfolded and incompletely assembled proteins are common side products of protein synthesis in the ER, and unlike correctly folded and assembled proteins, they are retained in the ER and eventually degraded (*1, 2*). Thus, when proteins exit the ER, they are not only sorted away from resident ER proteins but also from conformational variants of themselves. The transport-competent form usually corresponds to the compactly folded native conformation that has undergone correct co- and posttranslational processing. For most oligomeric proteins, a correct quarternary structure is also required.

It is clear that QC is crucial for securing the fidelity of gene expression at the posttranslational level. It works through multiple and partly overlapping mechanisms. Some of the controls apply to all proteins (primary QC); others are specific for selected proteins and protein families (secondary QC). The main strategies include retention in the ER, ER-associated degradation (ERAD), retrieval to the ER from downstream organelles, and rerouting from the Golgi complex to lysosomes or vacuoles (Fig. 1). Selective cargo capture at the ER "exit sites" may also take place. Although the ER is the main compartment for QC, the vesiculotubular clusters (VTCs) [also called the ER Golgi intermediate compartment (ERGIC)] and the Golgi complex also play a role.

The mechanisms involved raise many intriguing questions. What are the molecular principles of the conformation-based recognition underlying QC? Are there specific folding sensors, and if so, how do they work? To what degree are deviations from the native conformations tolerated? Are the rules of QC the same in all cell types? Does a native conformer expose "signals" for forward transport or hide "signals" for retention and retrieval? How are proteins targeted for degradation? Here we focus on the basic principles of QC and describe emerging concepts.

Association with ER Chaperones

The most commonly observed primary QC mechanism involves the association of newly synthesized proteins with ER chaperones and folding enzymes such as BiP, calnexin (CNX), calreticulin (CRT), GRP94, protein disulfide isomerase (PDI), ERp57, and ERp72. These factors are not only responsible for assisting the folding and assembly process, but also serve as retention anchors for immature proteins. Binding to any one of these resident ER proteins, even if only in an on-and-off cycle, seems to be sufficient to prevent forward transport.

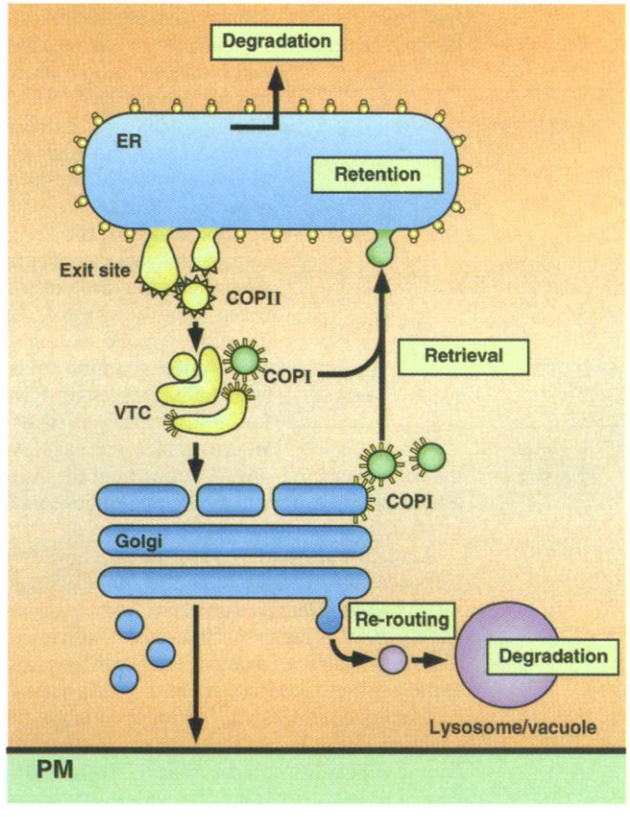

Fig. 1. Schematic overview of the organelles of the secretory pathway and their role in QC. Proteins are synthesized in the ER, where membrane-associated ribosomes are shown in yellow. In the ER, most proteins are retained until they are correctly folded and assembled. Persistently misfolded proteins are retrotranslocated to the cytosol and degraded by the proteasome. Once folded, proteins leave the rough ER and enter so-called ER "exit sites." Here, the components of the COPII coat, shown as yellow triangles, associate with the cytosolic surface of the membrane. They are responsible for budding off vesicles and tubular elements for export to the Golgi complex. Inclusion of proteins into COPII vesicles is selective and may contribute to QC. Once the COPII vesicles have detached from the ER and formed VTCs, the COPII coat is replaced by the COPI coat (a distinct coat material shown as yellow rectangles). COPI vesicles mediate the formation of return vesicles that bring some of the membrane and contents back to the ER. Because uptake into these retrograde vesicles is also selective, the VTCs and the cis-Golgi complex (where similar return vesicles form) may also serve as additional sites for QC. Finally, the trans-Golgi network, from which cargo moves to the plasma membrane (PM) and through endosomes to lysosomes (or the vacuole in fungi and plants), serves as a location for the rerouting of some misfolded proteins that are to be degraded.

