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Forum Review

ERp29, an Unusual Redox-Inactive Member of the Thioredoxin Family

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ABSTRACT

Oxidative folding in the endoplasmic reticulum is accomplished by a group of oxidoreductases where the protein disulfide isomerase (PDI) plays a key role. Structurally, redox-active PDI domains, like many other enzymes utilizing cysteine chemistry, adopt characteristic thioredoxin folds. However, this structural unit is not necessarily associated with the redox function and the current review focuses on the interesting example of a loss-of-function PDI-like protein from the endoplasmic reticulum, ERp29. ERp29 shares a common predecessor with PDI; however in the course of divergent evolution it has lost a hallmark active site motif of redox enzymes but retained the characteristic structural fold in one of its domains. Although the functional characterization of ERp29 is far from completion, all available data point to its important role in the early secretory pathway and allow tentative categorization as a secretion factor/escort protein of a broad profile. *Antioxid. Redox Signal.* 8, 325–337.

INTRODUCTION

IN VITRO STUDIES demonstrated that the amino acid sequence *per se* is sufficient to determine the native conformation of polypeptides (3, 20). However, in the highly congested environment of the protein-synthesizing cellular compartments, nascent polypeptide chains are prone to nonspecific aggregations, which necessitated the evolution of a network of special folding assistants, molecular chaperones. They protect newly synthesized or misfolded proteins by transient noncovalent associations to exposed hydrophobic patches and thereby increase the efficiency of folding (28, 30, 31). In addition to such noncatalytic interactions, the tertiary structure of many proteins is stabilized via the oxidation of cysteines and formation of the intramolecular disulfide bridges (24, 87, 94). In eukaryotes, such oxidative folding occurs predominantly in the endoplasmic reticulum (ER), the compartment with a main responsibility for biosynthesis, folding, and posttranslational modifications of the secretory and membrane proteins.

Disulfide bond formation in the ER is catalyzed by a number of protein:thiol oxidoreductases, such as protein disulfide isomerase (PDI), ERp72, ERp57, and P5-like PDIs, with a PDI as an enzyme of key importance (26, 102). Extensive isomerization and even reduction of disulfide bonds is required for some complex proteins in the ER in order to attain the native conformation. PDI catalyzes such reactions by virtue of its active site double cysteine motif (Cys-X-X-Cys), which is constantly reoxidized by Ero1p (66, 94) and its assistant, ERp44 (2). PDI consists of four domains (***a-b-b´-a´***), of which two (***a, a´***) are redox-active (containing the double cysteine motif) and two (***b, b´***) lack the active site sequence (41).

The ***a*** domain and even the redox-inactive ***b*** domain of PDI show significant structural similarity to thioredoxin, a small protein involved in many cytoplasmic redox functions (6, 35). The central core of thioredoxin is made of a four-stranded mixed β-sheet, surrounded by two α-helices from both sides of the β-sheet. This fold is characteristic for the majority of redox enzymes dealing with reactive dithiols, such as thioredoxins, glutaredoxins, PDIs, and bacterial Dsb family proteins (54).

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Although PDI was initially characterized as an oxidoreductase, it is a multifunctional protein that may also act as a general chaperone assisting in folding and stabilization of nascent polypeptides (27, 96, 97). PDI is a subunit of prolyl 4-hydroxylase (70) and microsomal triglyceride isomerase (101) where its function is not well understood but it appears to play a stabilizing rather than enzymatic role. Even the *Escherichia coli* thioredoxin is involved in redox-independent interactions, functioning, for instance, as a processivity factor of a phage T7 DNA polymerase (38).

It may be thus concluded that redox function and characteristic thioredoxin structural motif are not always tightly associated. Indeed, there are a number of thioredoxin fold-containing proteins with diverse functions, entirely unrelated to the redox activity (Table 1). It seems that the thioredoxin unit may serve as a basic module for a variety of biological functions confirming the parsimonious approach of nature in creating new structural elements. Discussions on this theme suggest that during evolution, possibly following gene duplications, the gene product may be released from functional constraints to evolve a new function, retaining, however, the conserved three-dimensional structure (19, 64, 72, 91).

This review will further focus on one of the members of this "odd" family, ERp29, a putative endoplasmic reticulum escort chaperone/secretion factor, the apparent product of divergent evolution that has lost a hallmark sequence motif of the redox enzymes but retained the characteristic structural fold in one of its domains. Although a structural relative of PDI, functionally ERp29 seems to have little in common not only with this foldase but also with the other members of the protein folding/secretion machinery of the ER. The ubiquitous expression, high degree of conservation in mammals and other features suggest an important housekeeping function of ERp29, apparently establishing a new class of ER folding/secretory assistants. Here we present the analysis of the known traits of ERp29 and its close relative, Wind, that provides interesting insights into the evolutionary aspects and structure–function relationships of these ER proteins.

ERp29, INITIAL FINDINGS

A proteomic study was the first to reveal the existence of ERp29 as a novel 20 amino acid-long N-terminal sequence from a human liver 2-D gel spot (39). Later, three laboratories almost simultaneously reported isolation of ERp29 from the rat liver, rat dental enamel cells, and human liver (18, 23, 25, 59). The electrophoretic migration of the protein (~29 kDa) determined its name (Endoplasmic Reticulum protein 29 kDa), however the SDS-PAGE behavior does not reflect accurately the molecular mass of the mature protein (24.5 kDa, 228 amino acids, rat sequence). This discrepancy might be attributed to the specifics of SDS-PAGE rather than to post-translational modifications (36). *In silico* methods predicted the existence of two domains nearly identical in size and intracellular topology of the protein. Localization was suggested by the ER-targeting hydrophobic N-terminal leader sequence and the C-terminal tetrapeptide, KEEL, the conserved variant of the KDEL retrieval signal, characteristic for the soluble ER luminal proteins. This tetrapeptide is recognized by the Golgi-localized receptor that recycles them back to the ER (63, 84). The predicted ER localization of ERp29 was confirmed by immunofluorescent microscopy and biochemical cell fractionation (25, 37, 59).

The primary structure failed to suggest the biological function of ERp29 despite the similarity between the N-terminal domain of ERp29 and the thioredoxin domains of various PDIs, due to the absence of the characteristic double cysteine active site motif. Consequently, the disulfide reductase and isomerase assays could not reveal any PDI-like enzymatic activity (36). Similarly no activity related to the thioredoxin-like enzymes utilizing cysteine chemistry, such as glutathione-S-transferase, glutathione peroxidase, and glutaredoxin were observed with recombinant (50) nor with native ERp29 (36). After publication of the first papers on ERp29, a developmental study in *Drosophila melanogaster* (fruit fly) identified one of the essential genes, *windbeutel*, responsible for the formation of the dorsal-ventral polarity of the embryo (47). *Windbeutel* is expressed for a short period of time in the ER of the ovarian follicle cells, at around the time when the dorsoventral axis of the egg chamber is first established. The gene product of *windbeutel*, Wind, is responsible for the escorting of Pipe, a glycosaminoglycan-modifying enzyme to Golgi where it directs the ventral activation of an extracellular serine proteolytic cascade defining the ventral side of the embryo (85). Wind and ERp29 share >30% of sequence identity and have similar domain structures. Despite the presence of the PDI-like double cysteine motif (albeit not in a canonical position), Wind does not display any redox activity and therefore was suggested to have a dedicated escort chaperone role rather than enzymatic function (10).

Certain posttranslational modifications, such as *N*-glycosylation, ATP-dependency and calcium binding have functional significance for many ER luminal proteins. However, *in vitro* translation studies did not reveal any glycosylated forms of ERp29 (59). ERp29 is neither retained on ATP-agarose nor possesses ATP-ase activity (25). Although the recombinant protein is easily phosphorylated *in vitro*, labeling of rat hepatoma cells and immunoprecipitation of ERp29 failed to demonstrate *in vivo* phosphorylation (our unpublished observations). Finally, Ca^{45}-overlay assay showed no calcium binding by ERp29 (25, 36).

TISSUE DISTRIBUTION

However symptomatic, co-localization of ERp29 with the members of the ER folding/secretion machinery is not enough to deduce its biological role. More informative is the tissue distribution, which showed the ubiquitous pattern of ERp29 expression and especially high levels in actively secreting tissues, such as pituitary, adrenal, mammary, thyroid and salivary glands, prostate, pancreas, and additionally, liver and kidney (80). Such a pattern of expression is quite typical for the constitutively expressed genes of ER chaperones (13, 89). Apparently, ERp29 expression commences at the early stages of development, as mRNA was detected in all of the tested fetal tissues (80). In comparative expression analysis of ERp29, PDI, and BiP, the major ER molecular chaperone showed nearly equimolar levels of all these proteins in the se-

cretory tissues (37). These data implicitly suggest the functional role of ERp29 in the early secretory pathway.

Although ERp29 levels are not particularly high in brain, three recent proteomic and immunochemical studies found that it is enriched in cerebellum especially in Purkinje neurons (52, 86, 93). Cerebellum is not conventionally regarded as a neurosecretory tissue and ERp29 correlated poorly with classical markers of neurosecretion, but strongly with a variety of major membrane proteins. Thus, the authors hypothesize that ERp29 is involved primarily in the production of endomembrane proteins rather than proteins destined for the cell exterior (52).

