The thiol-dependent reductase ERp57 interacts specifically with N-glycosylated integral membrane proteins.

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The lumen of the endoplasmic reticulum contains a number of distinct molecular chaperones and folding factors, which modulate the folding and assembly of newly synthesized proteins and protein complexes. A subset of these luminal components are specific for glycoproteins, and, like calnexin and calreticulin, the thiol-dependent reductase ERp57 has been shown to interact specifically with soluble secretory proteins bearing N-linked carbohydrate.

Calnexin and calreticulin also interact with glycosylated integral membrane proteins, and in this study we have examined the interaction of ERp57 with these substrates. As with soluble proteins, the binding of ERp57 to an integral membrane protein is dependent upon the protein bearing an N-glycan that has undergone glucose trimming. Furthermore, ERp57 binds to newly synthesized glycoproteins in combination with either calnexin or calreticulin. We propose that ERp57 acts in concert with calnexin and calreticulin to modulate glycoprotein folding and enforce the glycoprotein specific quality control mechanism operating in the endoplasmic reticulum.

The endoplasmic reticulum (ER) is a major site of protein synthesis, producing both secretory and integral membrane proteins. After insertion into, or translocation across, the membrane of the ER, newly synthesized proteins often require the assistance of folding enzymes and molecular chaperones to assist subsequent folding and oligomeric assembly (1, 2). Many chaperones specifically associate with newly synthesized proteins, apparently by recognizing specific features present in the incompletely folded or assembled polypeptide (3, 4).

Calnexin, an integral membrane protein, and calreticulin, its luminal homologue, are two ER-resident molecular chaperones that have been shown to bind selectively and transiently to glycoproteins that carry asparagine-linked carbohydrate side chains (5–7). More precisely, calnexin and calreticulin interact specifically with the monoglucosylated form of the oligosaccharide (8–10), leading to retention of the glycoprotein within the ER (11). The monoglucosylated glycans are generated by the action of glucosidases I and II, which rapidly remove two of the three glycans from the mannose-rich oligosaccharide core (12). Slow removal of the final glucose by glucosidase II allows the release of the glycoprotein by calnexin and calreticulin. The re-addition of a single, terminal, glucose residue by UDP-glucose:glycoprotein glucosyltransferase (13, 14) occurs when a glycoprotein has not attained its correctly folded state, thus regenerating a monoglucosylated glycan. In this way a cycle of de- and reglucosylation acts to modulate the association of calnexin and calreticulin (8, 12), allowing the selective binding and retention of incompletely folded or assembled glycoproteins within the ER. Hence, calnexin, calreticulin, and UDP-glucose:glycoprotein glucosyltransferase are believed to constitute a "quality control" step for newly synthesized glycoproteins prior to their exit from the ER (11, 12, 15).

We have recently identified a third ER-resident protein that binds specifically to secretory glycoproteins containing trimmed N-linked oligosaccharides (16). This soluble protein, ERp57 (also known as GRP58 (17), ERp61 (18), ER60 (19), HIP-70 (20), Q2 (21), and P58 (22), was originally believed to be phosphoinositide-specific phospholipase C-α (23). However, subsequent studies failed to identify any functional phospholipase activity (21, 22, 24–26). Several mammalian ERp57 cDNAs have been identified, and the amino acid sequences encoded all share significant homology with protein-disulfide isomerase (PDI) (27). Although ERp57 has been suggested to be a cysteine-dependent protease (19, 28), a carnitine palmitoyltransferase (29), and a thiol-dependent reductase (21, 22, 30, 31), its precise function remains to be established. Consistent with its thiol-dependent reductase activity is the presence of two WCGHCK motifs identical to those found in PDI and Erp72, and very similar to those in thioredoxin (27).

We previously found that ERp57 interacts specifically with glycosylated secretory proteins (16). In this study we show that ERp57 can bind integral membrane proteins only when they bear N-linked oligosaccharide side chains. Furthermore, this N-linked carbohydrate must be "trimmed" by the removal of a terminal glucose residue(s), and ERp57 appears to interact in combination with either calnexin or calreticulin. Hence, ERp57 may be a generic component of a glycoprotein-specific folding machinery operating in the ER.