Institute of Biochemistry, Swiss Federal Institute of Technology (ETH), Universitätstrasse 16, CH-8092 Zürich, Switzerland.

*To whom correspondence should be addressed. E-mail: ari.helenius@bc.biol.ethz.ch

The chaperones and folding enzymes are localized in the ER because they possess retention and retrieval signals. Most lumenal ER proteins have COOH-terminal Lys-Asp-Glu-Leu (KDEL) sequences that ensure retrieval from VTCs and the Golgi complex if they escape (*3*). Membrane proteins of type I carry a COOH-terminal KKXX (X is any amino acid) sequence for a similar purpose (*4*). Sequences that mediate ER retention also exist but are less well defined (*5*). There is evidence that the resident ER proteins form a dynamic network stabilized by weak interactions modulated by the high lumenal Ca^{2+} concentration (*6*). Such a matrix may contribute to the retention of incompletely folded proteins (*7*).

Association with ER folding factors provides a primary QC mechanism that applies to newly synthesized proteins, regardless of whether they are of endogenous, heterologous, or viral origin. Primary QC is retention based and depends on general biophysical properties shared by incompletely folded proteins; these include the presence of hydrophobic surface patches, mobile loops, and a lack of compactness (that is, any structural feature recognized by the molecular chaperones and folding enzymes present in the ER). During normal protein biogenesis, such features are only transiently exposed, resulting in transient association of newly synthesized proteins with QC factors.

In mammalian cells, the primary level of QC displays a high level of stringency because ER chaperones and folding enzymes constitute a highly redundant system in which individual members recognize their substrates differently. If one chaperone fails to interact with an incompletely folded protein, another one most likely will (*8*). Among the abundant lumenal chaperones, BiP binds to hydrophobic determinants exposed on the protein's surface (*9*), an interaction that may be promoted by co-chaperones from the DnaJ family (*10*). The GRP94 protein has peptide binding activity (*11*), whereas CNX and CRT bind, as described below, to glycoproteins that carry specific N-linked oligosaccharide tags diagnostic of incomplete folding. Whether additional polypeptide binding sites in CNX (*12*) contribute to the productive interaction with substrate glycoproteins in vivo remains to be seen. Thiol oxidoreductases of the PDI family catalyze the process of disulfide bond oxidation and isomerization through the transient formation of mixed disulfides with substrate proteins (*13, 14*). In addition, PDI and its homologs possess chaperone-like activity (*15*).

It is important to stress that transmembrane proteins containing folded domains on both sides of the ER membrane associate with lumenal as well as cytosolic folding factors (*16*). The cystic fibrosis transmembrane conductance regulator (CFTR) engages, for example, CNX through glycans on the lumenal side. On the cytosolic side, it binds chaperones such as heat shock protein 90 and heat shock cognate 70, the latter with the assistance of an ER membrane–bound DnaJ homolog, Hdj-2 (*17*). Whether association with cytosolic chaperones can cause ER retention is not yet clear. However, it is known that the QC process of certain transmembrane channels relies on the masking or unmasking of signals present in cytosolic domains, as described below.

The Role of N-linked Oligosaccharides

Retention-based, chaperone-mediated QC in the ER is particularly well characterized in the case of glycoproteins. Two homologous ER-resident lectins, CNX and CRT, bind to almost all soluble and membrane-bound glycoproteins synthesized in this compartment [see (*18*)]. They specifically associate with glycoproteins that have monoglucosylated trimming intermediates of the N-linked core glycans (*19*). Together with ERp57, a thiol oxidoreductase, with which they form complexes (*20*), they mediate retention and promote proper folding of glycoprotein substrates. Oligosaccharide trimming in the ER is thus tightly linked to glycoprotein folding, to QC, and (as described below) to degradation of misfolded glycoproteins.

Unlike other molecular chaperones, CNX and CRT cooperate with several independently acting enzymes that regulate substrate binding. The most important of these are uridine 5′-diphosphate (UDP)–glucose:glycoprotein glucosyltransferase (GT) (an association-promoting enzyme) and glucosidase II (a releasing enzyme). By adding and removing glucose residues to high-mannose N-linked oligosaccharides (Fig. 2A), these enzymes drive the cycle of substrate binding to, and release from, CNX and CRT (Fig. 2B). For many glycoproteins, this cycle is essential for efficient folding. Whereas glucosidase II is insensitive to the folding state of the glycoproteins, GT serves as the folding sensor by selectively reglucosylating those that have not acquired their native folded conformation [reviewed in (*21*)]. Only native conformers that are no longer reglucosylated by GT can exit the CNX/CRT cycle and move further along the secretory pathway.