GENE STRUCTURE AND EXPRESSION

Human ERp29 gene is mapped to chromosome 12. Additionally, an intronless pseudogene is located on chromosome 20. The rat gene is found on chromosome 12 and another ERp29-like gene is mapped to chromosome 17. The latter gene product is predicted to be shorter (205 aa) than the functional polypeptide and is either not translated at all or is rapidly degraded as it has not been detected immunochemically (12, 58, 59, 82). The other possibility is that similar to the human pseudogene it may not be even transcribed, which is supported by a single band in Northern blot (18, 59). The 5′-flanking region of the functional gene contains a conserved CpG island (80) that is frequently found close to the transcription initiation sites of the housekeeping genes (49). Canonical TATA-like sequences and CAAT boxes, the elements that commonly specify the transcription start site, are absent from the promoter. However, two GC boxes are mapped immediately upstream of one of the putative transcription start sites. Similar TATA-less, GC-rich promoters are often found in constitutively active genes (21). Sp1 binding to GC boxes in such promoters is critical for transcription initiation (73, 74), which is often directed from multiple sites. Both features were also demonstrated for the ERp29 gene (80). The basic regulation of ERp29 gene expression seems to be well conserved in mammals judging by nearly 60% identical promoters of the human, mouse, and rat genes and gene reporter data (80).

Search for alternative splice sites did not reveal any such motifs, which is consistent with the single transcript band in Northern blot. The absence of significant genetic variations in the exons and splice junctions affecting the primary structure of the polypeptide (as judged by the searches in the comprehensive SNP databases of NCBI and Celera (http://www.celera.com)) implies the importance of the sequence conservation for the predicted housekeeping function of ERp29.

EVOLUTIONARY EXPRESSION

ERp29 has an interesting pattern of evolutionary expression. ERp29-like genes are absent in prokaryotic organisms and monocellular eukaryotes and first emerge in Bilateria, the branch of Metazoa (multicellular organisms). Screening of the nearly-complete genomes of metazoans identifies ERp29s in arthropods (fruit fly, mosquito, honeybee) and mammals (Fig. 1). A gene strikingly similar to the mammalian ERp29s is present even in such a simple bilaterian as *Schmidtea mediterranea*, a freshwater flatworm that belongs to the phylum Platyhelminthes (Figs. 1 and 2). However, ERp29 is absent from the *C. eleganse* (nematodes) genome. Similarly, no obvious ERp29-like sequences can be traced in the sequenced fish genomes. This is quite surprising as mammals and fish belong to the same taxonomic unit Euteleostomi (bony vertebrates). Nevertheless, we found an interesting gene in the *Danio rerio* (zebrafish) genome (GenBank, accession AAH76464) that encodes a protein with unknown function with a KEEL ER-retrieval signal and an ER-targeting N-terminal signal peptide. It is clustered with human PDI on the dendrogram (Fig. 2) apparently due to the presence of four thioredoxin-like domains, of which the fold recognition methods predict characteristic PDI-like ***a*** and ***b*** domains. A similar gene is found in the freshwater puffer fish, *Tetraodon nigroviridis* (GenBank, CAF93956). Interestingly, these proteins contain no active redox motifs, which implicitly suggest their intermediate position between the redox-active PDIs and ERp29.

The phylogenetic tree suggests common origin of the ERp29 family and a group of PDIs from various organisms (Fig. 2). Several P5-like PDIs (26) from plants (represented by the protein from alfalfa) and protists (represented by the amoebal PDI), have a similar domain structure to ERp29s with one or two thioredoxin domains and a unique C-terminal domain, which position them closer to ERp29 than the mammalian PDIs.

The strong similarity between the mammalian and flatworm genes, the existence of ERp29-like genes in arthropods, and its absence in nematodes seemingly present an evolutionary puzzle. According to textbook phylogeny, all these metazoan species are organized in the hierarchical groups reflecting the increased complexity of their body plans, such as coelomata (arthropods and chordata), acoelomata (flatworms), and pseudocoelomata (nematodes). However, current evolutionary models based on molecular phylogeny present a more simple tree of metazoans, comprising only a small number of major branches (1). Thus, acoelomates and pseudocoelomates are included in Lophotrochozoa, which is a sister group to Ecdysozoa (arthropods and nematodes) within a large assemblage of Protostome invertebrates. According to this model, platyhelminths emerged later than it was thought previously, after the split of protostomes from deuterostomes (the metazoan taxon that includes vertebrates) (9). This explains better why, for instance, such simple organisms as flatworms share large sets of genes (including ERp29) with vertebrates, which they apparently inherited from the common ancestor. According to the same model, the absence of some of these genes in nematodes might simply reflect certain degenerative processes depending on the particular mode of development of these organisms (1).

In summary, phylogenetic analysis argues in favor of the hypothetical secretory role of ERp29 assuming that the protein export function is most extensively developed in multicellular organisms. It is conceivable that the split of ERp29 from PDI occurred due to the further differentiation of the secretory apparatus and rising demand for a novel, more

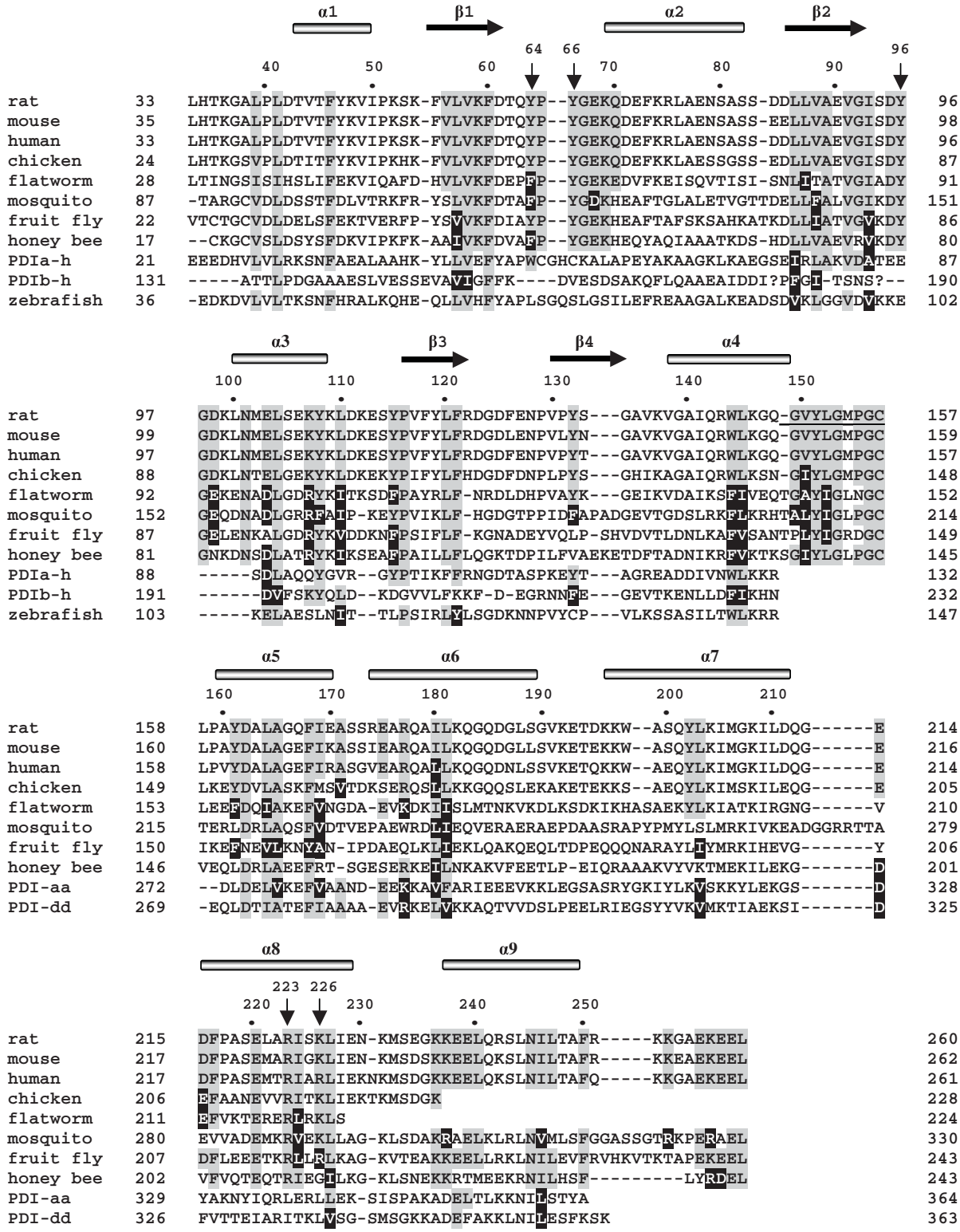

FIG. 1. Alignment of ERp29-like proteins from different species. *Bars* on the top indicate the positions of regular secondary structure elements in the rat ERp29 (50). Alignments are shown for ERp29s from mammals (rat, *Rattus norvegicus*, accession P52555, mouse, *Mus musculus*, P57759, human, *Homo sapiens*, P30040), chicken (*Gallus gallus*, CAG32394) insects (fruit fly, *Drosophila melanogaster*, AAC02944, mosquito, *Anopheles gambiae*, XP_310706, honey bee, *Apis mellifera*, XP_392944), flatworm (*Schmidtea mediterranea*, AY068176), ***a*** and ***b*** domains of human PDI (PDB entries 1mek and 2bjx), C-terminal domains of the P5-like PDIs from the plant alfalfa (PDI-aa, *Medicago sativa*, P38661) and soil-living amoeba (PDI-dd, *Dictyostelium discoideum*, AAB86685), and PDI-like protein from zebrafish (*Danio rerio*, AAH76464). Light shading indicates identical residues, dark shading (white letters), conservative changes. The GenBank entries for the flatworm and chicken ERp29s are missing parts of their C-terminal sequences. The linker residues between the N- and C-terminal domains in ERp29 are *underlined*. Arrows indicate conserved residues involved in the interaction between the ERp29-like protein from *Drosophila,* Wind and its partner Pipe.