EXPERIMENTAL PROCEDURES

Materials—Restriction endonucleases were purchased from New England Biolabs (Hitchin, Hertfordshire, United Kingdom (UK)). SP6 RNA polymerase and transcription buffers were supplied by Promega (Southampton, Hampshire, UK), and Protein A-Sepharose was from Zymed (Cambridge Bioscience, Cambridge, UK). The cross-linking reagent bismaleimidohexane (BMH) was purchased from Pierce and Warriner (Warrington, UK), while succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) was obtained from M & G (Stockport, Cheshire, UK). l-[35S]Methionine was purchased from New England Nuclear (DuPont, Stevenage, Hertfordshire, UK). Proteinase K, cyclo-

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heximide, puromycin, emetine, phenylmethylsulfonyl fluoride (PMSF), and castanospermine (CST) were obtained from Sigma (Dorset, UK). 1-Deoxynojirimycin (dMM) was purchased from Oxford GlycoSystems (Oxford, UK). Polyclonal rabbit anti-calreticulin serum was from Affinity BioReagents (Cambridge Bioscience, Cambridge, UK), while anti-calnexin serum raised against the carboxyl terminus of calnexin was a gift from Professor Ari Helenius (Department of Cell Biology, Yale University, New Haven, CT). Rabbit antiserum recognizing canine PDI was a gift from Dr. N. Bullied (School of Biological Sciences, University of Manchester, Manchester, UK). Polyclonal rabbit antiserum recognizing the NH2 terminus of Glcy was raised against a peptide representing the first 12 amino acids. Antibodies recognizing canine ERp57 were affinity-purified from total rabbit serum as described previously (16). Chicken antiserum raised against rat Erp57 (anti-Erp57-2) was the generous gift of Dr. J. Holtzman (Department of Pharmacology and Medicine, University of Minnesota, Minneapolis, MN).

Constructs—The 5’-noncoding region of the cDNA encoding human glycoporphin C (GlyC) (32) was replaced with the sequence 5’-AGATCT-TCG-3’. This sequence contains a consensus "BamHI" site, allowing the glycoporphin C coding region to be subcloned into pSPUT-K (Stratagene, Cambridge, UK) as a BglII/HpaI fragment, resulting in pSK-GlyC. Point mutants of glycoporphin C were generated using the Transformer™ site-directed mutagenesis kit (CLONTECH, Cambridge, UK). GlyC-Cys contains a cysteine residue rather than a serine at position six of GlyC. In GlyC-Cys ΔCHO the site of glycosylation, an asparagine residue at position eight, has been replaced with a serine.

The cDNA for the human glucose transporter (Glut 1) was provided by Dr. S. Baldwin (Department of Biochemistry and Molecular Biology, University of Leeds, UK). A mutant with a cysteine residue replacing the tyrosine at position 44, adjacent to the glycosylated asparagine, was generated as described above (cf. Ref. 32). This mutant construct, Glut 1-Cys*8, was used in all subsequent experiments. All mutant constructs were checked by DNA sequencing prior to use.

Transcription and Translation—pSK-GlyC was linearized with BamHI and used as a template in an SP6 RNA polymerase transcription system according to the manufacturers instructions (Promega). The resulting mRNA encoded full-length GlyC. mRNA encoding the NH2-terminal 155 amino acids of Glut 1-Cys*8 was prepared as described previously for wild type Glut 1 (32). The mRNAs were translated at 26 °C in a wheat germ lysate system (33) in the presence of L-15S-methionine and canine pancreatic microsomes. After 20 min, further initiation was inhibited by adding the 7-methylguanosine 5’-monophosphate to a final concentration of 4 mM. Following an additional 10 min, translation was terminated by the addition of cycloheximide to a final concentration of 2 mM, and samples were placed on ice.

