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The Endoplasmic Reticulum As the Extracellular Space Inside the Cell: Role in Protein Folding and Glycosylation

Miklós Csala, Éva Kereszturi, József Mandl, and Gábor Bánhegyi

Abstract

Significance: Proteins destined to secretion and exposure on the cell surface are synthesized and processed in the extracellular-like environment of the endoplasmic reticulum (ER) of higher eukaryotic cells. Compartmentation plays a crucial role in the post-translational modifications, such as oxidative folding and N-glycosylation in the ER lumen. Transport of the required intermediates across the ER membrane and maintenance of the luminal redox conditions and Ca^{2+} ion concentration are indispensable for appropriate protein maturation. ***Recent Advances:*** Cooperation of enzymes and transporters to maintain a thiol-oxidizing milieu in the ER lumen has been recently elucidated. Ca^{2+}-dependence of certain ER chaperones is a subject of intensive research. ***Critical Issues:*** Mounting evidence supports the existence of a real barrier between the ER lumen and the cytosol. The unique set of enzymes, selection of metabolites, and characteristic ion and redox milieu of the luminal compartment strongly argue against the general permeability of the ER membrane. ***Future Directions:*** Alterations in the luminal environment can trigger the unfolded protein response, a common event in a variety of pathological conditions. Therefore, redox and calcium homeostasis and protein glycosylation in the ER provide novel drug-targets for medical treatment in a wide array of diseases. *Antioxid. Redox Signal.* 16, 1100–1108.

Introduction

Several intracellular proteins are translated without interruption by free cytosolic ribosomes. These polypeptides remain in the cytosol or, the ones containing appropriate targeting sequences, are sorted to other cellular compartments. Their targeting involves post-translational transport through the nuclear pores or across the membranes of mitochondria, peroxisomes, or the endoplasmic reticulum (ER) to their final destinations (9). However, the ER is not only a possible target of protein sorting but also an organelle responsible for the synthesis, processing, and further targeting of proteins, which are finally secreted from the cell or are situated in the ER, Golgi apparatus, plasma membrane, or lysosomes. These proteins are usually translated by ribosomes attached to the surface of (rough) ER and are co-translationally transported into the ER lumen and ER membrane.

The luminal compartments of the endomembrane system can be regarded as extracellular space within the cell. Once transported into the ER lumen, secretory and plasma membrane proteins do not need to cross any further barrier to leave the cell or get exposed on the cell surface. In other words, the ER membrane is the only barrier that a protein needs to cross in order to reach the extracellular space. Since the ER lumen is a separate metabolic compartment in the eukaryotic cells, the processing, maturation, and quality control of these proteins occur in a special environment (17). Certain features of the ER lumen, most importantly the calcium concentration and thiol redox state, resemble the extracellular conditions (17). The proteins synthesized and processed in the ER, therefore, achieve their native conformation in an extracellular-like milieu, and get prepared to secretion or exposure on the cell surface (Fig. 1).

Besides remarkable differences between the luminal and cytosolic calcium ion concentrations and redox conditions, the ER is equipped with distinct chaperones and protein processing enzyme systems catalyzing characteristic post-translational modifications in the luminal compartment. The cysteinyl thiol groups are mostly oxidized to disulfides, and oligosaccharide moieties are linked to specific suitable aminoacyl side chains in most proteins processed in the ER (10). These post-translational modifications are also tightly

Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest, Hungary.

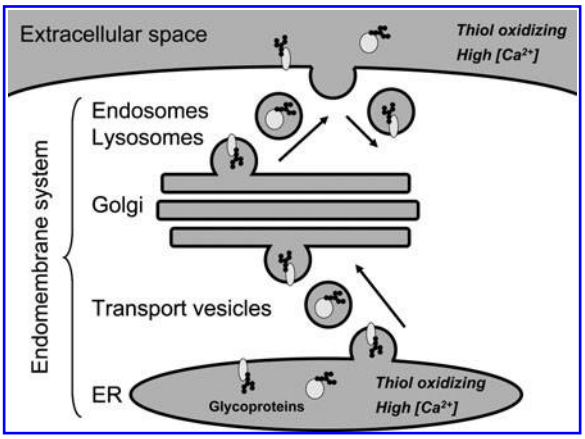

FIG. 1. Compartments of the endomembrane system. The compartments of the secretory pathway and endosomes are equivalent to the extracellular space. The calcium concentration and thiol redox state of the ER resemble the extracellular conditions to prepare proteins to secretion or exposure on the cell surface. ER, endoplasmic reticulum.

connected to the special features of the luminal environment. This review focuses on the specialties of the ER lumen, which are relevant from the aspect of protein folding.

