

Review

ERp57/GRP58: A PROTEIN WITH MULTIPLE FUNCTIONS

CARLO TURANO*, ELISA GAUCCI, CATERINA GRILLO
 and SILVIA CHICHIARELLI

Istituto Pasteur-Fondazione Cenci Bolognetti, Dipartimento di Scienze
 Biochimiche "A. Rossi Fanelli", Sapienza – Università di Roma, Italy

Abstract: The protein ERp57/GRP58 is a stress-responsive protein and a component of the protein disulfide isomerase family. Its functions in the endoplasmic reticulum are well known, concerning mainly the proper folding and quality control of glycoproteins, and participation in the assembly of the major histocompatibility complex class 1. However, ERp57 is present in many other subcellular locations, where it is involved in a variety of functions, primarily suggested by its participation in complexes with other proteins and even with DNA. While in some instances these roles need to be confirmed by further studies, a great number of observations support the participation of ERp57 in signal transduction from the cell surface, in regulatory processes taking place in the nucleus, and in multimeric protein complexes involved in DNA repair.

Key words: Protein disulfide isomerases, Calcitriol, STAT3, Cellular stress, Signal transduction, DNA repair

* Author for correspondence. e-mail: carlo.turano@uniroma1.it, phone: +39 06 49910576, fax: +39 06 4440062

Abbreviations used: $1\alpha,25\text{-(OH)}_2\text{D}_3$ – $1\alpha,25$ -dihydroxycholecalciferol and calcitriol; APE/Ref-1 – apurinic (apyrimidinic) endonuclease/redox-factor 1; CRP – C-reactive protein; ER – endoplasmic reticulum; ERAD endoplasmic reticulum associated protein degradation; mTORC – mammalian target of rapamycin complex; PDI – protein disulfide isomerase; PKC – protein kinases C; PLA₂ – phospholipases A₂; Plc – peptide loading complex; PLC – phospholipase C; PrP^{SC} – scrapie prion protein (misfolded prions); STAT – signal transducer and activator of transcription; STAT3 – signal transducer and activator of transcription 3; SV40 virus – simian virus 40; VDR – vitamin D receptor

INTRODUCTION

The protein ERp57/GRP58 (hereafter simply called ERp57) is a member of the protein disulfide isomerase family, whose components are present mainly, but not exclusively [1], in the endoplasmic reticulum. These proteins are characterized by multiple domains, each structurally similar to thioredoxin, a ubiquitous and abundant small protein responsible for a variety of cellular redox processes. While thioredoxin has only one active site, formed by two vicinal cysteines, the members of the protein disulfide isomerase family may have two or even three similar active sites, located on some, but not all, of their domains. The higher structural complexity of these proteins with respect to thioredoxin has provided them with a catalytic activity not only for the reduction or formation of disulfide bonds in other proteins, but also for the isomerization of the disulfide bonds which takes place during the correct folding of newly synthesized proteins.

The first mammalian protein disulfide isomerase to be characterized, and probably still the most thoroughly investigated, is the well-known PDI. However, ERp57 is increasingly capturing the interest of many investigators, as documented by the ever-growing number of papers that mention it and also by some recent reviews [2, 3]. ERp57 has been found in many diverse subcellular locations and it has been proposed, and sometimes proved, to be involved in a remarkable variety of processes. Actually the first function to be attributed to ERp57 when it was sequenced was mistaken, since the protein was thought to be a phospholipase C and was accordingly named PLC alpha [4]. This definition still persists in some databases and, importantly, also in some old papers, which will be mentioned later on. Apart from this misleading name, ERp57 has also been called GRP58 (glucose-regulated protein 58), ER-60, Q2, ERp58, ERp61, and 1,25D3-MARRS. The name GRP58 is indicative of the fact that ERp57 is a stress-responsive protein, and as such was first identified together with GRP78 and GRP94 as being a protein whose expression increases after glucose depletion [5].

The human protein, coded by the gene *PDI3* on chromosome 15, is formed by 505 amino acids, the first 24 of which constitute the signal peptide. Its structure is characterized by four domains, called respectively a, b, b' and a', each one characterized by a thioredoxin-like fold with alternating alpha-helices and beta-strands [6, 7]. The first and the fourth domain, i.e. the a and a' domains, also carry a thioredoxin-like active site, each with a cys-gly-his-cys sequence, which provide ERp57 with its redox properties. The C-terminal contains a gln-glu-aspleu sequence that is similar to the canonical ER-retention sequence. Near the C-terminal, furthermore, there is a nuclear localization signal (Figure 1). The data from crystallographic studies and the analogy with the classic PDI show that the four domains give to the protein a sort of U-shaped structure, which allows the terminal a and a' domains to come close to each other and interact together with another protein. The two central domains, b and b', on the other

hand, not only are necessary for the full activity of ERp57, but also provide the binding sites for calreticulin or for its membrane-bound homologous calnexin. These are two abundant ER proteins, whose interaction with ERp57 has been described in detail, taking place through the binding of an arm of these proteins, i.e. the P domain, mainly to the b' domain of ERp57 [8, 9]. This binding is specific for ERp57, while the homologous PDI, which has been extensively studied for its binding to other ligands, has no such affinity for calreticulin. PDI has instead a different site for the interactions with proteins and small ligands also on the b' domain [10]. In fact the b' domain is the one with the lowest homology between ERp57 and PDI, and this explains the different binding specificity of the two proteins. The calreticulin/calnexin binding site in ERp57 is enriched in lysines and arginines, thus promoting the interaction of the negatively charged P domain of calreticulin/calnexin, while the site for binding the protein substrates of PDI is located on the opposite face of the b' domain [9]. This domain in PDI directly binds the substrates for its reductase or isomerase activities. ERp57, instead, needs to associate with calreticulin, which is a lectin and therefore binds glycoproteins, so that ERp57 can in turn subject these to catalysis. However, also ERp57 has been found to interact with high affinity to a number of small ligands [11] and even macromolecules [12, 13], so that other sites must be present. In some cases they have been identified in the a' and a domains (Fig. 1), those containing the thioredoxin-like active sites, although the intervention of the cysteines at these active sites is not always required, since interactions have been detected even with ligands unable to form mixed disulfides.

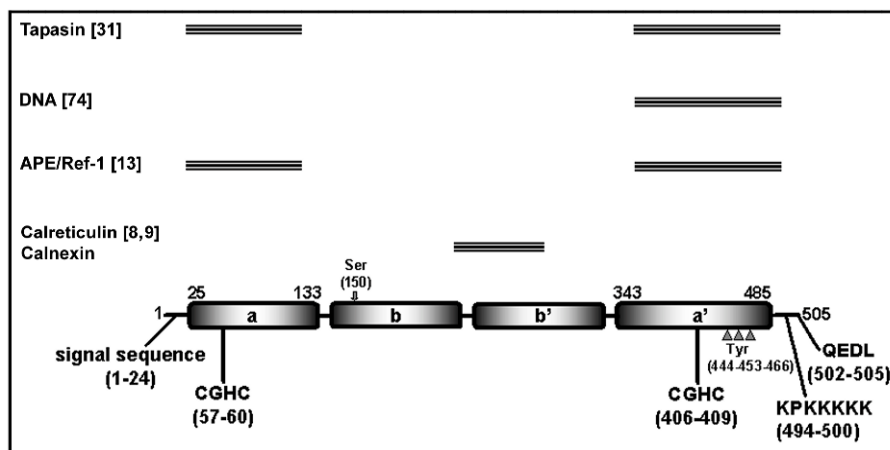


Fig. 1. A schematic representation of the domain structure of ERp57. The most important functional sequences and the experimentally detected phosphorylation sites are shown. The numbering refers to the human protein. The domains interacting with other macromolecules are also schematically indicated.

Post-translationally modified ERp57 has been described: tyrosines 444, 453 and 466 [14] or serine 150 [15] of the rat protein can be phosphorylated, although the effects of these modifications on the properties of the protein are unknown. The protein is very reactive towards xenobiotics and oxidizing reagents. Thus, it is among the few identified targets of the metabolites of aminoacetophen [16] and of halothane [17], and for this property it has been held responsible, at least in part, for the toxic effects of these substances. In regard to oxidizing agents, apart from their effects on the cysteines of the active sites [18], it readily undergoes oxidation also on other amino acid residues [19].

A conformational change has been described, which accompanies the change in the redox state of ERp57 [20], and affects the binding of ERp57 with some ligands, even if the binding does not involve the thioredoxin-like active sites. These sites, instead, participate in the formation of dimeric or polymeric forms of the protein, when this is present in its oxidized form. The biological importance of this phenomenon is not yet understood. However, the conformational change might provide a mechanism by which a variation of the redox potential of the environment affects some biological processes in which ERp57 is involved.