The membrane-associated fraction was isolated from the translation mixture by precipitation with 7% TRIS-acetate, centrifuged at 100,000 g for 1 hr at 4 °C. The resulting membrane pellet was resuspended in 0.67 volumes of the original translation reaction using low salt/sucrose cushion (250 mM sucrose, 500 mM potassium acetate, 5 mM magnesium acetate, 50 mM Hepes-KOH, pH 7.9) at 130,000 × g for 10 min at 4 °C. The resulting membrane pellet was resuspended in 0.67 volumes of the original translation reaction using low salt/sucrose cushion (250 mM sucrose, 500 mM potassium acetate, 5 mM magnesium acetate, 50 mM Hepes-KOH, pH 7.9) containing 1 mM emetine.

Cross-linking—Cross-linking was performed with either BMH or SMCC. SMCC is a heterobifunctional reagent, which reacts with suitable amino groups, i.e. the ε amino group of lysines and the primary amine found at the NH2-terminus of most proteins, and free sulfhydryls. BMH is a homobifunctional reagent specific for the free sulfhydryls of cysteine residues. The resuspended membrane fraction was incubated for 10 min at 26 °C in the presence of either 1 mM BMH or 1 mM SMCC added from a 50 mM stock in Me2SO. Control samples were treated with Me2SO alone. The reaction was quenched by adding either 0.1 volumes of 50 mM 2-mercaptoethanol and 500 mM glycine (for SMCC), or 0.1 volumes of 100 mM 2-mercaptoethanol (for BMH), and the samples were left for 20 min on ice. Following cross-linking the samples were subjected to trichloroacetic acid precipitation to yield the total membrane fraction, or trichloroacetic acid precipitated samples were immunoprecipitated as described below.

Protease Protection Assays—Membrane-associated GlyC was incubated with 250 μg/ml proteasine K for 30 min on ice. Where present, Triton X-100 was added to a final concentration of 1% prior to the incubation. Following the digestion, proteasine K was inhibited by the addition of 200 μg/ml PMSF, followed by precipitation with an equal volume of 20% trichloroacetic acid, 50% acetone. The resulting pellet was solubilized in 1% SDS, 100 mM Tris-HCl, pH 7.9, at 95 °C for 5 min.

![Fig. 1. Transmembrane orientation of Glycoporphin C and relative positions of potential cross-linking sites.](http://www.jbc.org)

ERp57 Interacts with Glycosylated Membrane Proteins

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**RESULTS**

**ERp57 Is Associated with Full-length Glycosylated GlyC**—We have previously shown that the “PDI-like” protein, ERp57 (see Ref. 27), associates specifically with newly synthesized secretory glycoproteins in the lumen of the ER (16). In this present study, the carbohydrate-dependent interactions between integral membrane proteins and components of the ER lumen were investigated. The glycosylated integral membrane protein glycoporphin C (GlyC) was used as a model glycoprotein for this analysis. GlyC is a single spanning membrane protein with an uncleaved signal-anchor sequence, i.e. the membrane protein glycophorin C (GlyC) could not be glycosylated at this position. Hence carbohydratespecific interactions, we generated a mutant GlyC-Cys position six but lacked an asparagine at position eight (GlyC-Cys ΔCHO) and hence could not be glycosylated at this position.

Before we investigated the interactions of GlyC with luminal ER proteins, we established that the point mutations we had introduced did not influence the integration or transmembrane...
Since the amino-group at the NH$_2$ terminus of the protein is expected, no such cross-linking products were observed when SMCC was omitted from the experiment (data not shown).

Since the membrane insertion of GlyC was unaffected by the introduction of the point mutations, we proceeded to analyze the interactions of these proteins with ER luminal components. Membrane fractions were incubated with or without proteinase K (250 µg/ml) and 1% Triton X-100 as indicated. After 30 min at 0 °C, the protease was inhibited by the addition of PMSF, the samples trichloroacetic acid-precipitated, and the products immunoprecipitated using antisera recognizing the NH$_2$ terminus of GlyC. Protected glycosylated (unfilled arrowhead) and unglycosylated (asterisk) GlyC are shown. The samples were analyzed on a 14% SDS-polyacrylamide gel.