The ER Compartment

The ER is a subcellular organelle in eukaryotic cells, which is composed of a membrane network forming tubules and cisternae and of its branching yet continuous lumen. Its variable volume is largely dependent on the type and state of the cells. In active protein secreting cells, it often comprises more than 10% of the total cell volume. Protein synthesis and processing are not the only, albeit major, functions of the ER. Enzymes of carbohydrate and lipid metabolism and biotransformation are also situated in the lumen (17). Intensive bidirectional traffic of metabolic intermediates across the ER membrane is, therefore, indispensable.

Mounting experimental and clinical evidence supports convincingly that the ER membrane is a real barrier allowing passage by selective transport. A wide array of molecules is known to be unable to cross this membrane at a significant rate, and this impermeability is an important determinant of the ER metabolism (17, 19). Genetic analysis of two ER-related human syndromes, namely glycogen storage disease type 1 and cortisone reductase deficiency, provided strong evidence for the separation of cytosolic and endoplasmic compartments. The former revealed that the lack of a specific transporter makes glucose 6-phosphate unable to enter the ER lumen (56). The latter proved that local NADPH generation is indispensable for cortisone reduction in the ER lumen, because the luminal NADPH pool is separated from the cytosolic one (23).

Nonselective passive diffusion across the ER membrane would wash away the concentration gradients (*i.e.*, all the characteristic differences between the cytosolic and ER luminal micro-environments). Compared to the cytosol, the ER lumen has about 10,000 times higher level of free calcium ion (44) and orders of magnitude lower ratio of glutathione (GSH) and glutathione disulfide (GSSG) (26, 31). These gradients are maintained or created by active calcium pumping (44) and luminal oxidation of thiols (54), respectively. However, they are also dependent on the poor permeability of the ER membrane to calcium ion and glutathione, because the active processes must surpass the passive trans-membrane fluxes.

It can be concluded that the ER lumen is a real separate metabolic compartment (17). Its characteristic redox and calcium homeostasis is tightly and mutually connected to the local protein maturation.

Folding-Related Redox Conditions in the ER Compartment

Generation of disulfide bonds in nascent proteins is one of the major post-translational modifications in the ER. Two electrons of the linking cysteinyl thiol groups are taken by protein disulfide isomerase (PDI), and are passed on to molecular oxygen by ER oxidase 1 (Ero1), which produces hydrogen peroxide (54). This process, also referred to as oxidative folding, is a major determinant of the redox conditions in the ER; which is, therefore, considered as an oxidizing compartment (Fig. 2).

A comprehensive review on the role of electron carriers in the main ER-related functions and in the cellular pathophysiology has been published recently (20). Among the major water-soluble redox competent molecules, glutathione (GSH), its oxidized form, glutathione disulfide (GSSG), ascorbate and its oxidized derivative, dehydroascorbic acid (DAA) are relevant to protein folding. Due to the lack of local synthesis, their concentration and redox state are determined by transmembrane transport and further metabolism in the ER lumen.

GSSG has been shown to oxidize PDI *in vitro* (38) and in Ero1-deficient cells (1). The hypothesis that protein disulfide formation is driven by GSSG uptake in the ER lumen was supported by findings indicative of selective microsomal transport of GSSG (31). However, this theory was challenged

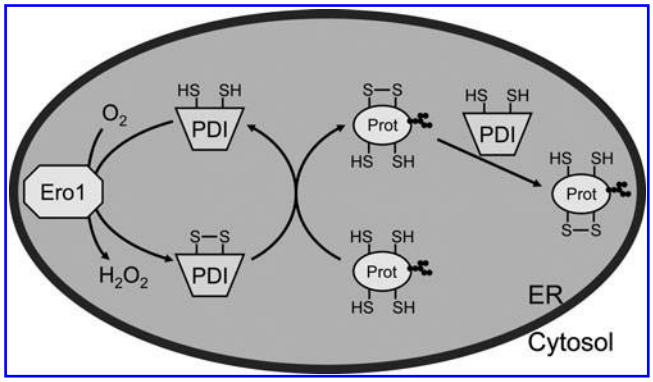

FIG. 2. The main electron transfer chain of oxidative folding. The ratio of disulfides and free protein thiols is characteristically high in the ER lumen. The Ero1 flavoenzyme plays a central role in the generation and maintenance of this thiol-oxidizing environment. It transfers the electrons from active cysteinyl thiols of PDI to oxygen molecules and produces hydrogen peroxide. Oxidized PDI forms disulfide bridges in nascent proteins by disulfide exchange. Reduced PDI, in turn, is able to catalyze the rearrangement of protein disulfides. ER, endoplasmic reticulum; Ero1, ER oxidase 1; PDI, protein disulfide isomerase.