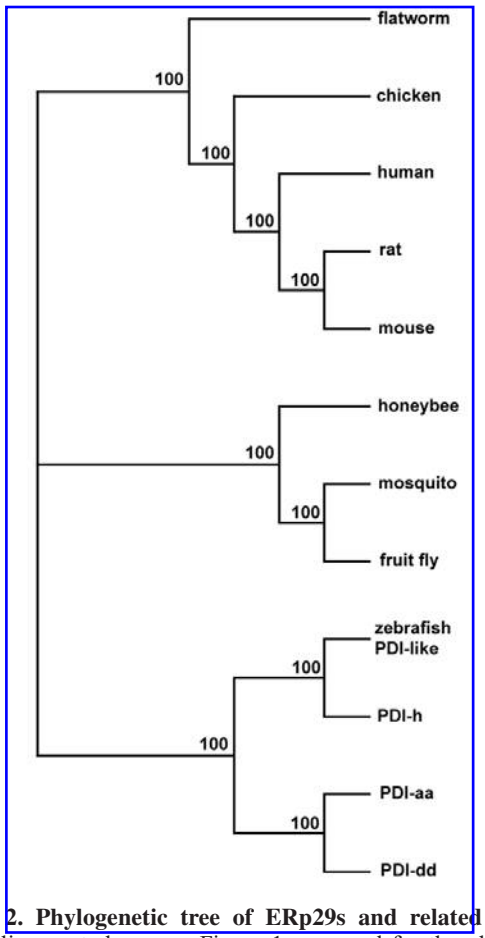

FIG. 2. Phylogenetic tree of ERp29s and related PDIs. The alignment shown on Figure 1 was used for the phylogenetic analysis. Phylogenies were inferred by distance matrix analysis using the PHYLIP program (http://evolution.genetics.washington.edu/phylip.html). The values supporting each node are derived from 100 resamplings. The image based on the PHYLIP output file was developed by the TreeView software (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

specialized functions, such as, more target-oriented assistance in folding, protection from premature associations, and the escort of proteins to their final destinations.

DOMAIN STRUCTURE AND CONFORMATION

ERp29 is comprised of two almost equal in size domains. The N-terminal domain shares up to 25%–30% of identical amino acids with the thioredoxin domains of several PDIs and the C-terminal domain displays about 30% of identity to the corresponding domain of P5-like PDIs, to Wind and to similar proteins from honey bee and mosquito (Fig. 1). The three-dimensional structure of the individual domains of ERp29 was determined by NMR spectroscopy (50) and the crystal structure of the full-length Wind has been published recently (51). Since the N-terminal domains of these proteins encompass ~130 residues and share 40 identical residues, it is not surprising that their overall fold is rather similar. 115 Cα atoms can be superimposed with the root mean square deviation of 3Å. This domain belongs to the thioredoxin family and the most similar structures are thioredoxins from various sources (79, 100), thiol-disulfide oxidoreductase from *Bacillus subtilis* and *E. coli* (17, 43), as well as ***a*** and ***b*** domains of human PDI (42) and also a number of redox-inactive proteins containing a thioredoxin fold in one or more domains (Table 1), of which the Ca^{2+}-binding protein calsequestrin is the nearest structural neighbor (98). The structural alignment performed for several proteins from the thioredoxin family including ERp29 and Wind demonstrated that there is no obvious difference in the 3D structures of the redox-active and -inactive proteins (results not shown).

Although the double cysteine motif is absent from the ERp29 sequence, the other hallmark conserved residue, Pro116 that corresponds to the Pro100 of human PDI (Fig. 1) is present in the N-terminal domain. Unlike most of the thioredoxin-fold proteins, Pro116 forms *trans*-peptide bond in ERp29 (28). However, in the redox-inactive thioredoxin domains this residue is often replaced (***b*** domain of PDI, spliceosomal protein U5 (26)), or present in *trans* conformation (glutathione peroxidase (25). Apparently this amino acid bears no functional significance for such proteins unlike the "true" thioredoxins that bind peptides via a disulfide bridge to the Cys-X-X-Cys sequence with the additional contacts taking place at a site located between this motif and the *cis*-proline.

The C-terminal domain of ERp29 contains a bundle of unusually arranged five helices, which represents a novel fold (Fig. 3) as compared to the 20 other five-helical folds found in the SCOP database. Based on the NMR structure 1G7D, a novel SCOP fold entry "ERP29 C domain-like" has been created that now contains two superfamilies, "Endoplasmic reticulum protein ERP29, C-domain" and "Helical domain of Sec23/24" (interestingly, the latter protein is an ER cargo receptor, a function that is close to the one projected for ERp29).

C-terminal domains of Wind and ERp29 differ more in the three-dimensional structures than their N-terminal domains. Only three out of five helices can be superimposed, whereas the last two helices are packed differently in the NMR structure of the C-terminal domain of ERp29 and the crystal structure of the full-length Wind.

The domain database INTERPRO (62) contains about 20 proteins with a similar domain. These are mostly, hypothetical P5-like PDIs from different genomes that have one or two redox-active N-terminal ***a*** domains. The alignment of all C-terminal domains (results not shown) revealed that helix 7 (Figs. 1 and 3) is not conserved at all whereas other helices contain from three to five conserved residues. Several of them are from the hydrophobic core of the domain; however, some conserved surface-localized residues might be important for the hypothetical common function of the C-terminal domain (see below).

Two domains of ERp29/Wind are connected by a flexible loop (~ residues 149–159). The ERp29 loop contains four conserved glycine residues, predicting the high domain mobility, whereas the loop from Wind is more rigid as there are only two glycines. However, the crystal structure of Wind

TABLE 1. PROTEINS CONTAINING THIOREDOXIN DOMAIN(S) WITHOUT THE REDOX ACTIVE CYS-X-X-CYS SEQUENCE AND NOT KNOWN TO BE INVOLVED IN ANY CYSTEINE CHEMISTRY-RELATED CATALYTIC REACTIONS

Protein	*Function*	*Domain structure*	*Thioredoxin domain function*	*Reference*
Phosducin	Regulation of G-proteins in light-adaptation	C-terminal domain is thioredoxin-like	Interaction with $G_{\beta v}$ protein	29
Human spliceosomal U5 protein (Dim1)	Regulation of cell cycle progressive via pre-mRNA splicing	One thioredoxin domain	Unknown	77
Calsequestrin	Ca^{2+} binding and storing	Three thioredoxin domains	Unknown	98
PICOT (PKC-binding protein)	Regulation of protein kinase C function	N-terminal thioredoxin domain	Unknown	103
RNA 3′-terminal phosphate cyclase	Conversion of the RNA 3′-phosphate to the 2′,3′-phosphodiester	Smaller thioredoxin domain is inserted into the larger domain	Unknown	67
Anterior gradient-2	p53 regulatory factor	One thioredoxin domain	Unknown	71
ERp29	Escort chaperone/ secretion factor (?)	N-terminal thioredoxin domain	Unknown	18, 50, 59, 82
Wind	Dedicated escort chaperone	N-terminal thioredoxin domain	Interaction with the substrate protein Pipe	47, 85