Due to these structural properties, most of which are also present in the other members of the protein disulfide isomerase family, ERp57 evidently shares with these homologous proteins its main functional properties, i.e. the ability to oxidize thiols or reduce disulfide bonds of other proteins, to reshuffle improperly formed disulfide bonds and to display chaperoning activity for the correct folding of proteins in the endoplasmic reticulum [21]. In fact these activities of ERp57 have been described and are now well understood in the endoplasmic reticulum, so only a brief mention of them will be provided in the next section, which will be followed by a description of the less well-known functions displayed outside this subcellular compartment. It should be noted that other enzymatic activities have been attributed to ERp57, i.e. transglutaminase [22], protease [23] and carnitine palmitoyl transferase [24] activities, although their physiological relevance has not been conclusively assessed.

The human protein has been the one most thoroughly investigated. ERp57, however, is present in a nearly identical form in all mammals, and as a highly homologous protein in chicken, in Teleostei and in Amphibia (*Xenopus*). A protein disulfide isomerase with lower homology (around 40% amino acid identity), but still more similar to ERp57 than to PDI, is present in various Arthropoda, such as *Anopheles*, and in Trematoda, such as *Schistosoma*, although the C-terminal lysine-rich sequence constituting the nuclear localization signal is lost. Surprisingly, in *Trichoplax adhaerens*, which is considered the simplest metazoan, ERp57 is present with a relatively high homology (more than 50% identity) and still has the C-terminal lysine-rich sequence.

ERp57 IN THE ENDOPLASMIC RETICULUM

The first specific function of ERp57 in the endoplasmic reticulum to be identified was its participation in the correct folding and in the quality control of neo-synthesized glycoproteins to be secreted or to be localized to the cell membrane. This activity requires the interaction of ERp57 with calreticulin or calnexin, which are responsible for recognizing and binding monoglucosylated proteins [25-27]. The latter are then subjected to the disulfide shuffling process performed by the ERp57 component of the complex. This process requires the intermediate formation of a mixed disulfide between the glycoprotein and the proximal cysteine of one of the two active sites of ERp57. The shuffling is then completed by the intervention of the distal cysteine present in the active sites. This mechanism was exploited for the identification of the glycoproteins which are the substrates of ERp57 *in vivo*, by employing a mutant ERp57 lacking the distal cysteines of each active site, so as to stabilize the mixed disulfide formed between the glycoprotein and ERp57 [28]. The authors thus identified nearly thirty proteins of human fibroblasts that are subjected to the quality control operated by ERp57, and demonstrated that the substrate specificity is provided not only by the glycosylation but also by the presence of common structural domains.

The interaction with calnexin or calreticulin, as mentioned before, takes place through the binding of an arm of these proteins, i.e. the P domain, mainly to the b' domain of ERp57, but also to the C-terminal segment of the b domain [9]. Thus this interaction leaves the a and a' domain free to react with their active sites for the disulfide isomerization process on the glycoproteins substrates.

As a second important function in the ER, ERp57 participates in the assembly of the major histocompatibility complex (MHC) class I [29], catalyzing disulfide formation in heavy chains of histocompatibility molecules and subsequently contributing to the formation of the peptide loading complex (Plc). This is composed of heavy chain class I proteins, β_2 microglobulin, calreticulin, ERp57, tapasin and the transporter associated with antigen presentation (TAP), which transports the peptides originated by the proteasome. In the Plc, ERp57 associates with tapasin [30] in a complex whose structure has been resolved at 2.6 Å resolution [31], providing the first complete 3D structure of ERp57, after a number of partial 3D structures have been obtained. Both a and a' domains of ERp57 interact with tapasin. In the a domain a disulfide bond is formed between cysteines 95 of tapasin and 57 of ERp57, while the a' domain-tapasin interaction is entirely non-covalent, confirming the potentiality of this domain to recognize other macromolecules (Fig. 1).

The tapasin-ERp57 complex already highlights the capability of ERp57 to act in different ways, since, contrary to its participation in glycoprotein folding, calreticulin does not constitute an absolute requirement [32], and the redox activity of ERp57 is not involved in the assembly of the peptide loading complex [32, 33] except for the formation of a stable disulfide bond between cys-57 of

ERp57 and cys-95 of tapasin. Actually a function of this strong complex seems to be the inhibition of the reduction of the disulfide bonds of the heavy chains, which are required for the proper formation of the MHC. Furthermore, the tapasin-ERp57 complex appears to intervene in the assembly and the stabilization of the Plc, so that ERp57 has a structural role rather than a catalytic one. In fact the suppression of ERp57 affects the stability of Plc and decreases both the expression of MHC on the cell surface and the peptide loading within the Plc [34]. ERp57 has also been found to modulate the activity of sarco/endoplasmic reticulum calcium ATPase (SERCA), which transports calcium ions into the endoplasmic reticulum, by regulating the redox state of the sulfhydryl groups in the intraluminal portion of SERCA [35]. Also this function is performed in conjunction with calreticulin.

Some evidence points to the involvement of ERp57 in response to cell infection. Schelhaas *et al.* [36] have shown that the protein participates in the process leading to the entry of SV40 virus into the cell. The virus, in fact, is transported by endocytosis from the cell surface into the endoplasmic reticulum, where it is processed by ERp57, which dissociates the capsid proteins and allows the entry of the virus into the cytosol and then into the nucleus. The capsid is formed by 72 homopentamers of the VP1 protein, which are associated with VP2 or VP3 proteins. This multi-pentameric structure is stabilized by interpentameric disulfide bonds and by calcium ions. When the intact virus reaches the endoplasmic reticulum, ERp57 catalyzes the isomerization of the disulfide bonds of the more external homopentamers, which now become intrachain disulfide bonds. This loosened viral structure is then retrotranslocated to the cytosol, possibly with the contribution of Derlin-1 (Degradation in endoplasmic reticulum protein 1), Sel1L (Protein sel-1 homolog 1) and PDI. In this compartment, the low concentration of calcium ions is likely to complete the dissociation of the capsid pentamers.

Furthermore, a recent review has underlined the ER recruitment onto the cell surface to supply membrane for the process of phagocytosis, and in particular for the formation of nascent phagosomes [37]. This ER-mediated phagocytosis seems to be a general mechanism of entry into macrophages, but it might also occur in other cell types. By a proteomic analysis, several endoplasmic reticulum-resident proteins, including ERp57, were revealed as components of phagosomes. Thus phagocytosis, beside being important for innate immunity through the degradation of pathogens, might also contribute to adaptive immunity, participating directly in the loading of peptides on the MHCs. In fact, it has been proposed that the peptides generated from foreign proteins inside the phagosome might be exported to the cytosol to be further digested by the proteasome, and then reimported into a phagosome, containing not only ERp57, but all the ER components needed for the peptide loading on the MHC class I molecules.

Thus, different types of processes appear to require the intervention of ERp57 in the ER, and, while in the first instance they appear to be widely different, all of

them exploit both the reactivity of the cysteines at the thioredoxin-like active sites and the propensity of ERp57 to associate with other proteins, which is an expected feature of a chaperone protein. As will be seen later, the association of ERp57 with other proteins is the basis for its roles also outside the ER, while the involvement of the thiol groups is not always equally evident in these other locations. It is worth pointing out that the ERp57-calreticulin complex, which has a dissociation constant in the micromolar range [9, 38], has been occasionally observed also outside the endoplasmic reticulum, although with functions that are less well understood than those displayed in the latter location.

ERp57 ON THE CELL SURFACE

Hirano *et al* [39] observed for the first time the exit of ERp57 from the ER, and noticed that the protein was being secreted from 3T3 cells. A number of observations subsequently pointed out that ERp57 could be found on the cell surface or in complexes with cell membrane proteins. This was unexpected, since the gln-glu-asp-leu (QEDL) sequence at the C-terminal of ERp57 resembles the true ER retention signal, which is lys-asp-glu-leu (KDEL), whose presence should hinder the exit of a protein from the endoplasmic reticulum compartment. However, many proteins carrying this signal sequence are in fact found not only on the cell surface but also in other subcellular locations. This might be explained by saturation of the ER retention machinery, or by removal of the lys-asp-glu-leu sequence or by complex formation with other macromolecules [40]. Retrotranslocation has been shown to occur for the exit of calreticulin from the ER [41].

ERp57 has been detected on the surface of the sperm head, and by the use of specific inhibitors it has been demonstrated that it is required for sperm-egg fusion [42]. Its intervention is likely to be related to the thiol-disulfide exchange reactions necessary for the gamete fusion process.