The ability of GT to sense the difference between folded and incompletely folded glycoproteins makes it an enzyme of particular interest. It is a 170-kD soluble glycoprotein present in the ER lumen and possibly in ER exit sites (*22*). It is fully functional in vitro without any other enzymes or chaperones. Work by Parodi and co-workers has shown that it recognizes both the oligosaccharide and the protein moiety of a misfolded glycoprotein (*23*). Thus, it does not interact with oligosaccharides or small glycopeptides, nor with unfolded proteins that do not have N-linked glycans. However, it does recognize misfolded glycoproteins containing only the innermost *N*-acetylglucosamine (GlcNAc) residue obtained from glycoproteins containing high-mannose N-linked oligosaccharides by digestion with endoglycosidase H (*24*). Because the first GlcNAc in an N-linked oligosaccharide is usually immobile in folded proteins because of interactions with the polypeptide chain (*25*), it may be the dynamic properties of this saccharide or its influence on the mobility of the polypeptide chain that serves as an indicator of local misfolding. Alternatively, GT recognizes exposed hydrophobic features on misfolded proteins in a way similar to many classical chaperones (*24*). Recent experiments indicate that, in multidomain proteins, GT reglucosylates only glycans present in misfolded domains and that it recognizes partially folded conformers more efficiently than random coil conformers of a model glycoprotein, ribonuclease B (*26*).

Thiol-Mediated Retention

A special mechanism of ER retention involves exposed free cysteines. This was first described for the retention of unassembled immunoglobulin (Ig) chains (*27*). In native Ig oligomers, individual Ig chains have cysteine residues that participate in interchain disulfide bonds. While still unassembled, some of these chains form intermolecular disulfide-bonded complexes with ER-resident thiol oxidoreductases such as PDI and ERp72, resulting in efficient ER retention (*28*). Thiol-mediated retention has also been shown for unassembled subunits of acetylcholinesterase (*29*). Mutation of the cysteine residue or addition of a low concentration of 2-mercaptoethanol to the cells results in secretion of unassembled polypeptides.

Retention by Aggregation

Newly synthesized proteins may also be retained in the ER, because of interactions with each other. It is well known from in vitro folding studies that incompletely folded proteins are prone to aggregation. In the ER lumen and membrane, this can lead to formation of large aggregates [see (*1*)]. Owing to their large size and the presence of trapped chaperones such as BiP and CNX, aggregates cannot diffuse freely within the ER and are transport incompetent. Many of the aggregates are cross-linked by nonnative interchain disulfide bonds. Although usually a dead-end pathway, aggregation is in certain cases part of the folding process. Thyroglobulin, major histocompatibility complex (MHC) class II, and procollagen, for example, form transient aggregates before acquiring their native structure (*30*).

Structural Considerations

It makes sense that primary QC in the ER works by structural rather than by functional

criteria, because many newly synthesized proteins leave the ER as inactive precursors. However, the purely "architectural" nature of selection can lead to problems. This occurs when potentially functional mutant proteins are retained because of minor structural defects. For instance, some patients with α_1-antitrypsin deficiency produce mutant molecules that, although functionally intact, are retained in the ER and degraded (*31*). In fact, a large number of diseases are known in which QC in the ER plays a role. Typically, they involve expression of mutant proteins that are targeted to the ER but fail to reach their intended cellular location, often displaying an ER storage phenotype with aggregated material accumulating in the ER (*32*). The CFTR ΔF508 mutant, which causes cystic fibrosis, is a well-studied example (*33*).

It is apparent that even minor folding defects can result in ER retention. However, the precise structural determinants leading to detection by the QC system are poorly understood. The secretion efficiency for bovine trypsin inhibitor mutants expressed in *Saccharomyces cerevisiae* was recently found to correlate with in vitro stability of the protein (*34*). It did not correlate with folding and unfolding rates measured in vitro. Thus, it seems that ongoing structural fluctuations rather than the time-averaged structure observed by x-ray crystallography or nuclear magnetic resonance determines how this protein interacts with the QC system. In contrast, a destabilized mutant of β-lactoglobulin was found to display accelerated secretion in comparison with the wild-type protein (*35*), suggesting that the mutant might fold fast enough to evade detection by ER chaperones. Although still limited, these studies seem to emphasize the dynamic properties of incompletely folded structures as an essential factor in primary QC recognition.

Expression of recombinant proteins has provided extensive information regarding which types of mutations are likely to cause retention. Obviously, mutations that have severe effects on folding or on posttranslational modifications are at the top of the list. It is, for example, commonly observed that truncated proteins and chimeras are only transport competent if the sites of truncation or ligation coincide with interphases between domains (*8*). Cysteine mutations are frequently damaging, reflecting the central role that intra- and interchain disulfides play in protein folding in the ER and in stabilizing folded structures (*36*). Mutations that affect signal peptide cleavage, glycosyl-phosphatidylinositol anchor addition, glycosylation, ligand binding, and metal ion association (Ca^{2+}, Cu^{2+}, and so forth) also often lead to retention.

Protein-Specific Quality Control

Once folded, cargo molecules are no longer retained by elements of the primary QC machinery. They can now enter the exit sites and leave the ER for the Golgi complex. The exact process by which export of proteins from the ER occurs is not clear. There are two main models: bulk flow and cargo capture (*37*). The former implies departure by default with fluid and membrane as vesicles leave the ER; the latter invokes selective receptor-mediated loading of cargo into the transport vesicles. An in-depth coverage of this subject lies outside the scope of this review [instead, see (*3, 38*)]. Although helpful as guidelines, neither model alone is likely to reflect realities in the cell. Instead, the situation may resemble that observed during endocytosis in which bulk transport of fluid and membrane coexists with receptor-mediated import of specific ligands (*39*).