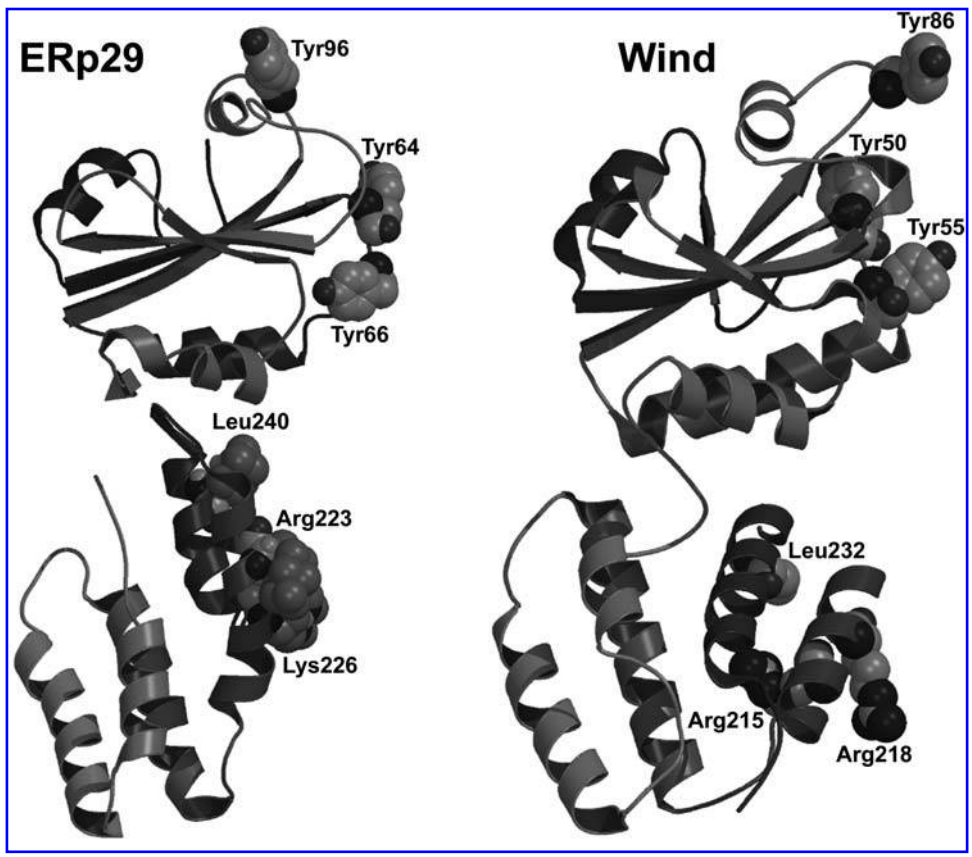

FIG. 3. Three-dimensional structures of ERp29 and Wind. Ribbon representation of two domains of rat ERp29 and Wind shown in the same orientation. The N-terminal thioredoxin domain is on the top of the C-terminal domain. Three tyrosine residues of the N-terminal domain and three residues from the C-terminal domain of Wind that are essential for Pipe transport and corresponding residues from ERp29 are shown as CPK models. The Wind dimerization interface is located below the plane of the figure, behind the monomer, and the dimer interface suggested for ERp29 is above the plane of the figure.

shows that N- and C-terminal domains have different orientation in different subunits of Wind, mostly due to the rotation around Ile144-Gly145. It is also noteworthy that this region contains the only cysteine residue of the molecule that is highly conserved and solvent-exposed. Along with other conserved amino acids it might be potentially important for the function of ERp29.

Size-exclusion chromatography, cross-linking, and dynamic light scattering suggested that ERp29 is a dimer (25, 58). Although the full-length ERp29 structure is not yet available, the line widths of the NMR signals of the separate domains suggested that dimerization is mediated exclusively by the N-terminal domain (50). Additional NMR experiments indicated that the dimerization site is formed by Phe118, Arg122, Asp123, Tyr132, and Trp144 (50).

ERp29 is the first example of a protein in which a thioredoxin-like domain was found to act as a homodimerization module without supporting interactions by covalent bonds or additional contacts by other domains. For example, human thioredoxin requires an intermolecular disulfide bridge for dimer formation (75, 100) and the intermolecular contacts observed in the single crystal of calsequestrin involve more than two thioredoxin-like domains (98). The intramolecular contacts observed between the two covalently linked thioredoxin-like domains of *Pyrococcus furiosus* PDI are also different from the interface identified for ERp29 (76). GST (5) and DsbC (56) also form dimers, but show no extensive contacts between the thioredoxin-like domains.

The dimerization mode in the crystal structure of Wind (51) is distinct from the NMR model for ERp29, although in both cases the dimerization interface is located in the N-terminal domain. The dimer of Wind buries 950 Å^2 of surface area from each subunit and only four out of 19 residues involved in the dimer interface are conserved between Wind and ERp29. The most drastic replacement is the Phe42 (Wind) to Ser53 (ERp29). The side chain of Phe42 contributes to 7.5% of the surface area interface and such replacement for a much smaller hydrophilic residue might result in reduced binding. Moreover, there are two gaps in the chain at the interface, Lys36 is inserted into the ERp29 sequence and His70 of Wind which contributes to 7% of the buried interface is not present in ERp29 (Fig. 1). Correspondingly, the residues, which were suggested to form dimerization surface in ERp29 are not conserved in Wind. Superposition of Wind and ERp29 shows that it is not only side chains which are different in this part of the protein, but the main chain conformation and orientation are different as well.

In addition to the formation of dimers, a fraction of ERp29 oligomerizes into the high-molecular weight complexes as demonstrated by the dynamic light scattering and cross-linking experiments *in vitro* and *in vivo* (50, 58). The NMR study identified residues 67, 68, 97, and 98 as potential sites of such multimerization. These residues are located near the ends of a groove that does not participate in the dimer interface, suggesting that it might be involved in oligomerization. Furthermore, residues 67 and 68 are located more than 20 Å apart from residues 97 and 98, indicating the presence of more than one binding mode as expected for nonspecific self-association.

Homodimerization and multimer formation has been observed for many chaperones [BiP (15), GRP94 (99), HSP70 (83), small heat shock proteins (7), and DsbC (55)]. The role of aggregation for chaperone function is still uncertain, although oligomerization obviously increases the surface area available for the binding of extended polypeptide chains and may generate new binding clefts at the dimer interface.

ERp29 PARTNERS IN THE ENDOPLASMIC RETICULUM

The aforementioned structural, genetic, and evolutionary aspects of ERp29 constitute a substantial body of indirect evidence suggesting its important role in the early secretory pathway. Obviously, the discovery of the putative partners and/or substrates of ERp29 would be an important step in deciphering the role of ERp29 in the ER. Early studies demonstrated that ERp29 associates with the hepatitis B small surface antigen (25) and co-immunoprecipitates with BiP in hepatoma cells (59). However, the most detailed investigation has been carried out in the thyroid epithelial cells (82). High levels of ERp29 gene expression in these cells (80) and its transcriptional upregulation by the thyroid-stimulating hormone (TSH) in concert with other ER chaperones (8, 48) suggested that ERp29 might be implicated in the maturation of the thyroid prohormone, thyroglobulin (Tg), a major secretory product of these cells. Indeed, ERp29 was found in the heterogeneous complexes with Tg, as well as with other ER chaperones, BiP and GRP94. Moreover, ERp29 associated with both native and denatured Tg in *in vitro* binding assay. However, it is not clear if this is a true chaperone-like interaction as recent work (36), as well as our unpublished data showed that both recombinant and native ERp29 lack the classical chaperone activity as judged by the inability to protect substrate proteins against thermal aggregation. It is also unlikely that ERp29 serves as a co-chaperone as no PDI or calreticulin-supporting activity was detected in the same study (36).

The same thyrocyte-based study revealed surprisingly high amounts of ERp29 in the extracellular medium, which prompted speculation about the escort protein function. The mechanisms for such unusual localization are not clear. One speculation is that the less conserved variant of the ERp29's retrieval signal (KEEL instead of KDEL) may also indicate weaker retrieval pressure and allows easier exit of ERp29 in association with its substrates. Similarly, another ER escort, receptor-associated protein (RAP) has even less effective signal, RNEL (14).

How essential is the association of ERp29 with thyroglobulin for the secretion of latter and what are the molecular mechanisms of such an interaction? No definite answers are known thus far; however, we have recently demonstrated substantial increase of Tg secretion in the thyroid cells overexpressing wild-type ERp29 while the siRNA-mediated arrest of ERp29 mRNA translation resulted in the attenuation of secretion (11). This would suggest that ERp29 function is vital for the export of Tg from thyroid cells.

As the functions of ERp29 and Wind seem to overlap, it was interesting to examine the possibility that the Wind–Pipe

interaction could serve as a prototype for that of ERp29–Tg. The putative substrate binding site of Wind has been identified in the thioredoxin domain (10). It is composed of a shallow pocket around Asp50 (Wind numbering) and two tyrosines, 53 and 55, localized in the turn before the α2 helix. Replacement of the Tyr86 also abrogates transport of Pipe to Golgi. All these residues are conserved between Wind and ERp29 and are sufficiently well superimposed structurally. Interestingly, mutations in this tyrosine cluster are not hindering, but on the contrary, stabilizing the interaction with Pipe, thus preventing its export to the Golgi. It is not clear if this substrate binding site bears the same significance also for ERp29-thyroglobulin interaction. Neither the full-length ERp29 nor the chimeric protein with the N-terminal domain of ERp29 and C-terminal domain of Wind can target Pipe to Golgi. However, the chimera composed of the N-terminal domain of Wind and the C-terminal domain of ERp29 is able to functionally replace Wind, although the N-terminal domain of Wind alone is not sufficient for the effective transport of Pipe. Interestingly, the isolated N-terminal domain of ERp29 is much more prone to aggregation and irreversible precipitation than the C-terminal domain and the full-length protein (48), suggesting that the C-terminal domain is largely responsible for the solubility of ERp29. For the P5-like PDIs, which lack the ER-retrieval signal, the importance of the C-terminal domain for the retention in the ER was demonstrated by the reduced retention of the deletion mutant (60).

However, mutational analysis of this domain in Wind (10) revealed a cluster of conserved residues (Arg215, Arg218 and Leu232) that form a second site responsible for the interaction with Pipe, which may indicate a more functional role of the C-terminal domain rather than merely retention and/or solubility of the full-length protein. Interestingly, the residues important for Pipe targeting and corresponding residues from ERp29 are located on the same side of both molecules as the essential tyrosines from the N-terminal domain of Wind (Fig. 3).

In general, these data suggest that although the general biological function of both proteins, broadly defined as escort chaperone/secretion factors, is likely to be similar, the mode of interaction and binding loci seem to be fairly diverged.