![Image](https://example.com/image1.png)

**Fig. 2.** Insertion of GlyC into microsomes. GlyC wild type or mutant mRNA was translated in a wheat germ system in the presence of canine pancreatic microsomes for 20 min at 26 °C. Isolated membrane fractions were incubated with or without proteinase K (250 µg/ml) and 1% Triton X-100 as indicated. After 30 min at 0 °C, the protease was inhibited by the addition of PMSF, the samples trichloroacetic acid-precipitated, and the products immunoprecipitated using antisera recognizing the NH$_2$ terminus of GlyC. Protected glycosylated (unfilled arrowhead) and unglycosylated (asterisk) GlyC are shown. The samples were analyzed on a 14% SDS-polyacrylamide gel.

The cysteine mutant (GlyC-Cys) was created to introduce a cross-linking site into GlyC that was closer to the site of carbohydrate addition than the NH$_2$-terminal amino group (see “Experimental Procedures”).

In the absence of protease treatment, two forms of GlyC-wt were observed, representing the glycosylated (GlyC-CHO) and non-glycosylated (GlyC) polypeptides (Fig. 2, lane 1). The 3-kDa difference in mobility is consistent with the addition of a single N-linked oligosaccharide side chain. GlyC-Cys behaved identically to GlyC-wt (Fig. 2, lane 7). As expected, the faster migrating non-glycosylated product was seen with GlyC-Cys ΔCHO (Fig. 2, lane 4), confirming that this protein was not glycosylated. Following proteinase K treatment, products that corresponded to the glycosylated (unfilled arrowhead) or unglycosylated (asterisk) NH$_2$ terminus of GlyC (Fig. 2, lanes 2, 5, and 8) could be immunoprecipitated. The bulk of the protease-protected GlyC-wt and GlyC-Cys was glycosylated (Fig. 2, lanes 2 and 8), while all of the protease-protected GlyC-Cys ΔCHO fragment was unglycosylated (Fig. 2, lane 5). No products were observed when Triton X-100 was present during proteinase K treatment, indicating complete digestion of GlyC (Fig. 2, lanes 3, 6, and 9). These results demonstrated that the mutant GlyC proteins used in this study are correctly inserted into canine pancreatic microsomes, with an orientation identical to the wild type protein.

Since the membrane insertion of GlyC was unaffected by the introduction of the point mutations, we proceeded to analyze the interactions of these proteins with ER luminal components. Following translation of GlyC-wt in the presence of canine pancreatic microsomes, the membrane-associated fraction was treated with the heterobifunctional cross-linking reagent SMCC. After cross-linking, the samples were subjected to immunoprecipitation with a variety of antibodies against ER components (data not shown). The incorporation of the cysteine into GlyC appeared to increase the efficiency of SMCC-dependent cross-linking to calnexin and calreticulin (cf. Figs. 3 and 4). An additional calnexin-containing adduct, barely visible in the GlyC-wt samples (Fig. 3, lane 4), was also considerably enhanced in GlyC-Cys (Fig. 4, lane 4, open arrowhead). An ~80-kDa product was observed with GlyC-Cys even in the absence of any added cross-linking reagent (Fig. 4, lane 1, filled circle). This product was not consistently observed (cf. Fig. 4, lane 1 and Fig. 5, lane 1) and may represent a small proportion of GlyC that has formed an aberrant inter-molecular disulfide linkage. When GlyC-wt was processed in the absence of SMCC, no products were observed following immunoprecipitation by the antisera recognizing ER components (data not shown). Introduction of an additional lysine, in place of the serine at position six of GlyC, had no effect and the cross-linking pattern observed with SMCC was identical to that seen with GlyC-wt (data not shown).

![Image](https://example.com/image2.png)

**Fig. 3.** GlyC associates with calnexin, calreticulin, and ERp57. mRNA encoding full-length GlyC-wt was translated in a wheat germ lysate system in the presence of canine pancreatic microsomes for 20 min at 26 °C, and the membrane-associated fraction was isolated. Samples were treated with 1 mM SMCC and immunoprecipitated under denaturing conditions with either: an unrelated anti-serum (lane 3), anti-calnexin (lane 4), anti-calreticulin (lane 5), anti-PDI (lane 6), or anti-ERp57 (lane 7) sera. Total products obtained after treatment with either M$_6$SO (lane 1) or 1 mM SMCC (lane 2) followed by trichloroacetic acid precipitation are also shown. Glycosylated (GlyC-CHO) and unglycosylated (GlyC) glycophorin C are indicated; cross-linking products between GlyC and calnexin, calreticulin, and ERp57 are identified. The samples were analyzed on a 9.6% SDS-polyacrylamide gel.