An unexpected function of ERp57 on the cell surface is the binding of the hydroxylated, hormonal form of vitamin D₃, i.e. 1 α ,25-dihydroxycholecalciferol (1 α ,25-(OH)₂D₃, calcitriol) [43], followed by fast activation of non-genomic processes and the internalization and nuclear import of ERp57 itself. This finding was the consequence of a search for a membrane receptor, whose existence was established by ligand binding studies. Rapid responses are not expected to be the result of the involvement of the classical vitamin D receptor (VDR), which operates through binding to DNA and the activation of gene expression, and therefore requires longer times to produce detectable events. The rapid cell response (seconds or minutes) after stimulation with 1 α ,25-(OH)₂D₃ activates numerous cascades of signal transduction, involving signaling proteins such as phospholipases C (PLC) and A₂ (PLA₂), protein kinases C (PKC) and ERK (extracellular response activated kinase), all of which have been shown to respond to the formation of the ERp57-1 α ,25-(OH)₂D₃ complex [44, 45]. PKC α and PKC β are both activated by 1 α ,25-(OH)₂D₃ binding to ERp57 in isolated

intestinal epithelial cells from chicks, with subsequent stimulation of phosphate uptake [43, 46]. ERp57 is also important for the steroid hormone-stimulated calcium uptake in mammalian intestinal cells [47]. ERp57 modulates the vitamin D-mediated anti-cancer activity, specifically in breast cancer; ERp57 knockdown selectively increases the sensitivity of MCF-7 cells to agents related to vitamin D [48]. $1,25(\text{OH})_2\text{D}_3$ stimulates PLA_2 -dependent rapid release of prostaglandin E_2 (PGE_2), activation of protein kinase C (PKC), and regulation of bone-related gene transcription and mineralization in osteoblast-like MC3T3-E1 cells via a mechanism involving ERp57 [45]. These data suggest that ERp57 is an important initiator of $1,25(\text{OH})_2\text{D}_3$ -stimulated membrane signaling pathways, which have both genomic and non-genomic effects during osteoblast maturation [45]. Boyan *et al.* have also shown that the process of extracellular matrix reorganization brought up by chondrocytes is regulated by $1,25(\text{OH})_2\text{D}_3$ interacting with ERp57, contained in the matrix vesicles [44]. In NB4 leukemia cells, ERp57 has been found to interact with NF κ B, possibly at the level of the cell membrane, but certainly in the cytoplasm, and this complex is induced to translocate to the nucleus by the action of $1,25(\text{OH})_2\text{D}_3$ and phorbol ester [49]. This process seems to correlate to the differentiation of leukemic cells.

Two relatively old studies involving cell membrane receptors have also highlighted the involvement of ERp57, although in both cases this protein was still erroneously considered as a phospholipase C. In these studies ERp57 has been shown to interact with the angiotensin II receptor [50] and the vasopressin receptor [51]. However, the fate of ERp57 after the formation of the hormone-receptor complex and its involvement in receptor function have not been investigated. In these papers the real presence of ERp57 is demonstrated by the use of its antibody, and phospholipase activity has been measured and shown to accompany the presence of ERp57. This activity, which is not present in purified or in recombinant ERp57, might be explained by a small amount of real phospholipase C accompanying the complex being investigated. In fact, the phospholipase activity is associated with ERp57 during its purification [52], but is not present in a pure ERp57 preparation [53]. The fact that the association of ERp57 with the angiotensin receptor is not trivial but is likely to possess functional significance is suggested by its phosphorylation following angiotensin binding, as demonstrated in a more recent paper [54].

A detailed description of ERp57 interaction with the all-trans retinoic acid receptor α has been reported [55]. In the Sertoli cells it has been shown that ERp57 associates with the receptor in the cytosol, presumably together with other proteins, and is required for the transport of the ligand-receptor complex into the nucleus, and subsequently into the ER to submit the receptor to the degradation process known as ERAD. The intervention of ERp57 in the receptor activity has been ascribed to a change in the receptor conformation, in such a way as to facilitate the binding of the ligand. In this study, immunofluorescence showed that the ERp57-receptor complex was initially located in the cytosol.

An important and detailed study by Sehgal and collaborators on human hepatoma cells, although not referring to an association of ERp57 with a receptor, nevertheless indicated the participation of this protein in a signal transduction pathway initiating at the cell surface and involving STAT3 [56-58]. STAT3 is a member of the family of STAT signaling and transcription factors, which are activated by kinases associated with receptors for cytokines. The activated forms, after entering into the nucleus, bind to their consensus sequences on DNA and modulate the expression of a variety of genes. STAT3, in particular, is attracting a lot of attention because it is often constitutively activated in a number of tumors, and, while its transcriptional regulatory activity is varied depending on tissue and cell types, its correlation with cancer growth can be explained by the increased expression of genes associated with proliferation, inflammation, and inhibition of apoptosis. When the status of STAT3 in regard to its monomeric or dimeric form was investigated in the cytosol, it was found that it was actually present in a multiprotein complex containing ERp57 [56]. Subsequently ERp57 was found associated with STAT3 also in the lipid raft fraction of cell membrane [57, 58] and, as will be discussed later, in the nucleus [59, 60].

Considering the association of ERp57 with receptors or with signal transduction proteins, and its status as a receptor itself, as in the case of calcitriol, it appears that this protein has an important role in signal transduction processes from the cell surface to the cell interior and/or the nucleus, and it is legitimate to foresee that other instances of such involvement await disclosure. For this function its propensity to bind other proteins, as expected from a chaperone, and to bind small molecules, as recently described, is well suited, while the influence of its redox properties in such processes is still unknown.

ERp57 has also been found to associate with a cell-membrane ion transporter in the tubule cells of the mammalian kidney cortex, where ERp57 is highly expressed [61]. This interaction enhances the activity of the membrane protein, which is the thiazide-sensitive sodium-chloride co-transporter, or solute carrier family 12 member 3.

Recently, an entirely different role of ERp57 on the cell surface has emerged. Studying immunogenic cell death, which is an important factor for a favorable outcome of chemotherapy of cancer cells, the presence of calreticulin on the cell membrane appeared to be essential for this process, which is actually dependent on the amount of this protein on the cell surface. ERp57 did not appear to be directly involved in the process, but was instead found to be necessary for the export of calreticulin from its major location, i.e. the endoplasmic reticulum, to the cell surface [62, 63]. The calreticulin-ERp57 complex, which as mentioned before has a clear functional role in the proper folding of glycoproteins in the endoplasmic reticulum, in this case is instead fundamental for the transit of calreticulin to the cell exterior. The exposure of calreticulin on the cell surface could function as an "eat me signal" for dendritic cells [63]. ERp57 is important for the translocation of CRT but does not possess any immunogenic properties.

ERp57 IN THE CYTOSOL

The presence of ERp57 in the cytosol has been pointed out before when the retinoic acid receptor [55] and STAT3 [56] have been mentioned, and is also required for the transit of ERp57 from the cell membrane to the nucleus upon the binding of calcitriol taking place in the cytosol and also on the cytosolic side of the ER membrane. Furthermore, as mentioned before, a complex of ERp57 and NF κ B has been detected in the cytosol of leukemic cells [49]. The fate of ERp57 bound to the receptors of angiotensin and vasopressin after their internalization has not been investigated, but it is possible that ERp57 remains associated with them in the process.

Considering its thioredoxin-like sites, ERp57 has been thought to be a participant in the mechanisms of cell protection against oxidative stress, regardless of its subcellular location. In view of its relative abundance in the cytosol, such function might indeed be important in this compartment.

A definite implication of the redox properties of ERp57 has emerged from the finding of its interaction with APE/Ref-1, which is a multifunctional protein, acting not only as a reductant for a variety of transcription factors, but also as a protein involved in DNA repair and a transcription factor itself [13]. The interaction appears to take place through the α and α' domains of ERp57 [13] (Fig. 1). Evidence has been presented for the maintenance of the reduced state of Ref-1 by ERp57. The ERp57-Ref-1 complex, furthermore, shuttles between the cytosol and the nucleus, where the reduction of transcription factors is expected to take place.

Further evidence of the importance of ERp57 as a redox-sensing protein involved in regulatory processes has resulted from a very recent study, showing the interaction of ERp57 with mTOR [64], taking place in the cytosol and on the cytosolic side of the ER membrane. mTOR is a serine-threonine protein kinase, found in two multiprotein complexes called mTORC1 and mTORC2. ERp57 interacts preferentially with mTORC1, which is formed by mTOR, Raptor (regulatory-associated protein of mTOR) and mLST8 (mTOR associated protein, LST8 homolog), and which phosphorylates the proteins 4E-BP1 (Eukaryotic translation initiation factor 4E-binding protein 1) and SGK1 (Serine/threonine-protein kinase Sgk1), thus regulating protein synthesis and cell growth. ERp57 contributes to the assembly of mTORC1, activates the kinase activity of mTOR, and also participates in the mechanism by which mTORC1 detects its upstream signals, such as stimulation by insulin or nutrients. Interestingly, ERp57 over-expression induces cellular proliferation, while ERp57 knockdown opposes the proliferation induced by insulin and nutrients. It appears that at least part of this behavior is related to the mTOR-ERp57 interaction, considering that mTOR is involved in the regulation of proliferation. mTORC1 was previously shown to be redox-regulated [65], and ERp57 can now be held responsible for this redox dependence [64]. mTOR is a known inhibitor of autophagy, and its activity is inhibited by starvation, with consequent

stimulation of the autophagic process. Starvation has been shown to induce oxidative stress [66]. Thus, the intervention of ERp57 in mTOR redox regulation might suggest the involvement of ERp57 in autophagy. ERp57 also interacts with mTORC2, although no significant functional alterations of this complex have been reported [64].