The Wind-Pipe and ERp29-Tg interactions are reminiscent of that between RAP and low-density lipoprotein (LDL) receptor (14). RAP prevents aggregation and premature ligand binding in the ER and escorts LDL receptor to the Golgi, acting as a dedicated escort chaperone. Wind, however has a limited temporal and spatial expression profile (47), as compared with the widespread tissue distribution of RAP. The comparable expression of ERp29 predicts broader substrate specificity. This would imply either the existence of different binding sites for a variety of putative ERp29 partners or a common binding locus for different substrates. There are many examples of the latter type of interactions, for example, with SH3-domains and plextrin homology domains. It was shown that at least *in vitro* these domains are rather promiscuous: most of them bind to a large number of targets with comparable affinities, and the domains from the same family recognize overlapping regions of a target protein (16). However, there are other examples, such as lectin C-type receptors of NK cells. Here different receptors from the same family recognize the same targets at different sites (65).

Association of ERp29 with another common secretory product, immunoglobulins, has been observed in a large ER-localized multiprotein complex that is comprised of incompletely folded immunoglobulin heavy chains and a variety of molecular chaperones, folding enzymes and minor co-chaperones including BiP, GRp94, GRp170, PDI, ERp72, ERp29, ERdj3, cyclophilin B, UDP-glucosyltransferase, and SDF2-L1 (57). Similarly, ERp29 has been found to associate with the misfolded κ isoform of immunoglobulin light chains (our unpublished observation). Interaction of ERp29 with immunoglobulins seems to be important for the differentiation of antigen-stimulated B lymphocytes into antibody-secreting plasma cells. ERp29 was detected in a comprehensive proteomic study of differentiating B cells along with other ER chaperones, foldases, and secretion factors whose expression was increased apparently to enhance the folding capacity of the ER (95).

Another proteomic analysis identified ERp29 in the milk fat globule (MFG) produced during lactation by mammary gland (104). ERp29 was localized in both constituents of MFG, the cytoplasmic lipid droplet (CLD) and MFG membranes (MFGM) that surround CLD. The authors conclude that ERp29 may be instrumental in lipid secretion from the mammary epithelial cells. However, as MFGM was also enriched with soluble milk proteins including casein, one can speculate that the presence of ERp29 in MFG might be explained by its putative secretory protein escort function. This is indirectly supported by our unpublished observation of the strong induction of ERp29 in mammary epithelium during lactation.

IS ERp29 A UPR TARGET?

The unfolded protein response (UPR) is an intracellular signaling pathway that regulates the protein folding and processing capacity of the endoplasmic reticulum (ER). UPR includes downregulation of protein synthesis to reduce the protein load on the ER and upregulation of molecular chaperones and other genes involved in the processing of secretory proteins (40, 61, 69). ERp29 was found among such upregulated ER proteins in rat hepatoma cells under the conditions that typically invoke UPR (treatment with the glycosylation blocker tunicamycin, the calcium ionophore A23187, the ER Ca^{2+}-ATPase inhibitor thapsigargin and the inhibitor of the ER-to-Golgi transport, brefeldin A) (59). However, the induction of ERp29 was not as strong as compared to the major ER chaperones and additionally, it could not be reproduced in all cell types (18, 25). ERp29 gene is lacking the ER stress response element (ERSE), the DNA motif characteristic for the UPR target genes. The ERp29 induction could be compared with the similarly weak ER stress-induced upregulation of PDI, which also lacks exact matches to full ERSE despite the presence of the multiple CCAAT boxes, a characteristic core of ERSE (90).

The UPR-triggered activation of chaperone genes is mediated principally by ATF6, a membrane-anchored transcription factor, and IRE1, an ER transmembrane kinase that catalyzes the splicing of mRNA of the X box-binding protein 1 (XBP1) to produce an active transcription factor. Both ATF6 and XBP1 have strong affinity to ERSE.

Besides its role as an ER adaptation reaction towards different environmental stressors (ER stress), UPR is activated under the conditions of physiological protein overload in order to restore the folding and secretory capacity of the ER. XBP1 seems to be a key physiological UPR regulator upregulating an additional cluster of genes by binding to DNA element(s) other than the ERSE. As mentioned above, the B lymphoblast transformation into the antibody-producing plasma cell is accompanied by the massive remodeling of the ER and requires concomitant activation of the genes involved in protein folding, exocytosis and proteasomal degradation (95). ERp29 was demonstrated to be one of these genes and moreover, it is also activated by the physiological UPR caused by the massive influx of the nascent Tg in the ER of thyroid epithelial cells (81).

These data and the absence of ERSE in the promoter of ERp29 prompted us to speculate that ERp29 expression is controlled primarily via the XBP1/IRE1 pathway. Indeed, our unpublished data show that while in wild-type mouse embryonic cells (MEF) ERp29 mRNA was induced by tunicamycin or thapsigargin, in the XBP1−/− and IRE1−/− MEFs such upregulation was abolished. Interestingly, another ER protein, EDEM (an essential ER quality control factor that targets terminally misfolded proteins to the degradative pathway), was also found to be regulated in a similar manner (105). The kinetics of ERp29 induction coincides with that of the EDEM and is substantially delayed as compared with the ATF6 target protein, BiP. Altogether, this would indicate that the XBP1-dependent subset of ER proteins including ERp29 is needed for the later stages of UPR in order to facilitate the traffic of increased amounts of correctly folded proteins and/or to direct misfolded species to the proteasomal machinery.

ERp29 AND DISEASE

In addition to the environmental stressors and physiological protein overload, UPR is initiated by the excessive accumulation of mutant secretory proteins due to their failure to acquire correct conformation and couple to the export machinery. A group of phenotypically diverse inherited pathologies, termed endoplasmic reticulum storage diseases (ERSD) with similar molecular pathogenesis includes such disorders as cystic fibrosis, neurodegenerative diseases, and others (for detailed reviews see 4, 45, 78). Disease pathology may be triggered by the cytotoxic effects of the protein overload or simply by the lack of the functional protein in the target organelle or cell exterior.

ERp29 was upregulated in one of such ERSDs, congenital hypothyroid goiter, with missense mutations that affect two cysteine residues of the thyroglobulin gene (12). This defect makes the mutant Tg molecules unable to leave the ER with the ensuing impairment of the synthesis of thyroid hormones.

Development of ERSDs is closely connected with the ER quality control, the "proof-reading" of newly synthesized proteins, so that only native conformers reach their final destinations (22). Quality control monitors not only the folding of single polypeptide chains but also the assembly of oligomers (such as Tg dimers). The subunits that fail to assemble are retained in the ER with further degradation by ERAD. However, as shown for cystic fibrosis, significant amount of the mutant transmembrane conductance regulator, entrapped in the ER is still functional (68). The same holds true for the mutant Tg in the human patient with a C1264R mutation (46), suggesting that the Tg defect is not severe enough to render the molecule absolutely useless for hormonogenesis. Therefore, quality control (and ERp29 as a potential member of it) may itself contribute to the disease pathogenesis by preventing the export of a considerable fraction of functionally competent Tg molecules.

Finally, our unpublished observations identified ERp29 as one of the genes important for the interaction of cancer cells with the surrounding stromal tissue (fibroblasts). The tumor growth rate was drastically reduced upon subcutaneous implantation of the breast epithelial cancer cells stably transfected with the dominant-negative ERp29 mutant form. This data supports the role suggested for ERp29 as an essential secretion/escort factor.

CONCLUSIONS

The post-genomic era presents researchers with a daunting challenge of decoding biological functions of hundreds if not thousands of hypothetical gene products without any clear consensus motifs that could facilitate the functional predictions. ERp29 is an archetypal example of such a polypeptide where the primary structure can predict merely the organelle-specific targeting and remote similarity to PDI. Although the information accumulated since the initial discovery of ERp29 is not sufficient for determining in details its biological function, it nevertheless allows tentative definition of ERp29 as a secretory assistant/escort protein.

How unique is this function and are there any functional relatives of ERp29? Most of the proteins populating ER can be defined as secretion factors in the sense that they all facilitate export of secretory or membrane proteins. Nevertheless, these accessory proteins are tentatively divided into three broad functional classes (34): "outfitters" (true molecular chaperones, folding catalysts), "escorts" (dedicated escort chaperones such as RAP) and "guides" or transport receptors (proteins selecting cargo molecules for the secretory vesicles). A few other classes or subclasses can be added such as enzymes responsible for posttranslational modifications (glycosylation), and co-chaperones. The information accumulated thus far rules out any enzymatic or classical chaperone activities of ERp29, thus suggesting a role of an ER escort or guide. The only known ER guides or cargo receptors [such as ERGIC-53, p24, BAP31 (32, 44, 88)] are membrane proteins and most of them have lectin domains that interact with secretory glycoproteins. The few known ER escort chaperones (such as the already mentioned RAP) possess quite characteristic narrow substrate specificity. It seems that even if tentatively classified as such an escort protein, the ubiquitous expression of ERp29 and the ensuing broad substrate specificity would make it unique.

However, the essential information that would put all the dots on the i's is still missing: the molecular mode of action of ERp29 remains open. Although no immediate answers are available, the existing three-dimensional structures of ERp29

domains and the crystal structure of Wind provide an excellent basis for finding the potential binding sites in the ERp29 molecule. Ongoing investigations in this direction in our and other laboratories are already yielded promising preliminary results pinpointing amino acids involved in putative ERp29-substrate protein(s) interactions (11, 33).