by the observations that the ER membrane is practically impermeable to GSSG while GSH can pass this barrier by a protein-mediated bidirectional passive transport (5). Elimination of cytosolic GSH enhances oxidative folding and abolishes disulfide isomerization due to hyperoxidation of PDI; normal disulfide formation can be restored by the addition of GSH (46). In line with these observations, a correlation between Ero1 activity and GSH oxidation has been found in yeast (16), although Ero1 can hardly oxidize GSH *in vitro* (54). This indicates that GSH is oxidized indirectly by Ero1 through PDI and hydrogen peroxide. In accordance with the proposed mechanism (16), GSH and protein thiols compete for the thiol oxidizing power in the ER. These findings support that GSH entering the ER lumen participates in the disulfide rearranging function of PDI by reducing the active center. From another point of view, the Ero1–PDI electron transfer chain oxidizes GSH to GSSG, as well as protein thiols to disulfide bridges in the ER. GSSG is entrapped in the luminal compartment due to the lack of efficient transport (Fig. 3). GSH or GSSG can also react directly with protein disulfides or thiols even in the absence of oxidoreductases (35). Consequently, a remarkable amount of glutathione-protein mixed disulfide is formed in the ER lumen, which may be an important component of this redox buffer system as a GSH reserve (7). However, the contribution of these nonenzymatic reactions to the overall activity of oxidative protein folding is negligible. Compartmentation of GSSG–GSH couple and maintenance of its characteristic redox state in the ER lumen is a prerequisite for appropriate activity of PDI and normal oxidative protein folding.

The ascorbate–DAA redox couple can also contribute to the oxidative protein folding in the ER. DAA can take electrons from thiol groups of PDI and other proteins (50) or even GSH. In addition, DAA can pass the ER membrane by protein-dependent (possibly a GLUT family transporter mediated) passive diffusion, while the membrane is impermeable to ascorbate (6). Addition of DAA enhances protein thiol oxidation in rat liver microsomes. In fact, the antioxidant ascorbate can also drive oxidative folding in microsomes *in vitro* (18) due to an ascorbate oxidase activity, which produces DAA at the outer surface of the membrane (53). According to the proposed mechanism of an alternative electron transfer chain involved in oxidative protein folding (4), ascorbate is converted to DAA, which enters the ER lumen and oxidizes protein (and GSH) thiol groups. Luminal ascorbate is prone to reoxidation, and hence a cyclic interaction of ascorbate and DAA with ROS and thiols, respectively, can mediate ROS-induced oxidative folding (Fig. 4). Ascorbate entrapped in the lumen can leave the cell through the secretory pathway, and contribute to the maintenance of vitamin C level in blood. Although direct measurements on ascorbate and DAA concentrations in the ER have not been reported, it has been demonstrated that other compartments of the secretory pathway contain ascorbate at high concentrations (22, 58).

There are several advantages of ascorbate accumulation in the ER. Despite its prooxidant role in disulfide formation, ascorbate is still a major scavenger of ROS and other reactive species, which also interacts with the major lipid-soluble antioxidant, tocopherol in the ER (21). Therefore, ascorbate can significantly strengthen the antioxidant defense capacity of the luminal compartment, which is largely compromised by the high [GSSG]:[GSH] ratio. In addition, ascorbate also serves as a cofactor in special enzymatic

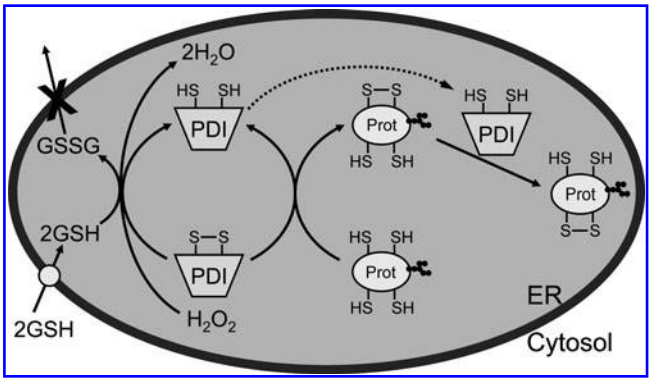

FIG. 3. Redox homeostasis of glutathione in the ER. The high (approx. 1:1) ratio of [GSSG] and [GSH] is due to local oxidation of GSH and entrapment of GSSG in the ER lumen. GSH can enter the compartment by protein-mediated passive transport. In addition to serving as a ROS scavenger, GSH is also a substrate of oxidized PDI and competes with protein thiols for the oxidizing power. GSSG, produced in either way, is retained in the luminal compartment due to the lack of efficient transport across the ER membrane. Therefore, glutathione participates in oxidative folding as a reducing agent and it helps to keep PDI in reduced form, so it can catalyze the rearrangement of protein disulfides. ER, endoplasmic reticulum; Ero1, ER oxidase 1; GSH, glutathione; GSSG, glutathione disulfide; PDI, protein disulfide isomerase.