With respect to QC, it is useful to consider the full spectrum of secondary mechanisms that modulate export of specific proteins from the ER. We have listed a set of protein-specific factors that are responsible for effecting maturation, folding, and assembly of proteins in the ER and for accelerating or inhibiting their forward transport (Table 1).

Although the factors listed are quite heterogenous and often poorly characterized, certain categories can be discerned. One group serves as chaperones and assembly factors. Their deletion most often results in the accumulation of specific substrate molecules in the ER, without effects on secretion of other proteins. One such factor is the Vma12p–Vma22p complex, which transient-

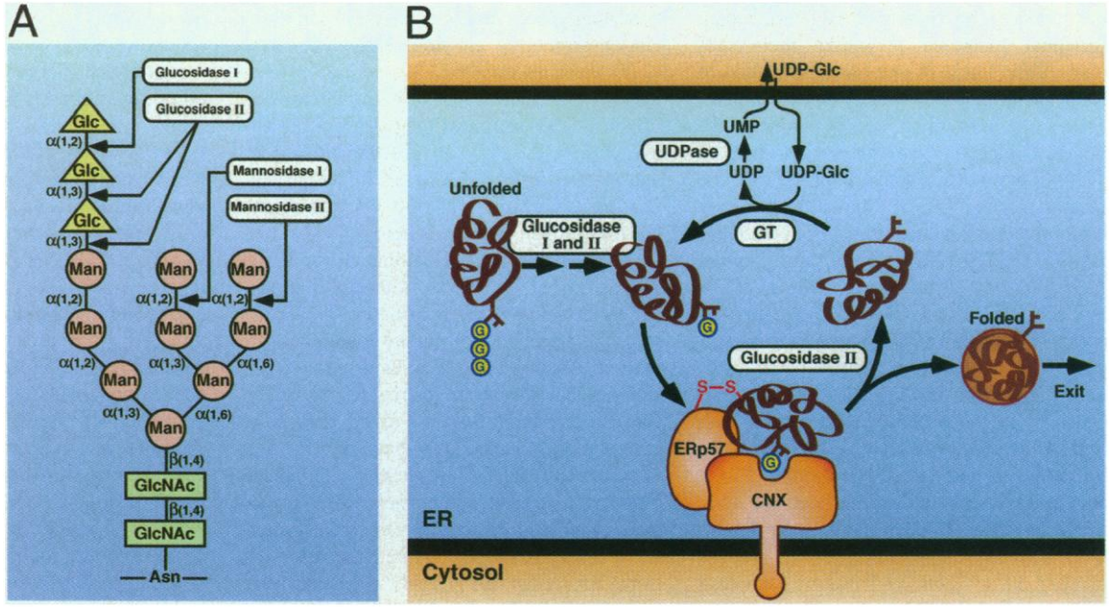

Fig. 2. The calnexin cycle. (**A**) The N-linked core oligosaccharide added to the polypeptide chains contains 14 saccharide units in a branched configuration. Enzymes involved in the trimming of the oligosaccharide in the ER are shown in white boxes. (**B**) A model for the role of CNX in the folding and QC of a newly synthesized protein (brown ribbon) containing an N-linked oligosaccharide. Two of the three glucose residues (G) are rapidly trimmed by glucosidases I and II. The monoglucosylated protein, thus generated, binds to CNX, CRT, or both (here only CNX is shown for simplicity). It is thereby exposed to ERp57, an associated thiol oxidoreductase, with which it forms transient mixed disulfides (S–S, shown in red). The remaining glucose residue is trimmed by glucosidase II, and the complex dissociates. If the protein is correctly folded, it can exit the ER. However, if it is not correctly folded, it is recognized by GT and reglucosylated, thereby allowing it to reassociate with CNX (and CRT). The cycle is repeated until the protein is either folded or degraded. Binding to CNX and CRT prevents exit from the ER of immature glycoproteins, promotes correct folding, inhibits aggregation, blocks premature oligomerization, and regulates ERAD (see Fig. 3). The UDP-glucose needed for reglucosylation is imported from the cytosol in exchange for uridine 5′-phosphate (UMP). This species is generated from the product of the reaction, UDP, by a special uridine diphosphatase (UDPase) enzyme (*69*).

ly interacts with the Vph1p subunit of the *S. cerevisiae* vacuolar adenosine triphosphatase in the ER to assist correct complex assembly (*40*).

Others work as escort proteins. A well-studied example is the receptor-associated protein (RAP), which interacts with proteins of the low density lipoprotein (LDL) receptor family and other transmembrane receptors. The protein prevents aggregation and premature ligand binding by its target molecules by escorting them out of the ER to the Golgi (*41*). Here, the complex dissociates, presumably because of the lower pH of this compartment, and RAP is retrieved to the ER by the KDEL receptor.