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NOTE ADDED IN PROOF

The search for the physiological function of ERp29 is getting an interesting twist with the upcoming publication implicating ERp29 as a critical component of a viral infection pathway (53). It was demonstrated that ERp29 triggers conformational changes in polyomavirus, which forms a hydrophobic viral particle capable of binding to and crossing the ER membrane. Thus, polyomavirus is hijacking an unknown endogenous function of ERp29, which nevertheless appears to be related to the transport/escorting function of this ER protein suggested above. This is the first indication of not only a structural but also a functional linkage of ERp29 with PDI, which was shown to unfold another toxic agent, cholera toxin, facilitating its retro-translocation from the ER (92).

ABBREVIATIONS

CLD, cytoplasmic lipid droplet; EDEM, ER degradation-enhancing alpha-mannosidase-like protein; ERSE, ER stress response element; ER, endoplasmic reticulum; ERSD, endoplasmic reticulum storage diseases; GST, glutathione-*S*-transferase; HSP70, heat shock protein 70 kDa; LDL, low-density lipoprotein; MEF, mouse embryonic cells; MFG, milk fat globule; MFGM, MFG membranes; PDI, protein disulfide isomerase; RAP, receptor-associated protein; Tg, thyroglobulin; UPR, unfolded protein response; XBP1, X box-binding protein 1.

REFERENCES

1. Adoutte A, Balavoine G, Lartillot N, and de Rosa R. Animal evolution. The end of the intermediate taxa? *Trends Genet* 15: 104–108, 1999.
2. Anelli T, Alessio M, Mezghrani A, Simmen T, Talamo F, Bachi A, and Sitia R. ERp44, a novel endoplasmic reticulum folding assistant of the thioredoxin family. *EMBO J* 21: 835–844, 2002.
3. Anfinsen CB. Principles that govern the folding of protein chains. *Science* 181: 223–230, 1973.
4. Aridor M and Balch WE. Integration of endoplasmic reticulum signaling in health and disease. *Nat Med* 5: 745–751, 1999.
5. Armstrong RN. Structure, catalytic mechanism, and evolution of the glutathione transferases. *Chem Res Toxicol* 10: 2–18, 1997.
6. Arner ES and Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. *Eur J Biochem* 267: 6102–6109, 2000.
7. Arrigo AP, Suhan JP, and Welch WJ. Dynamic changes in the structure and intracellular locale of the mammalian low-molecular-weight heat shock protein. *Mol Cell Biol* 8: 5059–5071, 1988.
8. Arvan P, Kim PS, Kuliawat R, Prabakaran D, Muresan Z, Yoo SE, and Abu Hossain S. Intracellular protein transport to the thyrocyte plasma membrane: potential implications for thyroid physiology. *Thyroid* 7: 89–105, 1997.
9. Balavoine G. The early emergence of platyhelminths is contradicted by the agreement between 18S rRNA and Hox genes data. *C R Acad Sci* III 320: 83–94, 1997.
10. Barnewitz K, Guo C, Sevvana M, Ma Q, Sheldrick GM, Soling HD, and Ferrari DM. Mapping of a substrate binding site in the protein disulfide isomerase-related chaperone wind based on protein function and crystal structure. J *Biol Chem* 279: 39829–39837, 2004.
11. Baryshev M, Sargsyan E, and Mkrtchian S. ERp29 is an essential endoplasmic reticulum factor regulating secretion of thyroglobulin. *Biochem Biophys Res Commun* 340: 617–624, 2006.
12. Baryshev M, Sargsyan E, Wallin G, Lejnieks A, Furudate S, Hishinuma A, and Mkrtchian S. Unfolded protein response is involved in the pathology of human congenital hypothyroid goiter and rat non-goitrous congenital hypothyroidism. *J Mol Endocrinol* 32: 903–920, 2004.
13. Beckmann RP, Lovett M, and Welch WJ. Examining the function and regulation of hsp 70 in cells subjected to metabolic stress. *J Cell Biol* 117: 1137–1150, 1992.
14. Bu G and Marzolo MP. Role of rap in the biogenesis of lipoprotein receptors. *Trends Cardiovasc Med* 10: 148–155, 2000.
15. Carlino A, Toledo H, Skaleris D, DeLisio R, Weissbach H, and Brot N. Interactions of liver Grp78 and *Escherichia coli* recombinant Grp78 with ATP: multiple species and disaggregation. *Proc Natl Acad Sci USA* 89: 2081–2085, 1992.
16. Castagnoli L, Costantini A, Dall'Armi C, Gonfloni S, Montecchi-Palazzi L, Panni S, Paoluzi S, Santonico E, and Cesareni G. Selectivity and promiscuity in the interaction network mediated by protein recognition modules. *FEBS Lett* 567: 74–79, 2004.
17. Crow A, Acheson RM, Le Brun NE, and Oubrie A. Structural basis of Redox-coupled protein substrate selection by the cytochrome c biosynthesis protein ResA. *J Biol Chem* 279: 23654–23660, 2004.
18. Demmer J, Zhou C, and Hubbard MJ. Molecular cloning of ERp29, a novel and widely expressed resident of the endoplasmic reticulum. *FEBS Lett* 402: 145–150, 1997.
19. Deo RC, Schmidt EF, Elhabazi A, Togashi H, Burley SK, and Strittmatter SM. Structural bases for CRMP function in plexin-dependent semaphorin3A signaling. *EMBO J* 23: 9–22, 2004.
20. Dobson CM and Karplus M. The fundamentals of protein folding: bringing together theory and experiment. *Curr Opin Struct Biol* 9: 92–101, 1999.

21. Dynan WS, Sazer S, Tjian R, and Schimke RT. Transcription factor Sp1 recognizes a DNA sequence in the mouse dihydrofolate reductase promoter. *Nature* 319: 246–248, 1986.
22. Ellgaard L and Helenius A. Quality control in the endoplasmic reticulum. *Nat Rev Mol Cell Biol* 4: 181–191, 2003.
23. Fang C, Mkrtchian S, and Ingelman-Sundberg M. Combination of direct DNA sequencing with degenerate primer-mediated PCR and 5′-/3′-RACE to screen novel cDNA sequences. *Biotechniques* 23: 52, 54, 56, 58, 1997.
24. Fassio A and Sitia R. Formation, isomerisation and reduction of disulphide bonds during protein quality control in the endoplasmic reticulum. *Histochem Cell Biol* 117: 151–157, 2002.
25. Ferrari DM, Nguyen Van P, Kratzin HD, and Soling HD. ERp28, a human endoplasmic-reticulum-lumenal protein, is a member of the protein disulfide isomerase family but lacks a CXXC thioredoxin-box motif. *Eur J Biochem* 255: 570–579, 1998.
26. Ferrari DM and Soling HD. The protein disulphide-isomerase family: unravelling a string of folds. *Biochem J* 339: 1–10, 1999.
27. Freedman RB, Hirst TR, and Tuite MF. Protein disulphide isomerase: building bridges in protein folding. *Trends Biochem Sci* 19: 331–336, 1994.
28. Frydman J. Folding of newly translated proteins in vivo: the role of molecular chaperones. *Annu Rev Biochem* 70: 603–647, 2001.
29. Gaudet R, Bohm A, and Sigler PB. Crystal structure at 2.4 angstroms resolution of the complex of transducin betagamma and its regulator, phosducin. *Cell* 87: 577–588, 1996.
30. Gething MJ. Role and regulation of the ER chaperone BiP. *Semin Cell Dev Biol* 10: 465–472, 1999.
31. Gething MJ and Sambrook J. Protein folding in the cell. *Nature* 355: 33–45, 1992.
32. Hauri H, Appenzeller C, Kuhn F, and Nufer O. Lectins and traffic in the secretory pathway. *FEBS Lett* 476: 32–37, 2000.
33. Hermann VM, Cutfield JF, and Hubbard MJ. Biophysical characterization of ERp29. Evidence for a key structural role of cysteine 125. *J Biol Chem* 280: 13529–13537, 2005.
34. Herrmann JM, Malkus P, and Schekman R. Out of the ER—outfitters, escorts and guides. *Trends Cell Biol* 9: 5–7, 1999.
35. Holmgren A. Thioredoxin. *Annu Rev Biochem* 54: 237–271, 1985.
36. Hubbard MJ, Mangum JE, and McHugh NJ. Purification and biochemical characterization of native ERp29 from rat liver. *Biochem J* 383: 589–597, 2004.
37. Hubbard MJ, McHugh NJ, and Carne DL. Isolation of ERp29, a novel endoplasmic reticulum protein, from rat enamel cells evidence for a unique role in secretory-protein synthesis. *Eur J Biochem* 267: 1945–1957, 2000.
38. Huber HE, Tabor S, and Richardson CC. *Escherichia coli* thioredoxin stabilizes complexes of bacteriophage T7 DNA polymerase and primed templates. *J Biol Chem* 262: 16224–16232, 1987.
39. Hughes GJ, Frutiger S, Paquet N, Pasquali C, Sanchez JC, Tissot JD, Bairoch A, Appel RD, and Hochstrasser DF. Human liver protein map: update 1993. *Electrophoresis* 14: 1216–1222, 1993.
40. Kaufman RJ. Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. *Genes Dev* 13: 1211–1233, 1999.
41. Kemmink J, Darby NJ, Dijkstra K, Nilges M, and Creighton TE. The folding catalyst protein disulfide isomerase is constructed of active and inactive thioredoxin modules. *Curr Biol* 7: 239–245, 1997.
42. Kemmink J, Dijkstra K, Mariani M, Scheek RM, Penka E, Nilges M, and Darby NJ. The structure in solution of the b domain of protein disulfide isomerase. *J Biomol NMR* 13: 357–368, 1999.
43. Kim JH, Kim SJ, Jeong DG, Son JH, and Ryu SE. Crystal structure of DsbDgamma reveals the mechanism of redox potential shift and substrate specificity(1). *FEBS Lett* 543: 164–169, 2003.
44. Kim KM, Adachi T, Nielsen PJ, Terashima M, Lamers MC, Kohler G, and Reth M. Two new proteins preferentially associated with membrane immunoglobulin D. *EMBO J* 13: 3793–3800, 1994.
45. Kim PS and Arvan P. Endocrinopathies in the family of endoplasmic reticulum (ER) storage diseases: disorders of protein trafficking and the role of ER molecular chaperones. *Endocr Rev* 19: 173–202, 1998.
46. Kim PS, Kwon OY, and Arvan P. An endoplasmic reticulum storage disease causing congenital goiter with hypothyroidism. *J Cell Biol* 133: 517–527, 1996.
47. Konsolaki M and Schupbach T. Windbeutel, a gene required for dorsoventral patterning in *Drosophila*, encodes a protein that has homologies to vertebrate proteins of the endoplasmic reticulum. *Genes Dev* 12: 120–131, 1998.
48. Kwon OY, Park S, Lee W, You KH, Kim H, and Shong M. TSH regulates a gene expression encoding ERp29, an endoplasmic reticulum stress protein, in the thyrocytes of FRTL-5 cells. *FEBS Lett* 475: 27–30, 2000.
49. Larsen F, Gundersen G, Lopez R, and Prydz H. CpG islands as gene markers in the human genome. *Genomics* 13: 1095–1107, 1992.
50. Liepinsh E, Baryshev M, Sharipo A, Ingelman-Sundberg M, Otting G, and Mkrtchian S. Thioredoxin fold as homodimerization module in the putative chaperone ERp29. NMR structures of the domains and experimental model of the 51 kDa dimer. *Structure* 9: 457–471, 2001.
51. Ma Q, Guo C, Barnewitz K, Sheldrick GM, Soling HD, Uson I, and Ferrari DM. Crystal structure and functional analysis of *Drosophila* Wind, a protein-disulfide isomerase-related protein. *J Biol Chem* 278: 44600–44607, 2003.
52. MacLeod JC, Sayer RJ, Lucocq JM, and Hubbard MJ. ERp29, a general endoplasmic reticulum marker, is highly expressed throughout the brain. *J Comp Neurol* 477: 29– 42, 2004.
53. Magnuson B, Rainey EK, Benjamin T, Baryshev M, Mkrtchian S, and Tsai B. ERp29 triggers a conformational change in polyomavirus to stimulate membrane binding. *Mol Cell* 20: 289–300, 2005.
54. Martin JL. Thioredoxin—a fold for all reasons. *Structure* 3: 245–250, 1995.
55. Martin JL, Bardwell JC, and Kuriyan J. Crystal structure of the DsbA protein required for disulphide bond formation in vivo. *Nature* 365: 464–468, 1993.