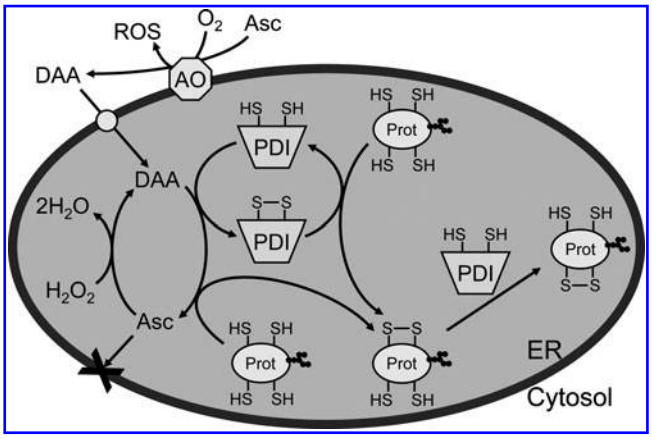

FIG. 4. Role of ascorbate and dehydroascorbic acid in oxidative folding. Ascorbate oxidase produces DAA on the cytosolic side of the ER membrane. DAA enters the lumen by a protein-mediated transport and oxidizes protein thiols (including those of PDI). Therefore, DAA contributes to disulfide formation in nascent proteins directly or through PDI activity. Ascorbate cannot leave the compartment due to the lack of efficient transport; nevertheless, it can be be reoxidized in the lumen by hydrogen peroxide or other ROS. Asc, ascorbate; AO, ascorbate oxidaseł; DAA, dehydroascorbic acid; ER, endoplasmic reticulum; Ero1, ER oxidase 1; GSH, glutathione; GSSG, glutathione disulfide; PDI, protein disulfide isomerase.

dioxygenase reactions in the ER. Prolyl and lysyl hydroxylase enzymes catalyze post-translational modifications in collagen. These metalloproteins consume nearly stoichiometric amounts of ascorbate when incorporating oxygen atoms in prolyl or lysyl sidechains and α-ketoglutarate cosubstrate (47).

Calcium Homeostasis and Folding in the ER

The ER is a major dynamic Ca^{2+} store in eukaryotic cells. The enormous calcium gradient between the two sides of the ER membrane is generated and maintained by the continuous activity of sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps. Although a slow permanent Ca^{2+} leakage can be demonstrated, fast Ca^{2+} release can only be triggered by the activation of Ca^{2+} channels—inositol trisphosphate receptors or ryanodine receptors—in the ER membrane.

The luminal ER proteins, including the local chaperones and foldases are accustomed to high (extracellular-like) Ca^{2+} concentrations. Therefore, Ca^{2+} release from the ER does not only modulate the cytosolic activities but also greatly affects protein maturation and secretion (25, 45). Several molecular chaperones, such as calreticulin, calnexin, immunoglobulin heavy chain-binding protein/78 kDa glucose-regulated protein (BiP/Grp78), or 94 kDa glucose-regulated protein (Grp94), serve as Ca^{2+} buffering proteins in the ER. Their functions and interactions and hence the formation of folding complexes are also dependent on Ca^{2+} concentrations (2).

Calreticulin is a highly abundant Ca^{2+}-binding chaperone found in all cell types of higher organisms, with the exception of erythrocytes. Calreticulin binds Ca^{2+} with high capacity and also participates in the folding procedure of the newly synthesized proteins (51). The proline-rich P-domain of this chaperone binds Ca^{2+} with high affinity and low capacity, while the C-domain contains low affinity, high capacity Ca^{2+}-binding sites (3). The Ca^{2+}-binding domains allow calreticulin to function as a modulator of Ca^{2+} homeostasis and are also responsible for the sensitivity of this chaperone to fluctuating Ca^{2+} levels. The frequent intraluminal Ca^{2+} fluctuations induce conformational changes in the protein. High Ca^{2+} levels protect calreticulin against proteolytic degradation, which indicates that these ion-induced conformational changes influence its ability to interact with other proteins in the ER. Calreticulin is rapidly and fully degraded when taking up its highly protease-sensitive conformation at low Ca^{2+} concentrations (14). Thermostability and three-dimensional structure of this chaperone are largely influenced by the luminal Ca^{2+} concentration. Optimal stability can be observed at physiological calcium concentrations, and changes in calcium levels in either direction lead to significantly reduced protein integrity (59). The rigid and compact conformation of the calcium-binding C-domain is severely perturbed at low Ca^{2+} concentration, which underlies the calcium sensor function of this calcium buffering protein (57). These results underscore the tight mutual relationship between Ca^{2+} concentrations and protein folding in the ER; Ca^{2+} levels affect protein folding and vice versa.