ERp57 IN THE NUCLEUS

The first evidence of the nuclear presence of ERp57 emerged from immunofluorescence detection in 3T3 cells and rat spermatids [67] and from a proteomic study of the nuclei of chicken hepatocytes, where ERp57 was found mainly localized in the internal nuclear matrix fraction [52]. The idea of a nuclear localization of ERp57 was at first not easily accepted, not only because the escape of a protein, provided with an ER retention signal, from the endoplasmic reticulum was considered unlikely, but also because of the proximity of the endoplasmic reticulum to the nucleus. The two organelles share a membrane, so contamination of the nucleus with the content of the endoplasmic reticulum, where the protein disulfide isomerases in general and ERp57 in particular are abundant, seemed a likely event. However, the presence of ERp57 in the nucleus has now been firmly established by a number of observations, carried out in different laboratories and with a variety of experimental techniques.

Treatments of various intact mammalian cultured cells with DNA-protein cross-linking agents (formaldehyde, cis-platinum and UV irradiation have been used) have demonstrated that ERp57 and DNA are indeed bound *in vivo*, in experimental conditions respecting the integrity of cellular structures [59, 68]. Immunofluorescence studies performed in a few cases where exceptionally the ERp57 nuclear content was very high confirmed the cross-linking results [52]. The nuclear import of ERp57 in the calcitriol- and phorbol ester-treated leukemia cells was also clearly visible with this technique [49].

Krynetski *et al.* [69] isolated from Nalm6 leukemia cells a nuclear protein complex which recognizes DNA modified by the action of anticancer nucleoside analogues. This complex, formed by HMGB1, HMGB2, HSC70, glyceraldehyde 3-phosphate dehydrogenase and ERp57, recognizes the incorporation of the modified nucleosides into the DNA and is responsible for the resulting genotoxic stress, eventually leading to apoptosis. In particular, ERp57 was found essential for the phosphorylation of histone H2AX, an indicator of genotoxic stress [70].

Interestingly, in a search of nuclear proteins interacting with purified ERp57, the latter was found in HepG2 cells to associate not only with APE/Ref-1, as mentioned before, but also with Ku80, Ku70 and nuclear matrix protein 200/hPso4 [13], all proteins involved in the process of DNA repair, so that somehow ERp57 appears to participate in more than one way in this process, albeit with a still unknown mechanism.

Other ERp57-containing complexes have been described following a proteomic comparative study of paclitaxel-resistant and sensitive ovary cancer cells, where

overexpressed ERp57 was identified as a major marker for chemotherapeutic resistance. ERp57 was found in multimeric nuclear complexes, containing, beside nucleophosmin and nucleolin and other components, structural proteins such as TUBB3 (a specific isotype of tubulin) and β -actin [71, 72]. The interaction of ERp57 with these two proteins appeared to be correlated with paclitaxel resistance.

Filter-binding and South-Western studies revealed that purified ERp57 binds *in vitro* to DNA, so the results from the cross-linking studies were not just due to the proximity of the protein to DNA, but rather to a true interaction [73]. No evidence was found for a high-affinity consensus sequence, but ERp57 interacted preferentially with A/T rich regions, and in general with DNA regions typical of the MARs (nuclear matrix associated regions) [68, 73], thus explaining the enrichment of the nuclear ERp57 in the nuclear matrix fraction [52]. The dissociation constant for a double stranded poly-dA.poly-dT polynucleotide was in the order of 10^{-7} M [73], which indicates a good affinity, although much lower than that usually shown by transcription factors. The DNA binds to the a' domain [74] (Fig. 1) and the binding, interestingly, requires the oxidized form of ERp57 [20, 73].

A chromatin-immunoprecipitation study suggested that this DNA-ERp57 interaction is not a trivial occurrence but has biological significance. In fact the DNA fragments immunoprecipitated with an anti-ERp57 antibody from HeLa and Raji cells cross-linked *in vivo* were enriched in sequences contained in known genes, either in introns or in 5'-flanking regions [75]. Significantly, some of these sequences had characteristics typical of regulatory regions, such as DNase hypersensitivity or structural features of MARs, which are thought to be associated with actively transcribing genes. Furthermore, when a number of enhancers, known to bind STAT3 for the activation of the corresponding genes, were examined, the consensus sequences for STAT3 were found to be associated both with this transcription factor and with ERp57 [60]. Thus, while ERp57 cannot itself be considered as a transcription factor owing to its relatively low affinity for DNA and its lack of stringent sequence specificity, it might act as an accessory protein for transcription regulation, possibly contributing to the formation of the multiprotein complexes present at the DNA regulatory sites or maintaining the transcription factors in their proper redox state. ERp57 is not unique among the protein disulfide isomerases in displaying such a function in the nucleus. PDI has been described as being associated with the estrogen receptor, bound to DNA, thus performing an auxiliary function in the mechanism of gene expression regulation [76].

Concerning the STAT3-ERp57 interaction, it should be noted that some conflicting results have been reported, so that a more extensive investigation is needed to clarify the origins of discrepancies. Thus, Coe *et al.* [77] reported that in mouse embryonic fibroblasts the interaction between the two proteins appeared to take place inside the endoplasmic reticulum. Sehgal and coworkers, instead, working on Hep3B hepatoma cells, offered ample evidence that STAT3

and ERp57 are associated at the level of the cell membrane and in the cytosol [56-58, 78]. However, they described this association as having a negative effect on the binding of STAT3 to DNA. Other evidence, as mentioned before, from M14 melanoma cells and HepG2 hepatoma cells, demonstrated the association of STAT3 and ERp57 also in the nucleus at the level of DNA interaction [59, 60], and showed a dramatic loss of expression of the STAT3-dependent gene *CRP* upon silencing of ERp57 in M14 cells [60]. The last result unequivocally indicates the possibility of positive involvement of ERp57 in the signaling and/or DNA binding of STAT3. Furthermore, *in vitro* experiments showed that inhibitors of ERp57 impaired the binding of STAT3 to its consensus sequence in the *CDKN1A* gene [60]. As a whole, these results show that in some way ERp57 and STAT3 do indeed interact, either functionally or structurally, but there is no agreement on the effects of interaction and on the subcellular locations where it takes place. It can be said that at least in some cell lines the interaction is definitely present on the plasma membrane, in the cytosol and in the nucleus. Further studies are required to understand the cause of these different results, which probably resides either in the experimental conditions used, such as cytokine stimulation, or in the cell type which is investigated. Also the type of interaction between the two proteins should be clarified, since it has not yet been proven that it is a direct one or that it takes place through another interacting protein.

Other data consistent with a role of ERp57 in gene expression are those referring to the nuclear import of ERp57 and NFκB following treatment with calcitriol and phorbol ester in NB4 leukemia cells [49]. The correlation between this nuclear import and the differentiation of the leukemic cells suggested the involvement of these proteins in the process of transcriptional regulation, although it has not been possible to prove the extent or the mode of the contribution of ERp57 to such regulation.

There is also an indirect way by which ERp57 intervenes in the regulation of gene expression, i.e. by means of regulating the redox state of a variety of transcription factors. The interaction of ERp57 with Ref-1 has been mentioned above, and the two proteins appear to cooperate in the activation of a variety of transcription factors, which need to be in their reduced form in order to bind to DNA [13]. The transcription factor E2A, instead, is active in its homodimeric form, stabilized by disulfide bonds. The reducing activity of ERp57, in this case, is responsible for the inactivation of the factor by reducing its disulfide bonds [79].

ERp57 IN MITOCHONDRIA

The presence of ERp57 has been detected in mitochondria, where it associates with a mitochondrial calpain. ERp57 appears to stabilize calpain, opposing its degradation by other mitochondrial proteases [80]. The ERp57-calpain complex acts by catalyzing a partial hydrolysis of the apoptosis-inducing factor (AIF),

which becomes detached from the internal mitochondrial membrane and can be released into the cytosol. Thus ERp57 appears to be involved in a process leading to apoptosis.