The luminal region of GlyC-Cys has two potential sites from which SMCC may cross-link to adjacent proteins: the amino group at the NH$_2$ terminus and the sulfhydryl of the cysteine. By using the homobifunctional, sulfhydryl-specific reagent...
BMH (see Ref. 36), we could limit the cross-linking targets in GlyC-Cys to the sulfhydryl of the cysteine alone.

The Interaction of ERp57 with GlyC Is Carbohydrate-dependent—Using BMH, in combination with GlyC-Cys and a nonglycosylated mutant (GlyC-Cys ΔCHO), we set out to establish the role of the N-linked carbohydrate in promoting specific interactions with glycosylated integral membrane proteins. Following treatment with BMH, a complex pattern of cross-linking products was observed with membrane inserted GlyC-Cys and GlyC-Cys ΔCHO (Fig. 5, lanes 2 and 9, respectively). With GlyC-Cys, three of these cross-linking products, with apparent molecular masses between 80 and 90 kDa (Fig. 5, lanes 6 and 7, arrow), were immunoprecipitated by two different antisera specific for ERp57. None of these ERp57-GlyC cross-links were observed with GlyC-Cys ΔCHO. In contrast, PDI was found to be cross-linked to both GlyC-Cys and GlyC-Cys ΔCHO (Fig. 5, lanes 2 and 9, filled diamond). The identity of the glycosylation-independent 91–94-kDa cross-linking product, observed with both GlyC-Cys and GlyC-Cys ΔCHO (Fig. 5, lanes 2 and 9, unfilled circle), remains to be established.

Thus, the BMH-dependent cross-linking of ERp57 to GlyC required an N-linked carbohydrate side chain. Since the immunoprecipitations were carried out after SDS denaturation of the samples, all three products visible must contain ERp57 (Fig. 5, lanes 6 and 7). The different products are most likely all GlyC-Cys-ERp57 adducts with different mobilities resulting from the cross-linking of GlyC-Cys to different cysteine residues within ERp57.

Association of ERp57 with GlyC Is Dependent on Glucose Trimming of the N-Linked Oligosaccharide—CST inhibits the ER glucosidases I and II, preventing the removal of the terminal glucose residues from N-linked oligosaccharides. CST has been used to establish that glucose trimming is required for several ER proteins to interact with nascent glycoproteins (7, 12, 16).

The effect of CST and dMM (a mannosidase inhibitor) on the BMH-dependent cross-linking of GlyC-Cys to ERp57 and PDI was assessed. A decrease in the mobility of glycosylated GlyC-Cys was observed following CST treatment, demonstrating that glucose trimming was efficiently inhibited (Fig. 6, lane 9, unfilled arrow).

In the presence of CST the amount of ERp57 cross-linking products obtained was dramatically reduced (by ~75%) (Fig. 6, lanes 4 and 6). In contrast, there was an ~50% increase in the PDI cross-linking product (Fig. 6, lanes 1 and 3). Treatment with dMM had little effect on either the ERp57 cross-linking products (Fig. 6, lanes 4 and 5) or the PDI cross-linking products (Fig. 6, lanes 1 and 2). These results confirm that ERp57 associates much more efficiently with GlyC polypeptides which bear a glucose-trimmed oligosaccharide.