Calnexin is a membrane-bound chaperone of the ER and is related to calreticulin regarding both structure and function. The unique and remarkable structure of calnexin has recently been revealed at 2.9 Å resolution by X-ray crystallography. It recruits ERp57, a member of the protein disulfide isomerase family, which specifically catalyzes disulfide bond exchange on glycoproteins bound to calnexin. Reduction in luminal Ca^{2+} concentration is accompanied by a progressive loss of native structure of calnexin. Decreasing free Ca^{2+} level decreases thermostability of the protein, while increasing its exposure to protease digestion (11).

BiP is one of the low affinity calcium binding proteins but—due to its abundance—is responsible for 25 % of the total Ca^{2+}-binding capacity of the ER (40). The protein is regulated by calcium both directly and indirectly through sigma-1 receptor (Sig-1R), one of its interaction partners. The chaperone functions of purified BiP are enhanced by calcium depletion (36). The calcium-sensitive ER membrane protein Sig-1R is normally associated with BiP; however they dissociate in case of calcium depletion from the ER. Since the luminal domain of Sig-1R has chaperone activity, calcium depletion may also increase the overall chaperone activity in the lumen (28).

Grp94 is also one of the most abundant Ca^{2+}-buffering proteins in the ER. The calcium-binding properties of Grp94 resemble those of calreticulin and calnexin (8). The N-terminal portion of the chaperone contains at least one high-affinity Ca^{2+}-binding site in the charged linker domain. The occupancy of this site at physiological Ca^{2+} concentration was shown to increase the peptide-binding activity of Grp94 (8).

Calreticulin, calnexin, BiP, and Grp94 are central players of protein maturation and glycoprotein quality control in the ER. BiP and Grp94 are general, albeit ER specific, chaperones, which recognize the hydrophobic side chains exposed on partially folded or misfolded proteins and prevent their aggregation. Calreticulin and calnexin are lectin chaperones. In addition to detecting non-native conformations, they bind nascent glycoproteins by recognizing their oligosaccharide moiety (13). Monoglucosylated N-glycoproteins (see next section) are considered as misfolded and are specifically retained by these lectins for further folding attempts in the ER lumen (Fig. 5). Malfunction of these chaperones leads to severe derangements in protein folding and quality control in the ER compartment. In light of their calcium sensitivity, it is not surprising that depletion of ER calcium stores leads to accumulation of immature proteins. In fact, the SERCA inhibitor thapsigargin, which attenuates active Ca^{2+} uptake, is one of the classic ER stressors widely applied to trigger the unfolded protein response (37).

Protein Glycosylation and Compartmentation in the ER

Most luminal proteins and luminal domains of membrane proteins undergo co- or post-translational glycosylation in the ER. This is a major post-translational modification in the eukaryotic cells, which can enhance solubility, improve folding, facilitate secretion, modulate antigenicity, and increase *in vivo* half-life of the glycoprotein. There are two major types of protein glycosylation: O- and N-glycosylations. The oligosaccharide moiety can be built up by glycosyltransferases from activated monosaccharides (nucleotide sugars) step-by-step on the hydroxyl-group of specific serine, threonine, or hydroxylysine residues through an O-glycosidic bond. O-glycosylation of secretory proteins takes place mostly in the Golgi apparatus but it can also be initiated in the ER (24). Alternatively, the oligosaccharide moiety can be transferred