ERp57 AS A STRESS-RESPONSE PROTEIN

ERp57 has been initially identified as a protein overexpressed in K12 cells, subjected to a stress caused by glucose depletion. This overexpression response has subsequently been confirmed in many cell types and with different stress-inducing agents [81]. The response is particularly noticeable following the unfolded protein response (UPR) and a protein overload in the endoplasmic reticulum. The origin of this stress response at the level of the ERp57 gene promoter is unclear, since no canonical UPR is present in 2 kbp of the 5'-flanking sequence of the gene. However, consensus sequences for Smad (mothers against decapentaplegic homolog) transcription factors, which are often involved in stress conditions, have been described by Nemere *et al.* [82] in the rat gene promoter, and are also present in the promoter of the human gene. The chaperone and redox activities of overexpressed ERp57 are expected to counteract some deleterious effects of cell stress, such as protein misfolding or damage caused by reactive oxygen species, and a number of studies have confirmed this behavior, although they have also revealed great differences in the response and in the protection provided, depending on the type of stress and on the cells which were examined.

In some cases the knowledge of these effects might even be exploited for a therapeutic intervention, as proposed in a recent study [83], in which the mechanism of action of antitumoral agents was investigated. It was shown that two proapoptotic drugs, bortezomib and fenretinide, act through an ER-stress mechanism, although fenretinide induces the stress by production of reactive oxygen species, while bortezomib does so by inhibition of proteasome. In both cases the homeostatic mechanism of ER, leading to overexpression of stress-response proteins, is only partially able to counteract the commitment to apoptosis induced by the drugs, which is a desirable outcome for their therapeutic action. Apoptosis is instead significantly incremented by the knockout of ERp57 or of other homologous protein disulfide isomerases [83, 84]. It should be noted from this study that ERp57 is a potential target for pharmacological intervention, so a search for its inhibitors appears to be highly desirable.

A further example of the cell-protecting activity of ERp57 emerged from the study of the neurodegenerative disease caused by misfolded prions (PrP^{SC}). There is evidence that PrP^{SC} acts by inducing ER stress in neuronal cells, and also by activating the ER caspase-12. It has been shown that prion replication is accompanied by increased expression of ERp57, and that the damage caused by the prion is inversely related to the amount of ERp57 [85]. As shown by

immunoprecipitation, ERp57 actually interacts with the misfolded prion, as expected from its chaperone properties.

This propensity of ERp57 to associate with unfolded and/or prone-to-aggregate proteins was confirmed by the finding that in the cerebrospinal fluid of normal subjects ERp57, together with calreticulin, forms a complex with the A β peptides [86]. These are derived by the partial splitting of the amyloid precursor protein in brain tissues and are responsible for the formation of the soluble fibrillar aggregates followed by the insoluble fibrillar precipitates, which constitute the plaques typical of Alzheimer's disease. Therefore ERp57 might have a protective role by preventing the self-association of the A β peptides.

However, in other situations, i.e. in other cells or under the effect of other agents, the role of ERp57 as a cell protective agent is not so clear, and the protein can even behave as a damaging molecule. Thus, in endothelial cells under hyperoxic conditions, suppression of ERp57 actually opposes apoptotic events [87].

The ER stress induced by dopamine or by 6-hydroxydopamine (6-OHDA) on neurological tissues or cells has been related to Parkinson's disease [88, 89]. As expected, in these conditions ERp57 is usually overexpressed [88, 90], although not necessarily able to counteract the damaging effects of the stress. In particular, it has been observed that in murine mesencephalic dopaminergic cells (MN9D), treated with 6-OHDA, ERp57 becomes oxidized and self-aggregated, leading to the formation of aggresomes [89], which might be at least in part responsible for the cellular damage found in Parkinson's disease.

CONCLUSIONS

There is now ample evidence that, in addition to its known functions in the ER, ERp57 is engaged in a number of different roles in other subcellular locations (Tab. 1). While its mechanisms of function in the ER are well understood, those involved elsewhere are usually unclear, and even the participation of its thioredoxin-like active sites is not always evident.

Some of these newly reported functions have been confirmed by a number of observations. Such is the case for the involvement of ERp57 in signaling processes, and instances of ERp57 association with regulatory proteins either at the level of the cell membrane or in the cytosol are being described with increasing frequency. A lot of evidence also points towards an indirect and possibly also a more direct mode to its participation in gene expression regulation.

There is no doubt that the role of ERp57 in such processes deserves further investigation. In this connection it must be pointed out that the phosphorylation of ERp57 has received scant attention, although it is likely to play an important role in signal transduction or in other regulatory events. ERp57 is phosphorylated

Tab. 1. Location, activities and functions of ERp57.

Locations	Activity	Function	Ref.
Endo-plasmic reticulum	Disulfide isomerase	Folding monoglucosylated proteins	[25-27]
	Disulfide isomerase	Dissociation of the capsid proteins of SV40	[36]
	Disulfide bond formation	Assembly of the major histo-compatibility complex (MHC) class I	[29]
	Redox activity	Redox state of the sulfhydryl groups in the intraluminal portion of SERCA	[35]
	Interaction with the all-trans retinoic acid receptor α	Submit the receptor to the degradation process known as ERAD	[55]
	Interaction with STAT3, in mouse embryonic fibroblasts	Modulation of STAT3 signaling from the lumen of the endoplasmic reticulum	[77]
Cell surface	Interaction with calcitriol	Fast activation of non-genomic processes of calcitriol (involving PLA_2 ; PKC; ERK) Stimulation of phosphate uptake. Internalization and nuclear import Regulation of bone-related gene transcription and mineralization in osteoblast-like MC3T3-E1 cells	[43-44, 46, 49] [45]
	Interaction with the angiotensin II receptor		[50]
	Interaction with vasopressin receptor		[51]
	Interaction with the all-trans retinoic acid receptor α		[55]
	Interaction with STAT3 in the lipid raft fraction of cell membrane	Participation of a signal transduction pathway initiating at the cell surface	[57, 58]
Cytosol	Interaction with STAT3 in the cytosol	Present in a multiprotein complex	[56-58, 78]
	Interaction with the all-trans retinoic acid receptor α	Transport of the ligand-receptor complex into the nucleus (in the Sertoli cells)	[55]
	Interaction with NF κ B in the cytosol of leukemic cells		[49]
	Redox activity; interaction with Ref-1	Maintenance of the reduced state of Ref-1 (complex, shuttles between the cytosol and the nucleus)	[13]
	Interaction with mTOR	Assembly and functioning of the multiprotein complex mTORC1; Appears to be at least in part responsible for the redox dependence of mTORC1 activity	[64]
Nucleus	Redox activity; interaction with Ref-1	Maintenance of the reduced state of Ref-1 (complex, shuttles between the cytosol and the nucleus)	[13]
	Interaction with STAT3	Binding to enhancers, known to bind STAT3 for the activation of the corresponding genes; involvement in the signaling and/or DNA binding of STAT3	[60]
	Protein complex with HMGB1, HMGB2, HSC70, glyceraldehyde 3-phosphate dehydrogenase and ERp57	Recognition of the incorporation of the modified nucleosides into the DNA (resulting in genotoxic stress, eventually leading to apoptosis); ERp57 is essential for the phosphorylation of histone H2AX	[69-70]
	Interaction with Ku80, Ku70 and nuclear matrix protein 200/hPso4	Participation in more than one process of DNA repair (unknown mechanism)	[13]

Locations	Activity	Function	Ref.
	Presence in multimeric nuclear complexes, containing nucleophosmin, nucleolin, TUBB3 (a specific isotype of tubulin) and β -actin	The interaction appeared to be correlated with paclitaxel resistance	[75-76]
	Interaction with A/T rich regions, and in general with MARs (nuclear matrix associated regions) of DNA; Binding requires the oxidized form of ERp57		[20, 68, 73]
	Redox activity on transcription factor E2A (stabilized by disulfide bonds)	Responsible for the inactivation of the factor by reducing its disulfide bonds	[79]
Mito-chondria	Interaction with calpain	Stabilize calpain, contrasting with its degradation by other mitochondrial proteases; ERp57-calpain complex- catalyzed partial hydrolysis of AIF	[80]

on one or two unidentified sites following angiotensin II binding to its receptor and on a serine by fasting and by leptin treatment in rats [15], and is known to be a substrate of the Lyn protein kinase [14]. While the role of these phosphorylations is still unknown, it is tempting to assume that they might contribute to the interaction of ERp57 with SH2 domains (Src homology 2) present in other proteins, such as STAT3.

Of great interest, although in need of further confirmation, are the observations relating ERp57 to some neurodegenerative diseases, such as Alzheimer's, prion and Parkinson's disease, where the protein appears to act either as a protective agent or, in the case of Parkinson's, as a noxious molecule contributing to the disease. Much is also to be learned about the behavior of ERp57 as a stress-responsive protein and as a protein involved in DNA damage recognition processes.