56. McCarthy AA, Haebel PW, Torronen A, Rybin V, Baker EN, and Metcalf P. Crystal structure of the protein disulfide bond isomerase, DsbC, from *Escherichia coli*. *Nat Struct Biol* 7: 196–199, 2000.
57. Meunier L, Usherwood YK, Chung KT, and Hendershot LM. A subset of chaperones and folding enzymes form multiprotein complexes in endoplasmic reticulum to bind nascent proteins. *Mol Biol Cell* 13: 4456–4469, 2002.
58. Mkrtchian S, Baryshev M, Matvijenko O, Sharipo A, Sandalova T, Schneider G, Ingelman-Sundberg M, and Mkrtchiana S. Oligomerization properties of ERp29, an endoplasmic reticulum stress protein. *FEBS Lett* 431: 322–326, 1998.
59. Mkrtchian S, Fang C, Hellman U, and Ingelman-Sundberg M. A stress-inducible rat liver endoplasmic reticulum protein, ERp29. *Eur J Biochem* 251: 304–313, 1998.
60. Monnat J, Neuhaus EM, Pop MS, Ferrari DM, Kramer B, and Soldati T. Identification of a novel saturable endoplasmic reticulum localization mechanism mediated by the C-terminus of a *Dictyostelium* protein disulfide isomerase. *Mol Biol Cell* 11: 3469–3484, 2000.
61. Mori K. Tripartite management of unfolded proteins in the endoplasmic reticulum. *Cell* 101: 451–454, 2000.
62. Mulder NJ, Apweiler R, Attwood TK, Bairoch A, Barrell D, Bateman A, Binns D, Biswas M, Bradley P, Bork P, Bucher P, Copley RR, Courcelle E, Das U, Durbin R, Falquet L, Fleischmann W, Griffiths-Jones S, Haft D, Harte N, Hulo N, Kahn D, Kanapin A, Krestyaninova M, Lopez R, Letunic I, Lonsdale D, Silventoinen V, Orchard SE, Pagni M, Peyruc D, Ponting CP, Selengut JD, Servant F, Sigrist CJ, Vaughan R, and Zdobnov EM. The InterPro Database, 2003 brings increased coverage and new features. *Nucleic Acids Res* 31: 315–318, 2003.
63. Munro S and Pelham HR. A C-terminal signal prevents secretion of luminal ER proteins. *Cell* 48: 899–907, 1987.
64. Nagano N, Orengo CA, and Thornton JM. One fold with many functions: the evolutionary relationships between TIM barrel families based on their sequences, structures and functions. *J Mol Biol* 321: 741–765, 2002.
65. Natarajan K, Dimasi N, Wang J, Margulies DH, and Mariuzza RA. MHC class I recognition by Ly49 natural killer cell receptors. *Mol Immunol* 38: 1023–1027, 2002.
66. Pagani M, Fabbri M, Benedetti C, Fassio A, Pilati S, Bulleid NJ, Cabibbo A, and Sitia R. Endoplasmic reticulum oxidoreductin 1-lbeta (ERO1-Lbeta), a human gene induced in the course of the unfolded protein response. *J Biol Chem* 275: 23685–23692, 2000.
67. Palm GJ, Billy E, Filipowicz W, and Wlodawer A. Crystal structure of RNA 3′-terminal phosphate cyclase, a ubiquitous enzyme with unusual topology. *Structure* 8: 13–23, 2000.
68. Pasyk EA and Foskett JK. Mutant (delta F508) cystic fibrosis transmembrane conductance regulator Cl^- channel is functional when retained in endoplasmic reticulum of mammalian cells. *J Biol Chem* 270: 12347–12350, 1995.
69. Patil C and Walter P. Intracellular signaling from the endoplasmic reticulum to the nucleus: the unfolded protein response in yeast and mammals. *Curr Opin Cell Biol* 13: 349–355, 2001.
70. Pihlajaniemi T, Helaakoski T, Tasanen K, Myllyla R, Huhtala ML, Koivu J, and Kivirikko KI. Molecular cloning of the beta-subunit of human prolyl 4-hydroxylase. This subunit and protein disulphide isomerase are products of the same gene. *EMBO J* 6: 643–649, 1987.
71. Pohler E, Craig AL, Cotton J, Lawrie L, Dillon JF, Ross P, Kernohan N, and Hupp TR. The Barrett's antigen anterior gradient-2 silences the p53 transcriptional response to DNA damage. *Mol Cell Proteomics* 3: 534–547, 2004.
72. Pons T, Gomez R, Chinea G, and Valencia A. Beta-propellers: associated functions and their role in human diseases. *Curr Med Chem* 10: 505–524, 2003.
73. Pugh BF and Tjian R. Transcription from a TATA-less promoter requires a multisubunit TFIID complex. *Genes Dev* 5: 1935–1945, 1991.
74. Purnell BA and Gilmour DS. Contribution of sequences downstream of the TATA element to a protein-DNA complex containing the TATA-binding protein. *Mol Cell Biol* 13: 2593–2603, 1993.
75. Qin J, Clore GM, Kennedy WP, Kuszewski J, and Gronenborn AM. The solution structure of human thioredoxin complexed with its target from Ref-1 reveals peptide chain reversal. *Structure* 4: 613–620, 1996.
76. Ren B, Tibbelin G, de Pascale D, Rossi M, Bartolucci S, and Ladenstein R. A protein disulfide oxidoreductase from the archaeon *Pyrococcus furiosus* contains two thioredoxin fold units. *Nat Struct Biol* 5: 602–611, 1998.
77. Reuter K, Nottrott S, Fabrizio P, Luhrmann R, and Ficner R. Identification, characterization and crystal structure analysis of the human spliceosomal U5 snRNP-specific 15 kD protein. *J Mol Biol* 294: 515–525, 1999.
78. Rutishauser J and Spiess M. Endoplasmic reticulum storage diseases. *Swiss Med Wkly* 132: 211–222, 2002.
79. Saarinen M, Gleason FK, and Eklund H. Crystal structure of thioredoxin-2 from Anabaena. *Structure* 3: 1097–1108, 1995.
80. Sargsyan E, Baryshev M, Backlund M, Sharipo A, and Mkrtchian S. Genomic organization and promoter characterization of the gene encoding a putative endoplasmic reticulum chaperone, ERp29. *Gene* 285: 127–139, 2002.
81. Sargsyan E, Baryshev M, and Mkrtchian S. The physiological unfolded protein response in the thyroid epithelial cells. *Biochem Biophys Res Commun* 322: 570–576, 2004.
82. Sargsyan E, Baryshev M, Szekely L, Sharipo A, and Mkrtchian S. Identification of ERp29, an endoplasmic reticulum lumenal protein, as a new member of the thyroglobulin folding complex. *J Biol Chem* 277: 17009–17015, 2002.
83. Schlossman DM, Schmid SL, Braell WA, and Rothman JE. An enzyme that removes clathrin coats: purification of an uncoating ATPase. *J Cell Biol* 99: 723–733, 1984.
84. Semenza JC, Hardwick KG, Dean N, and Pelham HR. ERD2, a yeast gene required for the receptor-mediated retrieval of luminal ER proteins from the secretory pathway. *Cell* 61: 1349–1357, 1990.
85. Sen J, Goltz JS, Konsolaki M, Schupbach T, and Stein D. Windbeutel is required for function and correct subcellular localization of the *Drosophila* patterning protein Pipe. *Development* 127: 5541–5550, 2000.
86. Shnyder SD and Hubbard MJ. ERp29 is a ubiquitous resident of the endoplasmic reticulum with a distinct role in