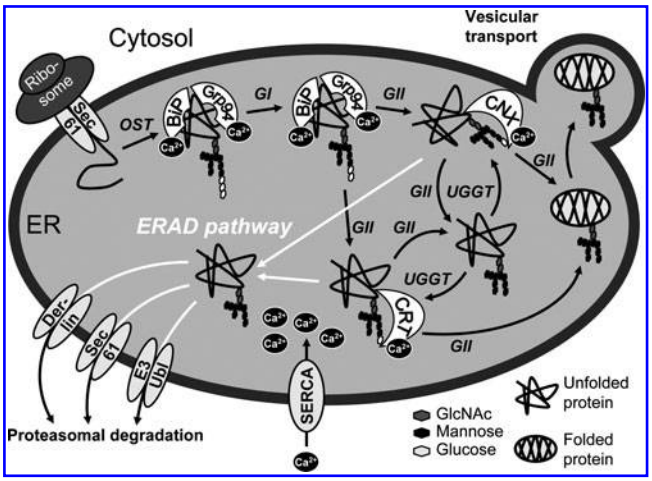

FIG. 5. Calcium-dependent chaperones in protein folding and quality control. Proteins are post- or cotranslationally imported into the ER through Sec61 translocon complex. Their folding is guarded in the ER by chaperones, such as BiP and Grp94, which recognize misfolded proteins and prevent their aggregation. Nascent proteins usually receive N-linked oligosaccharide moieties in the ER in a reaction catalyzed by oligosaccharyltransferase. Two glucosyl residues of the core glycan are removed by glucosidases I and II, respectively. The most proximal glucosyl residue is used as a label of immaturity, which is recognized by lectins (calnexin and calreticulin). Removal of this label by glucosidase II exposes the protein to a quality checking by UDP-glucose:glycoprotein glucosyltransferase (UGGT), which redirects immature proteins into the folding cycle by reglucosylation. Properly folded proteins can leave the ER by vesicular transport toward the Golgi apparatus. Permanently misfolded proteins are discarded by ERAD (*i.e.*, they get demannosylated and retrotranslocated to the cytosol for ubiquitylation and proteasomal degradation). Beside Sec61, Derlin and some E3 ubiquitin ligase family members have also been suggested to serve as the dislocon channel for the ERAD pathway. BiP, Grp94, calnexin, and calreticulin are Ca^{2+}-dependent chaperones; their optimal activity requires high calcium concentration in the compartment, which is generated and maintained by the continuous activity of SERCA pumps. BiP, immunoglobulin heavy chain-binding protein; CNX, calnexin; CRT, calreticulin; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum-associated degradation; GI, glucosidase I; GII, glucosidase II; Grp94, 94 kDa glucose-regulated protein; OST, oligosaccharyltransferase; UGGT, UDP-glucose:glycoprotein glucosyltransferase; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase.

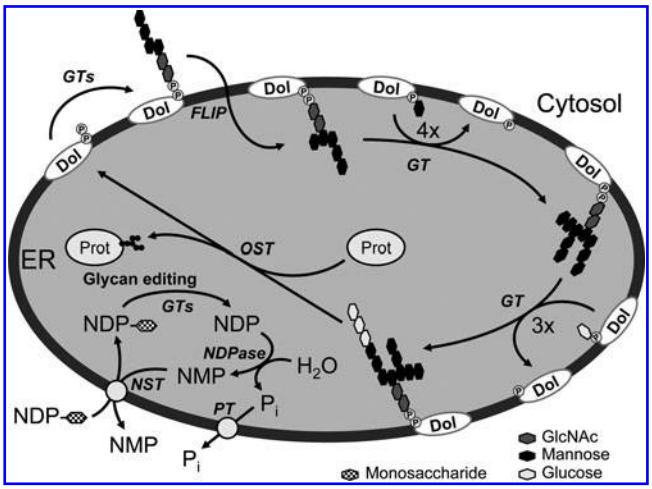

FIG. 6. Protein glycosylation and compartmentation in the ER. The core glycan, which is to be transferred to N-glycosylation sites of the native proteins, is partially assembled on dolichol pyrophosphate on the cytosolic side of the ER membrane by glycosyltransferases. The intermediate $Man_5GlcNAc_2$-PP-Dol is translocated across the membrane by a yet unidentified flippase. Its synthesis continues by the sequential addition of mannose and glucose residues by luminal glycosyltransferases using Dol-P-Man and Dol-P-Glc. Oligosaccharyltransferase links the core glycan en bloc to N-glycosylation sites of the nascent proteins. Glycan editing includes addition of further monosaccharide units. Nucleotide sugars (*e.g.*, UDP-glucose, UDP-N-acetylglucosamine, UDP-galactose, UDP-N-acetylgalactosamine and UDP-xylose) are imported into the ER lumen by NSTs, antiporters of nucleotide sugars and nucleoside monophosphates. Dol, dolichol; ER, endoplasmic reticulum; FLIP, flippase; GT, glucosyltransferase; NDP, nucleoside diphopshate; NMP, nucleoside monophopshate; NST, nucleotide sugar transporter; OST, oligosaccharyltransferase; PT, phosphate transporter.

by oligosaccharyltransferase en bloc from dolichol phosphate to the amino-group of a specific asparagine residue through an N-glycosidic bond (34).

This N-glycosylation is a characteristic post-translational modification in the ER. In addition to the above mentioned general functions, N-glycosylation has been shown to play a central role also in glycoprotein quality control in the ER. The initial oligosaccharide (core glycan) moiety to be transferred to N-glycosylation sites of the native proteins is partially assembled on the cytosolic side of the ER membrane. Sequential addition of monosaccharide units to membrane-embedded dolichol phosphate yields the intermediate, $Man_5GlcNAc_2$-PP-Dol, which is translocated across the membrane so that the oligosaccharide face the lumen of the ER, where the biosynthesis of core glycan continues to completion. Phosphorylated polyisoprenols, such as dolichol phosphate, partly destabilize the biological membranes, which might facilitate the 'flipping' (55). The translocation from the cytosolic to the luminal leaflet of the ER membrane is catalyzed by flippases, which have been functionally characterized but still remain to be identified (30).