Finally, a further important aspect of ERp57 function needs to be addressed: the possible participation of calreticulin in ERp57 activities performed outside the ER. The strong affinity between the two proteins, and the presence of calreticulin in different subcellular compartments, including the nucleus, raises the question of the importance of calreticulin in the processes that have been mentioned before, including the signaling events and gene expression regulation.

Acknowledgments. E.G. is recipient of a grant from the “Enrico ed Enrica Sovena” Foundation, Italy.

REFERENCES

1. Turano, C., Coppari, S., Altieri, F. and Ferraro, A. Proteins of the PDI family: unpredicted non-ER locations and functions. **J. Cell. Physiol.** 193 (2002) 154-163.
2. Khanal, R.C. and Nemere, I. The ERp57/GRp58/1,25D3-MARRS receptor: multiple functional roles in diverse cell systems. **Curr. Med. Chem.** 14 (2007) 1087-1093.
3. Coe, H. and Michalak, M. ERp57, a multifunctional endoplasmic reticulum resident oxidoreductase. **Int. J. Biochem. Cell Biol.** 42 (2010) 796-799.
4. Bennett, C.F., Balcarek, J.M., Varrichio, A. and Crooke, S.T. Molecular cloning and complete amino-acid sequence of form-I phosphoinositide-specific phospholipase C. **Nature** 334 (1988) 268-270.
5. Lee, A.S. The accumulation of three specific proteins related to glucose-regulated proteins in a temperature-sensitive hamster mutant cell line K12. **J. Cell. Physiol.** 106 (1981) 119-125.
6. Ferrari, D.M., Söling, H.D. The protein disulphide-isomerase family: unravelling a string of folds. **Biochem. J.** 339 (1999) 1-10.
7. Silvennoinen, L., Myllyharju, J., Ruoppolo, M., Orrù, S., Caterino, M., Kivirikko, K.I. and Koivunen, P. Identification and characterization of structural domains of human ERp57: association with calreticulin requires several domains. **J. Biol. Chem.** 279 (2004) 13607-13615.
8. Russell, S.J., Ruddock, L.W., Salo, K.E., Oliver, J.D., Roebuck, Q.P., Llewellyn, D.H., Roderick, H.L., Koivunen, P., Myllyharju, J. and High, S. The primary substrate binding site in the b' domain of ERp57 is adapted for endoplasmic reticulum lectin association. **J. Biol. Chem.** 279 (2004) 18861-18869.
9. Kozlov, G., Maattanen, P., Schrag, J.D., Pollock, S., Cygler, M., Nagar, B., Thomas, D.Y. and Gehring, K. Crystal structure of the bb' domains of the protein disulfideisomerase ERp57. **Structure** 14 (2006) 1331-1339.
10. Klappa, P., Ruddock, L.W., Darby, N.J. and Freedman, R.B. The b' domain provides the principal peptide-binding site of protein disulfide isomerase but all domains contribute to binding of misfolded proteins. **EMBO J.** 17 (1998) 927-935.
11. Gaucci, E., Chichiarelli, S., Grillo, C., Del Vecchio, E., Eufemi, M. and Turano, C. The binding of antibiotics to ERp57/GRP58. **J. Antibiot.** (Tokyo) 61 (2008) 400-402.
12. Dick, T.P., Bangia, N., Peaper, D.R. and Cresswell, P. Disulfide bond isomerization and the assembly of MHC class I-peptide complexes. **Immunity** 16 (2002) 87-98.
13. Grillo, C., D'Ambrosio, C., Scaloni, A., Maceroni, M., Merluzzi, S., Turano, C. and Altieri, F. Cooperative activity of Ref-1/APE and ERp57 in reductive activation of transcription factors. **Free Radic. Biol. Med.** 41 (2006) 1113-1123.

14. Donella-Deana, A., James, P., Staudenmann, W., Cesaro, L., Marin, O., Brunati, A.M., Ruzzene, M. and Pinna, L.A. Isolation from spleen of a 57-kDa protein substrate of the tyrosine kinase Lyn. Identification as a protein related to protein disulfide-isomerase and localisation of the phosphorylation sites. **Eur. J. Biochem.** 235 (1996) 18-25.
15. Kita, K., Okumura, N., Takao, T., Watanabe, M., Matsubara, T., Nishimura, O. and Nagai, K. Evidence for phosphorylation of rat liver glucose-regulated protein 58, GRP58/ERp57/ER-60, induced by fasting and leptin. **FEBS Lett.** 580 (2006) 199-205.
16. Zhou, L., McKenzie, B.A., Eccleston, E.D. Jr, Srivastava, S.P., Chen, N., Erickson, R.R. and Holtzman, J.L. The covalent binding of [¹⁴C]acetaminophen to mouse hepatic microsomal proteins: the specific binding to calreticulin and the two forms of the thiol:protein disulfide oxidoreductases. **Chem. Res. Toxicol.** 9 (1996) 1176-1182.
17. Martin, J.L., Pumford, N.R., LaRosa, A.C., Martin, B.M., Gonzaga, H.M., Beaven, M.A. and Pohl, L.R. A metabolite of halothane covalently binds to an endoplasmic reticulum protein that is highly homologous to phosphatidylinositol-specific phospholipase C-alpha but has no activity. **Biochem. Biophys. Res. Commun.** 178 (1991) 679-685.
18. Laragione, T., Gianazza, E., Tonelli, R., Bigini, P., Mennini, T., Casoni, F., Massignan, T., Bonetto, V. and Ghezzi, P. Regulation of redox-sensitive exofacial protein thiols in CHO cells. **Biol. Chem.** 387 (2006) 1371-1376.
19. van der Vlies, D., Pap, E.H., Post, J.A., Celis, J.E. and Wirtz, K.W. Endoplasmic reticulum resident proteins of normal human dermal fibroblasts are the major targets for oxidative stress induced by hydrogen peroxide. **Biochem. J.** 366 (2002) 825-830.
20. Grillo, C., D'Ambrosio, C., Consalvi, V., Chiaraluce, R., Scaloni, A., Maceroni, M., Eufemi, M. and Altieri, F. DNA-binding activity of the ERp57 C-terminal domain is related to a redox-dependent conformational change. **J. Biol. Chem.** 282 (2007) 10299-10310.
21. Freedman, R.B., Hirst, T.R. and Tuite, M.F. Protein disulphide isomerase: building bridges in protein folding. **Trends Biochem. Sci.** 19 (1994) 331-336.
22. Okudo, H., Kito, M., Moriyama, T., Ogawa, T. and Urade, R. Transglutaminase activity of human ER-60. **Biosci. Biotechnol. Biochem.** 66 (2002) 1423-1426.
23. Urade, R., Nasu, M., Moriyama, T., Wada, K. and Kito, M. Protein degradation by the phosphoinositide-specific phospholipase C-alpha family from rat liver endoplasmic reticulum. **J. Biol. Chem.** 267 (1992) 15152-15159.
24. Murthy, M.S. and Pande, S.V. A stress-regulated protein, GRP58, a member of thioredoxin superfamily, is a carnitine palmitoyltransferase isoenzyme. **Biochem. J.** 304 (1994) 31-34.