Potential cargo receptors are also listed in Table 1. It is clear that cells have mechanisms for accelerated transport of selected proteins from the ER and for cargo concentration before Golgi arrival (*42*). Thus, it is possible that proteins such as ERGIC-53, a mannose lectin, or the family of p24 proteins serve a cargo receptor function (*43, 44*). Finally, egasyn and carboxylesterase represent retention molecules, which limit the export of specific proteins from the ER (*45, 46*).

The unifying theme is that these proteins, unlike those working at the primary level of QC, exert their effect on selected protein species or protein families. Accordingly, their expression is often limited to the cell types that produce the respective substrates. Like chaperones, they serve important QC functions by securing the fidelity of maturation and deployment of specific proteins, without themselves being part of the final functional protein. The number of such specialized factors identified is rapidly increasing, emphasizing the complexity of QC processes in the ER.

Masking and Unmasking of Signals

In the case of membrane proteins, secondary QC sometimes involves masking and unmasking of signals. These are short peptide sequences in cytosolic and transmembrane domains that determine retention, retrieval, or degradation.

The first case described was that of charged residues in the transmembrane domain of T cell receptor α chain, which were found to serve as a signal for selective ER degradation of the unassembled subunit (*47*). For cell surface IgM, two hydrophilic residues in the membrane-spanning region of the μ heavy chain have been shown to confer ER retention in the absence of light chain (*48*). In the case of the human high-affinity receptor for IgE, a somewhat different masking phenomenon has been observed. Premature export of the unassembled subunits to the plasma membrane is prevented by an exposed ER retrieval signal [a coat protein complex I (COPI)–interacting KKXX motif] in the cy-

Table 1. Overview of protein-specific QC factors and their target molecules and functions.

Factor	Organism or cell type	Target molecules	Function
p24 family	*Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and mammalian cells	Various secreted proteins, for example, invertase and Gas1p in *S. cerevisiae*	Potential cargo receptors for a subset of secretory and membrane proteins (*71*)
ERGIC-53	Mammalian cells	Glycoproteins, for example, cathepsin C and blood clotting factors V and VIII	Potential cargo receptor for a subset of secreted glycoproteins (*43*)
LST1	*S. cerevisiae*	Plasma membrane H^+-ATPase Pma1p	Potential cargo receptor (*72*)
Erv14p	*S. cerevisiae*	Plasma membrane protein Axl2p	Potential cargo receptor (*73*)
Chs7p	*S. cerevisiae*	Chs3p catalytic subunit of chitin synthetase III	Ensures ER secretion competence by unknown mechanism (*74*)
Vma12p–Vma22p complex	*S. cerevisiae*	Vacuolar H^+-ATPase subunit Vph1p	Promotes H^+-ATPase complex assembly in the ER (*40*)
Gsf2p	*S. cerevisiae*	Hexose transporters Hxt1 and Gal2p	Ensures ER secretion competence by unknown mechanism (*75*)
Lag1p and Dgt1p	*S. cerevisiae*	GPI-anchored proteins Gas1p and Yap3p	Facilitate ER secretion of GPI-anchored proteins (*76*)
Shr3p	*S. cerevisiae*	Amino acid permeases, for example, Hip1p and Gap1	Ensures ER secretion competence by unknown mechanism (*77*)
ODR-4 and ODR-8	*C. elegans* olfactory neurons	Odorant receptors ODR-10 and STR-2	Ensures ER secretion competence by unknown mechanism (*78*)
Prolyl 4-hydroxylase	Mammalian cells	Procollagen	Recognizes and retains partially folded procollagen; assists its folding (*79*)
HSP47	Mammalian collagen-producing cells	Procollagen	Specialized procollagen chaperone (*80*)
BAP31	Mammalian cells	Cellubrevin	Ensures ER secretion competence possibly through a sorting function (*81*)
RAP	Mammalian cells	LDL receptor family and other transmembrane receptors	Prevents aggregation and premature ligand binding by acting as an escort (*41*)
Invariant chain	Antigen-presenting cells	MHC class II	Prevents aggregation and premature ligand binding by acting as an escort; directs endosomal targeting (*82*)
Tapasin	Mammalian cells	MHC class I	Prevents ER exit of MHC class I without bound antigenic peptide (*83*)
NinaA	*Drosophila* photoreceptor cells	Rh1 and Rh2 rhodopsins	Ensures ER secretion competence through direct interaction; possibly chaperone, escort, or both (*84*)
Microsomal triglyceride transfer protein	Primarily liver cells and intestinal cells	Apolipoprotein B (apoB)	Assists translocation, assembly, and secretion of apoB-containing lipoproteins (*85*)
Protective protein/ cathepsin A	Mammalian cells	Neuraminidase and β-galactosidase	Ensures ER secretion competence through direct interaction and directs lysosomal targeting (*86*)
β-catenin	Epithelial cells	E-cadherin	Targets E-cadherin to the basal-lateral membrane through direct interaction with cytosolic tail (*87*)
Egasyn	Mammalian cells	β-glucuronidase	Mediates ER retention of target molecule through KDEL-like ER retention signal (*45*)
Carboxylesterase	Hepatocytes	C-reactive protein	Mediates ER retention of target molecule through KDEL-like ER retention signal (*46*)

toplasmic COOH-terminal domain of the α subunit. Export occurs when the α subunit oligomerizes with the γ subunit and the KKXX signal is masked (*49*).