After flipping into the luminal side, the synthesis of core glycan continues by the sequential addition of four more mannose residues and three glucose residues by luminal glycosyltransferases using dolichol phosphate linked monosaccharides (Dol-P-Man and Dol-P-Glc, respectively) as substrates (Fig. 6). Dol-P-Man and Dol-P-Glc are synthesized on the cytosolic side of the membrane and are also flipped to the luminal side by yet unidentified transporters.

Once transferred to an asparagine residue of the glycoprotein, the core glycan is thoroughly remodeled through multiple trimming (glycosidases) and elongation (glycosyltransferases). The glucosyl residues are quickly removed by glucosidases I and II. However, the most proximal glucosyl residue is used as a label of immaturity and is recognized by calnexin and calreticulin, as it was mentioned above. Removal

of this label by glucosidase II exposes the protein to a quality checking by UDP-glucose:glycoprotein glucosyltransferase (UGGT), which redirects immature proteins into the folding cycle by reglucosylation (Fig. 5).

Compartmentation allows a careful timing in protein glycosylation. This is well represented, for example, in the separated action of class 1 α-mannosidases involved in the processing of N-glycans and quality control in the early secretory pathway. ER α1,2-mannosidase I (ERManI) and the ER degradation enhancing α-mannosidase-like proteins (EDEM proteins) play a central role in the initiation of ER associated degradation (ERAD) while Golgi α-mannosidase I (GolgiManI) is involved in glycan maturation in the Golgi complex (43). Endogenous human ERManI has been recently shown to be localized to the Golgi apparatus and therefore physically separated from the ER. Moreover, the Golgi localization seems to be necessary for its function in generating ERAD subsrates (49). Immature proteins likely recycle through the Golgi complex before reengagement with calnexin for subsequent conformational changes. This finding supports the hypothesis that the "timed" degradation of irreparable glycoproteins is due to vesicle recycling rather than to strict ER retention (39). This might also explain why some N-glycoproteins must cycle through the Golgi complex to get disposed by proteasomes (12). The advantage of placing ERManI in a common compartment with all the other α1,2-mannosidases instead of the ER lumen could be in preventing any direct competition between the systems of folding and degradation (49).

Both O- and N-glycosylations in the ER lumen require activated monosaccharides (*e.g.*, UDP-glucose, UDP-N-acetylglucosamine, UDP-galactose, UDP-N-acetylgalactosamine, and UDP-xylose), which in turn are all synthesized in the cytosol. Since the nucleotide sugars are polar and charged molecules, they can only enter the lumen by protein-mediated transport across the ER membrane (Fig. 6). The solute carrier family SLC35 consists of nucleotide sugar transporters (NSTs) localized in the Golgi and/or ER membrane (32). NSTs are antiporters of nucleotide sugars and the corresponding nucleoside monophosphates. The nucleoside diphosphate sugars imported into the ER lumen participate in protein (or lipid) glycosylation by delivering their monosaccharide moieties. The remaining nucleoside diphosphates are subsequently hydrolyzed by nucleoside diphosphatases to nucleoside monophosphates, which are exported through the nucleotide sugar antiport as counter-ligands (19).

The translocon peptide channels, which seem to contribute to the limited general permeability of the ER membrane, have been shown to transport a variety of charged and polar molecules. Low capacity nucleotide sugar transport through the translocon complexes might play an important role in priming the glycosylation-associated high capacity antiport activities (41).

Transport of Proteins into and from the ER

Although the permeability of the ER membrane to small molecules has been a subject of long debate, it is unequivocally accepted that proteins can only pass this barrier through controlled peptide channels. Inward (either co- or post-translational) transport of polypeptides across the ER membrane is mediated by the Sec61 complex and associated proteins (48). Sec61 translocon complex is a heterotrimeric transmembrane pore composed of Sec61$\alpha\beta\gamma$ subunits. It forms a gated channel, which allows peptide translocation into the ER lumen and lateral integration of transmembrane segments into the membrane (Fig. 5).

The hydrophilic nature (15) and size (27) of the translocon pores were investigated by using fluorescent quenchers. These studies revealed that Sec61 forms an aqueous pore in the ER membrane, with a dynamically changing diameter, which was estimated as 9–15 Å and 40–60 Å in ribosome-free or ribosome-bound states, respectively (33).