25. Oliver, J.D., van der Wal, F.J., Bulleid, N.J. and High, S. Interaction of the thiol-dependent reductase ERp57 with nascent glycoproteins. **Science** 275 (1997) 86-88.
26. Molinari, M. and Helenius, A. Glycoproteins form mixed disulphides with oxidoreductases during folding in living cells. **Nature** 402 (1999) 90-93.
27. Oliver, J.D., Roderick, H.L., Llewellyn, D.H. and High, S. ERp57 functions as a subunit of specific complexes formed with the ER lectins calreticulin and calnexin. **Mol. Biol. Cell.** 10 (1999) 2573-2582.
28. Jessop, C.E., Chakravarthi, S., Garbi, N., Hämmerling, G.J., Lovell, S. and Bulleid, N.J. ERp57 is essential for efficient folding of glycoproteins sharing common structural domains. **EMBO J.** 26 (2007) 28-40
29. Lindquist, J.A., Jensen, O.N., Mann, M. and Hämmerling, G.J. ER-60, a chaperone with thiol-dependent reductase activity involved in MHC class I assembly. **EMBO J.** 17 (1998) 2186-2195.
30. Dick, T.P., Bangia, N., Peaper, D.R. and Cresswell, P. Disulfide bond isomerization and the assembly of MHC class I-peptide complexes. **Immunity** 16 (2002) 87-98.
31. Dong, G., Wearsch, P.A., Peaper, D.R., Cresswell, P. and Reinisch, K.M. Insights into MHC class I peptide loading from the structure of the tapasin-ERp57 thioredoxin heterodimer. **Immunity** 30 (2009) 21-32.
32. Zhang, Y., Kozlov, G., Pocanschi, C.L., Brockmeier, U., Ireland, B.S., Maattanen, P., Howe, C., Elliott, T., Gehring, K. and Williams, D.B. ERp57 does not require interactions with calnexin and calreticulin to promote assembly of class I histocompatibility molecules, and it enhances peptide loading independently of its redox activity. **J. Biol. Chem.** 284 (2009) 10160-10173.
33. Peaper, D.R. and Cresswell, P. The redox activity of ERp57 is not essential for its functions in MHC class I peptide loading. **Proc. Natl. Acad. Sci. USA** 105 (2008) 10477-10482.
34. Garbi, N., Tanaka, S., Momburg, F., and Hammerling, G.J. Impaired assembly of the major histocompatibility complex class I peptide-loading complex in mice deficient in the oxidoreductase ERp57. **Nat. Immunol.** 7 (2006) 93-102.
35. Li, Y. and Camacho, P. Ca²⁺-dependent redox modulation of SERCA 2b by ERpERp57. **J. Cell Biol.** 164 (2004) 35-46.
36. Schelhaas, M., Malmström, J., Pelkmans, L., Haugstetter, J., Ellgaard, L., Grünewald, K. And Helenius, A. Simian Virus 40 depends on ER protein folding and quality control factors for entry into host cells. **Cell** 131 (2007) 516-529.
37. Desjardins, M. ER-mediated phagocytosis: a new membrane for new functions. **Nat. Rev. Immunol.** 3 (2003) 280-291.
38. Frickel, E.M., Riek, R., Jelesarov, I., Helenius, A., Wuthrich, K. and Ellgaard, L. TROSY-NMR reveals interaction between ERp57 and the tip of the calreticulin P-domain. **Proc. Natl. Acad. Sci. USA** 99 (2002) 1954-1959.

39. Hirano, N., Shibasaki, F., Sakai, R., Tanaka, T., Nishida, J., Yazaki, Y., Takenawa, T. and Hirai, H. Molecular cloning of the human glucose-regulated protein ERp57/GRP58, a thiol-dependent reductase. Identification of its secretory form and inducible expression by the oncogenic transformation. **Eur. J. Biochem.** 234 (1995) 336-342.
40. Johnson, S., Michalak, M., Opas, M. and Eggleton, P. The ins and outs of calreticulin: from the ER lumen to the extracellular space. **Trends Cell Biol.** 11 (2001) 122-129.
41. Afshar, N., Black, B.E. and Paschal, B.M. Retrotranslocation of the chaperone calreticulin from the endoplasmic reticulum lumen to the cytosol. **Mol. Cell. Biol.** 25 (2005) 8844-8853.
42. Ellerman, D.A., Myles, D.G. and Primakoff P. A role for sperm surface protein disulfide isomerase activity in gamete fusion: evidence for the participation of ERp57. **Dev. Cell.** 10 (2006) 831-837.
43. Nemere, I., Farach-Carson, M.C., Rohe, B., Sterling, T.M., Norman, A.W., Boyan, B.D. and Safford, S.E. Ribozyme knockdown functionally links a 1,25(OH)2D3 membrane binding protein (1,25D3-MARRS) and phosphate uptake in intestinal cells. **Proc. Natl. Acad. Sci. USA** 101 (2004) 7392-7397.
44. Boyan, B.D., Wong, K.L., Fang, M. and Schwartz, Z. 1 α ,25(OH)2D3 is an autocrine regulator of extracellular matrix turnover and growth factor release via ERp60 activated matrix vesicle metalloproteinases. **J. Steroid. Biochem. Mol. Biol.** 103 (2007) 467-472.
45. Chen, J., Olivares-Navarrete, R., Wang, Y., Herman, T.R., Boyan, B.D. and Schwartz, Z. Protein-disulfide isomerase-associated 3 (Pdia3) mediates the membrane response to 1,25-dihydroxyvitamin D3 in osteoblasts. **J. Biol. Chem.** 285 (2010) 37041-37050.
46. Tunsophon, S. and Nemere, I. Protein kinase C isoforms in signal transduction for the 1,25D3-MARRS receptor (ERp57/PDIA3) in steroid hormone-stimulated phosphate uptake. **Steroids** 75 (2010) 307-313.
47. Nemere, I., Garbi, N., Hämmerling, G.J. and Khanal, R.C. Intestinal cell calcium uptake and the targeted knockout of the 1,25D3-MARRS (membrane-associated, rapid response steroid-binding) receptor/PDIA3/Erp57. **J. Biol. Chem.** 285 (2010) 31859-31866.
48. Richard, C.L., Farach-Carson, M.C., Rohe, B., Nemere, I. and Meckling, K.A. Involvement of 1,25D3-MARRS (membrane associated, rapid response steroid-binding), a novel vitamin D receptor, in growth inhibition of breast cancer cells. **Exp. Cell Res.** 316 (2010) 695-703.
49. Wu, W., Beilhartz, G., Roy, Y., Richard, C.L., Curtin, M., Brown, L., Cadieux, D., Coppolino, M., Farach-Carson, M.C., Nemere, I. and Meckling, K.A. Nuclear translocation of the 1,25D3-MARRS (membrane associated rapid response to steroids) receptor protein and NFkappaB in differentiating NB4 leukemia cells. **Exp. Cell Res.** 316 (2010) 1101-1108.

50. Mah, S.J., Ades, A.M., Mir, R., Siemens, I.R., Williamson, J.R. and Fluharty, S.J. Association of solubilized angiotensin II receptors with phospholipase C-alpha in murine neuroblastoma NIE-115 cells. **Mol. Pharmacol.** 42 (1992) 217-226.
51. Aiyar, N., Bennett, C.F., Nambi, P., Valinski, W., Angioli, M., Minnich, M. and Crooke, S.T. Solubilization of rat liver vasopressin receptors as a complex with a guanine-nucleotide-binding protein and phosphoinositide-specific phospholipase C. **Biochem. J.** 261 (1989) 63-70.
52. Altieri, F., Maras, B., Eufemi, M., Ferraro, A. and Turano, C. Purification of a 57kDa nuclear matrix protein associated with thiol:protein-disulfide oxidoreductase and phospholipase C activities. **Biochem. Biophys. Res. Commun.** 194 (1993) 992-1000.
53. Srivastava, S.P., Fuchs, J.A. and Holtzman, J.L. The reported cDNA sequence for phospholipase C alpha encodes protein disulfide isomerase, isozyme Q-2 and not phospholipase-C. **Biochem. Biophys. Res. Commun.** 193 (1993) 971-978.
54. Tokutomi, Y., Araki, N., Kataoka, K., Yamamoto, E. and Kim-Mitsuyama, S. Oxidation of Prx2 and phosphorylation of GRP58 by angiotensin II in human coronary smooth muscle cells identified by 2D-DIGE analysis. **Biochem. Biophys. Res. Commun.** 364 (2007) 822-830.
55. Zhu, L., Santos, N.C. and Kim, K.H. Disulfide isomerase glucose-regulated protein 58 is required for the nuclear localization and degradation of retinoic acid receptor alpha. **Reproduction** 139 (2010) 717-731.
56. Ndubuisi, M.I., Guo, G.G., Fried, V.A., Etlinger, J.D. and Sehgal, P.B. Cellular physiology of STAT3: Where's the cytoplasmic monomer? **J. Biol. Chem.** 274 (1999) 25499-25509.
57. Sehgal, P.B., Guo, G.G., Shah, M., Kumar, V. and Patel, K. Cytokine signaling: STATS in plasma membrane rafts. **J. Biol. Chem.** 277 (2002) 12067-12074.
58. Guo, G.G., Patel, K., Kumar, V., Shah, M., Fried, V.A., Etlinger, J.D. and Sehgal, P.B. Association of the chaperone glucose-regulated protein 58 (GRP58/ER-60/ERp57) with Stat3 in cytosol and plasma membrane complexes. **J. Interferon Cytokine Res.** 22 (2002) 555-563.
59. Eufemi, M., Coppari, S., Altieri, F., Grillo, C., Ferraro, A. and Turano, C. ERp57 is present in STAT3-DNA complexes. **Biochem. Biophys. Res. Commun.** 323 (2004) 1306-1312.
60. Chichiarelli, S., Gaucci, E., Ferraro, A., Grillo, C., Altieri, F., Cocchiola, R., Arcangeli, V., Turano, C. and Eufemi, M. Role of ERp57 in the signaling and transcriptional activity of STAT3 in a melanoma cell line. **Arch. Biochem. Biophys.** 494 (2010) 178-183.
61. Wyse, B., Ali, N. and Ellison, D.H. Interaction with grp58 increases activity of the thiazide-sensitive Na-Cl cotransporter. **Am. J. Physiol. Renal Physiol.** 282 (2002) F424-430.