Quality control of adenosine 5′-triphosphate (ATP)–sensitive K^+ channels in *Drosophila* is also believed to involve masking (*50*). These are hetero-oligomeric transmembrane proteins that fold and assemble in the ER. Each subunit contains an Arg-Lys-Arg (RKR) sequence in the cytosolic domain. Improperly regulated and incompletely assembled channels were transported to the cell surface when these sequences were mutated (*51*). The RKR sequences probably serve as ER retention or retrieval signals that must be masked before the protein can be transported.

Related sequences (RXR) are also present in cytosolic domains of CFTR (*52*). Mutational analysis showed that these sequences block transport of the misfolded CFTR ΔF508 mutant to the cell surface. Because this misfolded protein is known to concentrate in the VTCs (*53*), it would not be surprising if the RXR sequences represent a retrieval signal that interacts with COPI components like the COOH-terminal KKXX sequences do. Given the importance of properly regulated ion channels, these two examples illustrate how dangerous life might be for a cell without stringent QC.

That selective retrieval from the VTCs and the cis-Golgi can indeed serve a QC function was demonstrated with a temperature-sensitive mutant of the vesicular stomatitis virus G (VSV-G). When overexpressed in Chinese hamster ovary cells, a large fraction of this misfolded viral glycoprotein started to cycle between the ER, the VTCs, and the cis-Golgi (*53*). A piggyback mechanism of retrieval in which the VSV-G protein was retrieved to the ER bound to BiP, which in turn associated with the KDEL receptor, was suggested.

Rerouting from the Golgi Complex

Some misfolded and incompletely assembled proteins that have escaped ER retention are diverted from the Golgi complex to the vacuole in yeast for degradation. Rerouting does not seem to be a major QC pathway in mammalian cells, but constitutes an important backup system in *S. cerevisiae,* where the QC machinery in the ER seems to be less stringent. Although the yeast ER clearly does play an important role in QC, it contains a rather stripped-down version of the lumenal machinery, in comparison with mammalian cells. It lacks, for example, homologs of GRP94 and CRT, and there is no evidence for a functional CNX cycle (*18*).

In *S. cerevisiae,* a transmembrane protein, Vps10p, that cycles between the late Golgi and the endosome has recently been shown to be required for the rerouting of certain unfolded proteins (*54*). It is possible that Vps10p serves as a general folding sensor and capture receptor for transport from the Golgi complex to the vacuole. Vps10p not only ferries misfolded proteins but also native vacuolar proteins such as carboxypeptidase Y (CPY) and proteinase A to the vacuole (*55*).

ER-Associated Degradation

Prolonged retention of misfolded and incompletely folded proteins in the ER leads to their degradation (*2*). ER-associated degradation is mainly carried out by the 26*S* proteasome located in the cytosol [for reviews, see (*56, 57*)]. The process occurs in several steps: terminally misfolded or unassembled proteins are recognized by ER chaperones such as calnexin, BiP, or by other factors such as specific mannose lectins. They are then retrotranslocated through the Sec61 channel into the cytosol (*58, 59*), deglycosylated (in the case of glycoproteins), and polyubiquitinated before proteasomal degradation. How proteins that are to be degraded are identified and targeted for retrotranslocation has not been established, but it is likely that the machinery responsible for protein folding again plays a role in the selection and preparation of aberrant products for disposal.

Because mannosidase inhibitors block degradation of misfolded glycoproteins, it has been suggested that the removal of mannose units by slow-acting ER-resident α-1,2-mannosidases might work as the timer for glycoprotein degradation (*60, 61*). More specifically, a $Man_8GlcNAc_2$ glycan with a mannose (Man) missing in the middle branch, generated by mannosidase I, was recently identified as a necessary signal for the degradation of misfolded CPY in yeast (*62*). Although yeast and mammalian cells are not fully comparable with respect to the role of glucose trimming, the effects of mannose trimming on degradation seem strikingly similar.