ERp57 Interacts in Combination with Calnexin or Calreticulin—The carbohydrate-dependent interactions of GlyC-Cys were further investigated by sequential immunoprecipitation. Membrane-associated GlyC-Cys was treated with BMH, and the cross-linking products were immunoprecipitated under nondenaturing conditions, i.e. without SDS denaturation, using antisera specific for calnexin, calreticulin, or PDI (Fig. 7, lanes 1, 4, and 7, respectively). Prior to a second round of immunoprecipitation, samples were denatured by heating with 1% SDS at 95 °C, and the resulting products were then re-
precipitated using a variety of antibodies (Fig. 7, lanes 2, 3, 5, 6, 8, and 9). Calnexin (asterisk), calreticulin (unfilled arrow), and PDI (filled diamond) were all shown to be cross-linked to GlyC-Cys by re-polymerization with the respective antisera (Fig. 7, lanes 3, 5, and 6, respectively). ERp57-GlyC-Cys cross-linking products had co-immunoprecipitated with anti-calnexin and anti-calreticulin sera under nondenaturing conditions (Fig. 7, lanes 3 and 6, respectively). However, no co-precipitation of ERp57 with PDI cross-linking products was observed (Fig. 7, lane 9). Hence, ERp57 appears to interact with glycosylated glycoprotein G in combination with calnexin or calreticulin.

Association of ERp57 with Glut 1, a Multiple Spanning Membrane Protein—To determine whether the association of ERp57 with glycosylated membrane proteins was more widespread, the carbohydrate-dependent interactions of the human Glut 1-glucose transporter were examined. A 155-residue amino-terminal fragment of Glut 1-Cys44, GT155-Cys44 (cf. Ref. 32), was translated in the presence of microsomes, with or without CST, and the membranes isolated by centrifugation through a high salt/sucrose cushion. The membrane fraction was then treated with 1 mM SMCC and cross-linking products immunoprecipitated under both non-denaturing and denaturing conditions (Fig. 8, A and B, respectively). In the absence of SDS treatment, the anti-calnexin and anti-calreticulin sera immunoprecipitated a major cross-linking product of approximately 74 kDa (Fig. 8A, lanes 3 and 4, arrow), which was not observed under denaturing conditions (Fig. 8B, lanes 3 and 4). However, a product of identical mobility was immunoprecipitated by anti-ERp57 serum under denaturing conditions, indicating that ERp57 interacts with GT155-Cys44 (Fig. 8B, lane 6). When CST was present during protein synthesis and cross-linking, the interaction with ERp57 was completely inhibited (Fig. 8B, lane 12). Hence, the previously characterized NH2-terminal fragment of Glut 1 (32) interacts with ERp57 only after glucose trimming of the N-linked carbohydrate side chain. The GT155-Cys44-ERp57 cross-linking product is co-immunoprecipitated with calnexin and calreticulin antisera, and we conclude that ERp57 is identical to the 60-kDa calnexin-associated protein (CAP-60) we previously identified in association with the wild type GT155 (16, 32). Hence, ERp57 interacts specifically with two glycosylated integral membrane polypeptides.

**DISCUSSION**

We recently established that the ‘PDI-like’ protein, ERp57, interacts specifically with secretory glycoproteins bearing glucose-trimmed, N-linked, oligosaccharides (16). In this study we have established that ERp57 shows a similar specificity for integral membrane glycoproteins.

We have used the cross-linking reagents SMCC and BMH to identify calnexin, calreticulin, and ERp57 as cross-linking partners of the single-spanning membrane glycoprotein, GlyC. ERp57 was also cross-linked to an NH2-terminal fragment of the multiple spanning membrane glycoprotein, Glut 1. Crucially, the interaction of ERp57 with both GlyC and Glut 1 was...
shown to require the presence of a glucose-trimmed, N-linked, oligosaccharide side chain. Calnexin and calreticulin are also specific for glucose-trimmed glycoproteins, binding preferentially to proteins with monoglucosylated N-linked carbohydrate side chains (11).

We have now established that ERp57 associates specifically with two different integral membrane glycoproteins (this work) and three soluble glycoproteins (16). On this basis it seems likely that ERp57 is a generic ER component with the potential to interact with all newly synthesized glycoproteins. Hence, ERp57 may play a specific role in modulating glycoprotein biosynthesis at the ER and constitute part of the quality control pathway devoted to these molecules (11).