62. Panaretakis, T., Joza, N., Modjtahedi, N., Tesniere, A., Vitale, I., Durchschlag, M., Fimia, G.M., Kepp, O., Piacentini, M., Froehlich, K.U., van Endert, P., Zitvogel, L., Madeo, F. and Kroemer, G. The co-translocation of ERp57 and calreticulin determines the immunogenicity of cell death. **Cell Death Differ.** 15 (2008) 1499-1509.
63. Obeid, M. ERP57 membrane translocation dictates the immunogenicity of tumor cell death by controlling the membrane translocation of calreticulin. **J. Immunol.** 181 (2008) 2533-2543.
64. Ramírez-Rangel, I., Bracho-Valdés, I., Vázquez-Macías, A., Carretero-Ortega, J., Reyes-Cruz, G. and Vázquez-Prado, J. Regulation of mTORC1 complex assembly and signaling by GRp58/ERp57. **Mol. Cell. Biol.** 31 (2011) 1657-1671.
65. Sarbassov, D.D. and Sabatini, D.M. Redox regulation of the nutrient-sensitive raptor-mTOR pathway and complex. **J. Biol. Chem.** 280 (2005) 39505-39509.
66. Scherz-Shouval, R., Shvets, E., Fass, E., Shorer, H., Gil, L. and Elazar, Z. Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. **EMBO J.** 26 (2007) 1749-1760.
67. Ohtani, H., Wakui, H., Ishino, T., Komatsuda, A. and Miura, A.B. An isoform of protein disulfide isomerase is expressed in the developing acrosome of spermatids during rat spermiogenesis and is transported into the nucleus of mature spermatids and epididymal spermatozoa. **Histochemistry** 100 (1993) 423-429.
68. Coppari, S., Altieri, F., Ferraro, A., Chichiarelli, S., Eufemi, M. and Turano, C. Nuclear localization and DNA interaction of protein disulfide isomerase ERp57 in mammalian cells. **J. Cell. Biochem.** 85 (2002) 325-333.
69. Krynetski, E.Y., Krynetskaia, N.F., Bianchi, M.E. and Evans, W.E. A nuclear protein complex containing high mobility group proteins B1 and B2, heat shock cognate protein 70, ERp60, and glyceraldehyde-3-phosphate dehydrogenase is involved in the cytotoxic response to DNA modified by incorporation of anticancer nucleoside analogues. **Cancer Res.** 63 (2003) 100-106.
70. Krynetskaia, N.F., Phadke, M.S., Jadhav, S.H. and Krynetskiy, E.Y. Chromatin-associated proteins HMGB1/2 and PDIA3 trigger cellular response to chemotherapy-induced DNA damage. **Mol. Cancer Ther.** 8 (2009) 864-872.
71. Cicchillitti, L., Di Michele, M., Urbani, A., Ferlini, C., Donat, M.B., Scambia, G. and Rotilio, D. Comparative proteomic analysis of paclitaxel sensitive A2780 epithelial ovarian cancer cell line and its resistant counterpart A2780TC1 by 2D-DIGE: the role of ERp57. **J. Proteome Res.** 8 (2009) 1902-1912.
72. Cicchillitti, L., Della Corte, A., Di Michele, M., Donati, M.B., Rotilio, D. and Scambia, G. Characterisation of a multimeric protein complex associated with ERp57 within the nucleus in paclitaxel-sensitive and -resistant

- epithelial ovarian cancer cells: the involvement of specific conformational states of beta-actin. **Int. J. Oncol.** 37 (2010) 445-454.
73. Ferraro, A., Altieri, F., Coppari, S., Eufemi, M., Chichiarelli, S. and Turano, C. Binding of the protein disulfide isomerase isoform ERp60 to the nuclear matrix-associated regions of DNA. **J. Cell. Biochem.** 72 (1999) 528-539.
74. Grillo, C., Coppari, S., Turano, C. and Altieri, F. The DNA-binding activity of protein disulfide isomerase ERp57 is associated with the a(') domain. **Biochem. Biophys. Res. Commun.** 295 (2002) 67-73.
75. Chichiarelli, S., Ferraro, A., Altieri, F., Eufemi, M., Coppari, S., Grillo, C., Arcangeli, V. and Turano, C. The stress protein ERp57/GRP58 binds specific DNA sequences in HeLa cells. **J. Cell. Physiol.** 210 (2007) 343-351.
76. Schultz-Norton, J.R., McDonald, W.H., Yates, J.R. and Nardulli, A.M. Protein disulfide isomerase serves as a molecular chaperone to maintain estrogen receptor alpha structure and function. **Mol. Endocrinol.** 20 (2006) 1982-1995.
77. Coe, H., Jung, J., Groenendyk, J., Prins, D. and Michalak, M. ERp57 modulates STAT3 signaling from the lumen of the endoplasmic reticulum. **J. Biol. Chem.** 285 (2010) 6725-6738.
78. Sehgal, P.B. Plasma membrane rafts and chaperones in cytokine/STAT signaling. **Acta Biochim. Pol.** 50 (2003) 583-594.
79. Markus, M. and Benezra, R. Two isoforms of protein disulfide isomerase alter the dimerization status of E2A proteins by a redox mechanism. **J. Biol. Chem.** 274 (1999) 1040-1049.
80. Ozaki, T., Yamashita, T. and Ishiguro, S. ERp57-associated mitochondrial micro-calpain truncates apoptosis-inducing factor. **Biochim. Biophys. Acta** 1783 (2008) 1955-1963.
81. Murray, J.I., Whitfield, M.L., Trinklein, N.D., Myers, R.M., Brown, P.O. and Botstein, D. Diverse and specific gene expression responses to stresses in cultured human cells. **Mol. Biol. Cell** 15 (2004) 2361-2374.
82. Rohe, B., Safford, S.E., Nemere, I., Farach-Carson, M.C. Regulation of expression of 1,25D3-MARRS/ERp57/PDIA3 in rat IEC-6 cells by TGF beta and 1,25(OH)2D3. **Steroids** 72 (2007) 144-150.
83. Corazzari, M., Lovat, P.E., Armstrong, J.L., Fimia, G.M., Hill, D.S., Birch-Machin, M., Redfern, C.P. and Piacentini, M. Targeting homeostatic mechanisms of endoplasmic reticulum stress to increase susceptibility of cancer cells to fenretinide-induced apoptosis: the role of stress proteins ERdj5 and ERp57. **Br. J. Cancer** 96 (2007) 1062-1071.
84. Lovat, P.E., Corazzari, M., Armstrong, J.L., Martin, S., Pagliarini, V., Hill, D., Brown, A.M., Piacentini, M., Birch-Machin, M.A. and Redfern, C.P. Increasing melanoma cell death using inhibitors of protein disulfide isomerases to abrogate survival responses to endoplasmic reticulum stress. **Cancer Res.** 68 (2008) 5363-5369.
85. Hetz, C., Russelakis-Carneiro, M., Wälchli, S., Carboni, S., Vial-Knecht, E., Maundrell, K., Castilla, J. and Soto, C. The disulfide isomerase Grp58 is

- a protective factor against prion neurotoxicity. **J. Neurosci.** 25 (2005) 2793-2802.
86. Erickson, R.R., Dunning, L.M., Olson, D.A., Cohen, S.J., Davis, A.T., Wood, W.G, Kratzke, R.A. and Holtzman, J.L. In cerebrospinal fluid ER chaperones ERp57 and calreticulin bind beta-amyloid. **Biochem. Biophys. Res. Commun.** 332 (2005) 50-57.
87. Xu, D., Perez, R.E., Rezaiekhalthigh, M.H., Bourdi, M. and Truog, W.E. Knockdown of ERp57 increases BiP/GRP78 induction and protects against hyperoxia and tunicamycin-induced apoptosis. **Am. J. Physiol. Lung Cell. Mol. Physiol.** 297 (2009) L44-51.
88. Dukes, A.A., Van Laar, V.S., Cascio, M. and Hastings, T.G. Changes in endoplasmic reticulum stress proteins and aldolase A in cells exposed to dopamine. **J. Neurochem.** 106 (2008) 333-346.
89. Kim-Han, J.S. and O'Malley, K.L. Cell stress induced by the parkinsonian mimetic, 6-hydroxydopamine, is concurrent with oxidation of the chaperone, ERp57, and aggresome formation. **Antioxid. Redox Signal.** 9 (2007) 2255-2264.
90. Akazawa, Y.O., Saito, Y., Nishio, K., Horie, M., Kinumi, T., Masuo, Y., Yoshida, Y., Ashida, H. and Niki, E. Proteomic characterization of the striatum and midbrain treated with 6-hydroxydopamine: alteration of 58-kDa glucose-regulated protein and C/EBP homologous protein. **Free Radic. Res.** 44 (2010) 410-421.