Upon proteasome inhibition in mammalian cells, a misfolded variant of α_1-antitrypsin with its glycans in the $Glc_1Man_8GlcNAc_2$ form (Glc, glucose) is retained in the ER and stably associates with CNX (*61*). The model that emerges from inhibition studies helps to rationalize how glucose and mannose trimming, CNX binding, and proteasome-mediated degradation may function together and how selective, timer-controlled targeting to the proteasome might occur (Fig. 3). It implies that the central role of GT as a folding sensor is important not only in the CNX/CRT cycle but also for targeting of misfolded proteins for degradation. Moreover, it suggests a

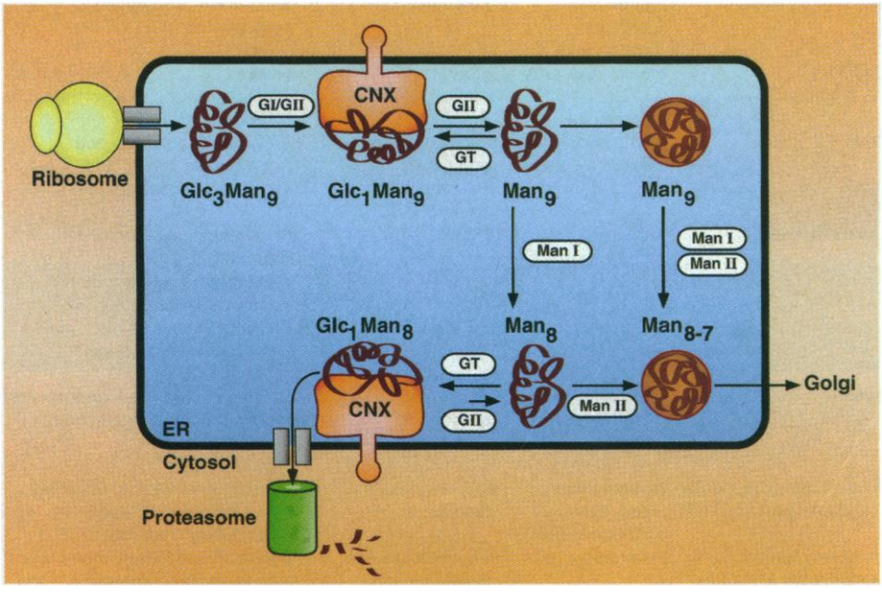

Fig. 3. ER-associated degradation of glycoproteins. This model shows the potential involvement of glucosidases I and II (GI and GII, respectively), GT, and ER mannosidases I and II (Man I and II, respectively) in ERAD of glycoproteins. The newly synthesized glycoprotein (folded or unfolded) and resident enzymes involved are depicted as in Fig. 2B. For simplicity, oligosaccharide structures are labeled. The fully folded glycoprotein containing trimmed mannoses is free to leave the ER because it has no glucose residue that could mediate its binding to CNX. However, if persistently unfolded, the glycoprotein gets marked for degradation by the action of mannosidase I, which generates the $Man_8GlcNAc_2$ form of the oligosaccharide (see Fig. 2A). After reglucosylation by GT, the protein rebinds CNX. Because the $Glc_1Man_8GlcNAc_2$ form is a poor substrate for GII, as compared to the $Glc_1Man_9GlcNAc_2$ form (*70*), the glycoprotein is no longer rapidly released from CNX. The misfolded glycoprotein is retrotranslocated and degraded by the proteasome. This figure was based on the model recently proposed by Liu *et al.* (*61*).

role for CNX in the targeting of some glycoprotein substrates for retrotranslocation.

It is likely that additional factors that mediate the interaction between the misfolded protein and the translocon may emerge as this intriguing pathway is further analyzed. For instance, BiP seems to be directly or indirectly involved in the process (*59, 63*). Furthermore, a number of genes have been identified in yeast associated with putative targeting, retrotranslocation, and degradation functions (*57*). Analysis of their functions may provide further insights.

General Considerations

Protein folding, oligomerization, and QC involve a series of interactions starting at the level of the growing nascent chain and ending after complete maturation. MHC class I molecules provide a particularly illustrative example of the complexity of such processes [reviewed in (*64*)]. The heavy chain undergoes stepwise binding to the general folding factors CNX, CRT, and ERp57. Two specific components are also involved: TAP (transporter associated with antigen processing) as a peptide translocator in the ER membrane and tapasin as a peptide editor. Together, these factors bind to the newly synthesized protein and ensure that it is correctly folded and assembled and that it does not leave the ER without a correct peptide in its peptide-binding pocket. In this case, the general (primary) and the specific (secondary) components of the QC system work intimately together in multimolecular complexes in the ER membrane.

Perhaps the most important insight that such an example offers is how dependent folding and assembly of a protein can be on the "welcoming committee" of chaperones, enzymes, and other factors that await it in the ER. It appears that some newly synthesized polypeptide chains associate with these factors throughout their maturation and may in fact never be entirely free in solution until fully folded. Yet, there is little doubt that the native folded structure is determined by the amino acid sequence as originally demonstrated by Anfinsen [for review, see (*65*)]. In addition, the primary sequence, together with covalent modifications encoded by specific sequence features, determines the choice of chaperones with which the protein interacts. For example, the presence of glycosylation consensus sequences close to the NH_2-terminus of a protein determines that it will interact cotranslationally with CNX and CRT instead of binding to BiP (*66*). This, in turn, defines ERp57 rather than PDI as the main thiol oxidoreductase that it will associate with.

By adjusting their primary sequence to the prevailing complement of folding factors, individual proteins have developed a variety of ways to make use of folding and QC mechanisms and thus fine-tune the structural requirements for their expression. It is even conceivable that, to be transport competent with partially unfolded domains, some proteins have evolved ways to make themselves, or parts of themselves, invisible to the QC machinery.