Both the ERp57-GlyC and ERp57-Glut 1 cross-linking products co-precipitate with either anti-calnexin or anti-calreticulin sera under non-denaturing conditions. This is consistent with the proposal that ERp57 interacts with glycoproteins in combination with calnexin or calreticulin (16). The carbohydrate specificity of the interaction may be mediated by the lectin-like proteins calnexin and calreticulin (7, 10, 37, 38), rather than by ERp57 itself. In this regard it is interesting to note the presence of an ~160-kDa cross-linking product immunoprecipitated by anti-calnexin sera (Fig. 4, lane 4, open arrowhead). This presumably represents a ternary complex between GlyC, calnexin, and an as yet unidentified protein, suggesting calnexin may be able to recruit components other than ERp57 into a ternary complex.

The exact role of ERp57 within the lumen of the ER remains to be established. ERp57 shares significant sequence homology with PDI, a soluble ER-resident protein that catalyzes disulfide interchange, promoting the formation of native disulfide bonds within newly synthesized proteins (27). The greatest homology occurs within two 110-amino acid repeats, which each contain a Trp-Cys-Gly-His-Cys-Lys motif. These motifs are completely conserved between a number of PDI-like proteins, and they occur within newly synthesized proteins (27). The carbohydrate specificity of the interaction may be mediated by the lectin-like proteins calnexin and calreticulin (7, 10, 37, 38), rather than by ERp57 itself. In this regard it is interesting to note the presence of an ~160-kDa cross-linking product immunoprecipitated by anti-calnexin sera (Fig. 4, lane 4, open arrowhead). This presumably represents a ternary complex between GlyC, calnexin, and an as yet unidentified protein, suggesting calnexin may be able to recruit components other than ERp57 into a ternary complex.

Although both calnexin and calreticulin have been described as molecular chaperones (11, 15) and have been shown to bind glycoprotein folding intermediates (6–8, 40), no direct effect of calnexin or calreticulin upon protein folding has been demonstrated. Since ERp57 has the potential to influence disulfide bond formation, it may be that the role of calnexin and calreticulin is to direct newly synthesized glycoproteins into specific folding pathways mediated by other components, such as ERp57.

In fact, the interaction of ERp57 with glycoproteins is not dependent upon the presence of a cysteine residue since the wild type GlyC, lacking any cysteines, is still cross-linked to ERp57. Likewise, the soluble glycoprotein, yeast pro-α-factor, which also has no cysteine residues, can be cross-linked to ERp57 in a CST-sensitive manner (16). Thus, ERp57 might function as a more general “molecular chaperone” in addition to

![Diagram](image-url)
a specific role in modulating disulfide bond formation. This is consistent with the idea that, in addition to its disulfide isomerase activity (27), PDI may play other roles within the lumen of the ER (41, 42). The proposal that ERp57 may act as a molecular chaperone is supported by the observation that, like BiP (GRP78) and GRP94, it is a glucose-regulated protein (17, 31), and its synthesis is rapidly increased upon glucose deprivation and the inhibition of protein glycosylation (43).

Furthermore, the levels of GRP94, BiP, ERp57, and calreticulin all show a 5–10-fold increase in the thyrocytes of mice when compared with the levels of these proteins in the thyrocytes of normal mice (44). Since BiP and GRP94 are functional molecular chaperones (1, 11), ERp57 may also have a similar function as part of the ER “stress response.” A second member of the PDI family, ERp72, has also been shown to be induced under conditions of stress and hence proposed to function as some form of molecular chaperone (42, 44, 45).

Significantly, when the association of ERp57, calnexin, and calreticulin with GlyC is impaired by blocking glucose trimming, the cross-linking of GlyC to PDI shows a reciprocal increase. This suggests that the association of one set of ER luminal proteins with a newly synthesized glycoprotein may restrict the access of alternative components.

We envisage that, following recruitment by calnexin or calreticulin, ERp57 acts to modulate the folding of newly synthesized glycoproteins and contributes to the recently described “quality control” pathway for these molecules (see Ref. 11). We are now trying to address the role of ERp57 upon glycoprotein folding directly.

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The thiol-dependent reductase ERp57 interacts specifically with N-glycosylated integral membrane proteins.

John G. Elliott, Jason D. Oliver, and Stephen High

Page 13852, Fig. 5: This figure was published incorrectly. The correct version is shown below:

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