The Sec61 complex undergoes conformational changes that open or close the channel. The closed conformation forms an hourglass-shaped pore with a narrow central constriction (pore ring) due to the tilt of the trans-membrane spans. In this state, the luminal side of the pore is occluded by an α-helical plug domain (42). BiP, a prominent ER chaperone, is also tightly attached to the luminal end of the channel, which was also suggested to block the leakage through the pore. Ribosome binding induces the opening of the channel; however, BiP is only released when the pore is occupied by the nascent peptide chain, which emerges at the luminal side. These mechanisms seem to prevent the formation of wide pores in large amounts in the ER membrane, which would destroy the concentration gradients and wash out the special features of the luminal milieu.

Nevertheless, ribosomes often remain associated to the translocons after translation has been completed. The components of the channel have also been shown to remain assembled and maintain their organization after translocating the nascent chain (52). These nontranslating and nontranslocating ribosome–translocon complexes seem to be responsible for a limited nonselective permeability, which can play a role in calcium leakage and contribute to the substrate supply of certain luminal enzymes.

Properly folded proteins remain compartmentalized and leave the ER by vesicular transport without crossing the membrane (Fig. 5). Quality control mechanisms prevent forwarding of aberrant proteins towards the Golgi apparatus by retaining misfolded polypeptides in the ER and redirecting them into the folding machinery. Intraluminal accumulation of defective cargo is prevented by diverting hopelessly misfolded proteins from futile folding attempts to degradation. The active process of ERAD involves the displacement of demannosylated defective proteins by retrograde translocation to the cytosol for poly-ubiquitylation and subsequent proteasomal degradation. Sec61 translocon has been proposed to mediate retrotranslocation too. However, other proteins, such as Derlin and some E3 ubiquitin ligase family members, have also been suggested to serve as the dislocon channel for the ERAD pathway (29) (Fig. 5).

Concluding Remarks

The ER membrane separates two characteristically different compartments of the eukaryotic cell, the cytosol and the ER lumen. The former one is strictly separated from the extracellular space too, and can be considered as the real interior of the cell. Nonetheless, the ER (and the whole endomembrane system) keeps a continuous dynamic contact with the extracellular fluid. Substances situated in the ER lumen can be secreted from the cell by vesicular

transport without passing any membrane barriers. Therefore, the ER lumen can be looked at as a piece of the outer space within the cell. Accordingly, secretory proteins and plasma membrane proteins to be exposed on the cell surface are synthesized and processed in the ER. It serves the optimal preparation of these proteins that certain major determinants of the luminal compartment, such as the calcium concentration and the thiol-disulfide redox state, resemble those of the extracellular fluid.

The cytosol and the luminal compartments of the ER are separated by the continuous ER membrane, a real barrier to proteins as well as metabolic intermediates and inorganic ions. Strictly controlled peptide channels, such as the Sec61 complex, mediate the entry and exit of proteins across the ER membrane, and the nonspecific permeability through their wide hydrophilic pore is minimized by careful mechanisms. These mechanisms allow the maintenance of great concentration gradients, such as those of Ca^{2+} and glutathione, between the two compartments. The price of this separation is the necessity of selective transporters for the intensive bidirectional traffic of metabolites across the ER membrane. For instance, N-glycosylation, a major post-translational modification in the lumen, depends on the transport of carbohydrate derivatives from the cytosol.

Compartmentation of protein maturation in the ER is indispensable for oxidative folding, which would be totally incompatible with the cytosolic redox and antioxidant homeostasis. Protein quality control and ERAD evolved on grounds of and their strategy is totally based on this compartmentation: immature proteins are retained in the ER lumen for further folding attempts while hopelessly misfolded proteins are discarded by being thrown out from the ER into the cytosol. Compartmentation doubtlessly plays a special role in the process of protein folding in the ER and also provides an interesting aspect of overview and discussion.

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Address correspondence to:
Dr. Miklós Csala
Department of Medical Chemistry, Molecular Biology and Pathobiochemistry
Semmelweis University
1444 Budapest
POB 260
Hungary

E-mail: csala.miklos@med.semmelweis-univ.hu

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Abbreviations Used

BiP/Grp78 = immunoglobulin heavy chain-binding protein/78 kDa glucose-regulated protein
DAA = dehydroascorbic acid
Dol = dolichol
ER = endoplasmic reticulum
ERAD = endoplasmic reticulum associated degradation
Ero1 = ER oxidase 1
GI = glucosidase I
GII = glucosidase II
Glc = glucose
GlcNAc = *N*-acetylglucosamine
GLO = gulonolactone oxidase
Grp94 = 94kDa glucose-regulated protein
GSH = glutathione
GSSG = glutathione disulfide
Man = mannose
NSTs = nucleotide sugar transporters
PDI = protein disulfide isomerase
ROS = reactive oxygen species
SERCA = sarco/endoplasmic reticulum Ca^{2+}-ATPase
Sig-1R = sigma-1 receptor
SLC35 = solute carrier 35
UGGT = UDP-glucose:glycoprotein glucosyltransferase