On the other hand, cells have evolved to optimally support proper maturation of the particular selection of proteins that they produce. That the secretory pathway is, in fact, "differentiated" in specific cell types is revealed by the slow and inefficient folding observed for many heterologously expressed proteins. It is by no means self-evident that a protein that folds properly in one cell type will do so in another. Incompatibility is most often observed between cells of distantly related species such as yeast and mammals, but it also occurs between mammalian cell types. One obvious reason can be the lack of essential secondary factors. Differences in the relative amounts of the ubiquitous folding factors and in the physiological conditions in the ER (such as Ca^{2+} and ATP concentrations and redox environment) may also contribute.

In view of these cellular differences, it is not surprising that QC plays a role in developmental processes. During B and T cell development, for example, some of the differentiation steps involve QC processes in the ER. A precursor B cell (pre-B cell) can only establish a viable lineage if prereceptor subunits are able to assemble into transport competent oligomers [reviewed in (*67*)]. Furthermore, membrane-bound heavy chains in pre-B cells are selectively retained in the ER when a switch to the gamma isotype occurs (*68*).

Perspectives

The challenge for the future is to define further the existing primary and secondary QC systems. It will be crucial to determine the structural and dynamic properties that result in retention of a protein. The structure of the ER lumen with respect to lateral mobility and protein networks requires more attention. It will also be essential to determine whether there are true cargo receptors in VTCs and, if so, whether they display conformation-dependent affinity for their cargo. The mechanisms for cargo concentration and retrieval must be defined, as well as the events that lead to rerouting of cargo proteins from the Golgi to the lysosome or vacuole.

For individual proteins and protein families, the essential secondary QC factors need to be defined and analyzed. The role of N-linked glycans in their different trimmed forms as indicators of folding and time spent in the ER must be further elucidated, with a focus on identifying additional mannose binding lectins involved in ERAD. It will also be important to pursue the analysis of alternative, proteasome-independent mechanisms of ERAD. The capacity to modulate folding and QC in the cell may prove essential in the treatment of diseases with an ER storage etiology such as cystic fibrosis and α_1-antitrypsin deficiency or in suppressing the generation of protein aggregates such as those resulting in neurodegenerative diseases. It may also lead to new ways of interfering with the expression of harmful proteins produced by viruses and other pathogens, and it may allow better control of heterologous protein expression. The cellular and physiological context of protein folding and assembly, in all its complexity, cannot be ignored.

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REVIEW

Posttranslational Quality Control: Folding, Refolding, and Degrading Proteins

Sue Wickner,[1] Michael R. Maurizi,[2] Susan Gottesman[1]*

Polypeptides emerging from the ribosome must fold into stable three-dimensional structures and maintain that structure throughout their functional lifetimes. Maintaining quality control over protein structure and function depends on molecular chaperones and proteases, both of which can recognize hydrophobic regions exposed on unfolded polypeptides. Molecular chaperones promote proper protein folding and prevent aggregation, and energy-dependent proteases eliminate irreversibly damaged proteins. The kinetics of partitioning between chaperones and proteases determines whether a protein will be destroyed before it folds properly. When both quality control options fail, damaged proteins accumulate as aggregates, a process associated with amyloid diseases.

The appearance and maintenance of functional proteins within cells depends on more than the fidelity of transcription and translation. The initial folding of proteins and assembly of multiprotein complexes can be helped and sometimes requires the participation of molecular chaperones—proteins that catalyze protein folding. By binding exposed hydrophobic patches on proteins, chaperones prevent proteins from aggregating into insoluble, nonfunctional inclusions and help them reach their stable native state. After initial folding and assembly, proteins may suffer damage in response to various stresses or insults. For such damaged proteins, as for proteins misfolded because of mutations in the gene encoding the protein, lack of fidelity in transcription, or translational errors, a number of fates are possible: rescue by chaperones, destruction by energy-dependent cytoplasmic proteases, or aggregation. The efficiency and cost of protein quality control depends on the balance among these processes.

There is significant overlap in the functional and physical features of the prokaryotic and eukaryotic chaperone and proteolytic machinery. Similar principles govern the mechanisms of substrate selection and unfolding by molecular chaperones and the adenosine triphosphatase (ATPase) components of proteases. In fact, the ATPase components of proteases can function as molecular chaperones [reviewed in (*1*)]. We propose a unified model for partitioning of nonnative proteins between chaperones (for remodeling) and proteases (for degradation), which we refer to as protein triage (*2*). In discussing quality control, we do not address the numerous biologically important regulatory functions of chaperones and proteases.

When Is Quality Control Necessary?

Most native cellular proteins probably do not interact with chaperones and are resistant to

[1]Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD 20892–4255, USA. [2]Laboratory of Cell Biology, National Cancer Institute, Bethesda, MD 20892–4255, USA.

*To whom correspondence should be addressed: E-mail: susang@helix.nih.gov

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