secretory protein production. *J Histochem Cytochem* 50: 557–566, 2002.
87. Sitia R and Molteni SN. Stress, protein (mis)folding, and signaling: the redox onnection. *Sci STKE* 2004: pe27, 2004.
88. Stamnes MA, Craighead MW, Hoe MH, Lampen N, Geromanos S, Tempst P, and Rothman JE. An integral membrane component of coatomer-coated transport vesicles defines a family of proteins involved in budding. *Proc Natl Acad Sci USA* 92: 8011–8015, 1995.
89. Subjeck JR and Shyy TT. Stress protein systems of mammalian cells. *Am J Physiol* 250: C1–C17, 1986.
90. Tasanen K, Parkkonen T, Chow LT, Kivirikko KI, and Pihlajaniemi T. Characterization of the human gene for a polypeptide that acts both as the beta subunit of prolyl 4-hydroxylase and as protein disulfide isomerase. *J Biol Chem* 263: 16218–16224, 1988.
91. Thornton JM, Orengo CA, Todd AE, and Pearl FM. Protein folds, functions and evolution. *J Mol Biol* 293: 333–342, 1999.
92. Tsai B, Rodighiero C, Lencer WI, and Rapoport TA. Protein disulfide isomerase acts as a redox-dependent chaperone to unfold cholera toxin. *Cell* 104: 937–948, 2001.
93. Tsugita A, Kawakami T, Uchida T, Sakai T, Kamo M, Matsui T, Watanabe Y, Morimasa T, Hosokawa K, and Toda T. Proteome analysis of mouse brain: two-dimensional electrophoresis profiles of tissue proteins during the course of aging. *Electrophoresis* 21: 1853–1871, 2000.
94. Tu BP, Ho-Schleyer SC, Travers KJ, and Weissman JS. Biochemical basis of oxidative protein folding in the endoplasmic reticulum. *Science* 290: 1571–1574, 2000.
95. van Anken E, Romijn EP, Maggioni C, Mezghrani A, Sitia R, Braakman I, and Heck AJ. Sequential waves of functionally related proteins are expressed when B cells prepare for antibody secretion. *Immunity* 18: 243–253, 2003.
96. Wang CC. Protein disulfide isomerase assists protein folding as both an isomerase and a chaperone. *Ann NY Acad Sci* 864: 9–13, 1998.
97. Wang CC and Tsou CL. Protein disulfide isomerase is both an enzyme and a chaperone. *FASEB J* 7: 1515–1517, 1993.
98. Wang S, Trumble WR, Liao H, Wesson CR, Dunker AK, and Kang CH. Crystal structure of calsequestrin from rabbit skeletal muscle sarcoplasmic reticulum. *Nat Struct Biol* 5: 476–483, 1998.
99. Wearsch PA and Nicchitta CV. Endoplasmic reticulum chaperone GRP94 subunit assembly is regulated through a defined oligomerization domain. *Biochemistry* 35: 16760–16769, 1996.
100. Weichsel A, Gasdaska JR, Powis G, and Montfort WR. Crystal structures of reduced, oxidized, and mutated human thioredoxins: evidence for a regulatory homodimer. *Structure* 4: 735–751, 1996.
101. Wetterau JR, Combs KA, Spinner SN, and Joiner BJ. Protein disulfide isomerase is a component of the microsomal triglyceride transfer protein complex. *J Biol Chem* 265: 9801–9807, 1990.
102. Wilkinson B and Gilbert HF. Protein disulfide isomerase. *Biochim Biophys Acta* 1699: 35–44, 2004.
103. Witte S, Villalba M, Bi K, Liu Y, Isakov N, and Altman A. Inhibition of the c-Jun N-terminal kinase/AP-1 and NF-kappaB pathways by PICOT, a novel protein kinase C-interacting protein with a thioredoxin homology domain. *J Biol Chem* 275: 1902–1909, 2000.
104. Wu CC, Howell KE, Neville MC, Yates JR, 3rd, and McManaman JL. Proteomics reveal a link between the endoplasmic reticulum and lipid secretory mechanisms in mammary epithelial cells. *Electrophoresis* 21: 3470–3482, 2000.
105. Yoshida H, Matsui T, Hosokawa N, Kaufman RJ, Nagata K, and Mori K. A time-dependent phase shift in the mammalian unfolded protein response. *Dev Cell* 4: 265–271, 2003.

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2. Danmei Gao, I Fon Bambang, Thomas C Putti, Yuan Kun Lee, Des R Richardson, Daohai Zhang. 2011. ERp29 induces breast cancer cell growth arrest and survival through modulation of activation of p38 and upregulation of ER stress protein p58IPK. *Laboratory Investigation* . [CrossRef]
3. U. Krugel, H.-X. He, K. Gier, J. Reins, I. Chincinska, B. Grimm, W. X. Schulze, C. Kuhn. 2011. The Potato Sucrose Transporter StSUT1 Interacts with a DRM-Associated Protein Disulfide Isomerase. *Molecular Plant* . [CrossRef]
4. E. Farmaki, S. Mkrtchian, I. Papazian, A.G. Papavassiliou, H. Kiaris. 2011. ERp29 regulates response to doxorubicin by a PERK-mediated mechanism. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1813**:6, 1165-1171. [CrossRef]
5. Daohai Zhang, Des R. Richardson. 2011. Endoplasmic reticulum protein 29 (ERp29): An emerging role in cancer. *The International Journal of Biochemistry & Cell Biology* **43**:1, 33-36. [CrossRef]
6. Daohai Zhang, Thomas C. Putti. 2010. Over-expression of ERp29 attenuates doxorubicin-induced cell apoptosis through up-regulation of Hsp27 in breast cancer cells. *Experimental Cell Research* **316**:20, 3522-3531. [CrossRef]
7. Guennadi Kozlov, Pekka Määttänen, David Y. Thomas, Kalle Gehring. 2010. A structural overview of the PDI family of proteins. *FEBS Journal* **277**:19, 3924-3936. [CrossRef]
8. I Fon Bambang, Songci Xu, Jianbiao Zhou, Manuel Salto-Tellez, Sunil K Sethi, Daohai Zhang. 2009. Overexpression of endoplasmic reticulum protein 29 regulates mesenchymal–epithelial transition and suppresses xenograft tumor growth of invasive breast cancer cells. *Laboratory Investigation* **89**:11, 1229-1242. [CrossRef]
9. Wolfgang Hoehenwarter, Yajun Tang, Renate Ackermann, Klaus-Peter Pleissner, Monika Schmid, Robert Stein, Ursula Zimny-Arndt, Nalin M. Kumar, Peter R. Jungblut. 2008. Identification of proteins that modify cataract of mouse eye lens. *PROTEOMICS* **8**:23-24, 5011-5024. [CrossRef]
10. Souren Mkrtchian, Mikhail Baryshev, Ernest Sargsyan, Ioulia Chatzistamou, Aspasia-Athina Volakaki, Nick Chaviaras, Agatha Pafiti, Aggeliki Triantafyllou, Hippokratis Kiaris. 2008. ERp29, an endoplasmic reticulum secretion factor is involved in the growth of breast tumor xenografts. *Molecular Carcinogenesis* **47**:11, 886-892. [CrossRef]
11. April A. Dukes, Victor S. Van Laar, Michael Cascio, Teresa G. Hastings. 2008. Changes in endoplasmic reticulum stress proteins and aldolase A in cells exposed to dopamine. *Journal of Neurochemistry* **106**:1, 333-346. [CrossRef]
12. P. A. Fowler, N. J. Dora, H. McFerran, M. R. Amezaga, D. W. Miller, R. G. Lea, P. Cash, A. S. McNeilly, N. P. Evans, C. Cotinot, R. M. Sharpe, S. M. Rhind. 2008. In utero exposure to low doses of environmental pollutants disrupts fetal ovarian development in sheep. *Molecular Human Reproduction* **14**:5, 269-280. [CrossRef]
13. Dr. , Adam M. Benham , Roberto Sitia . 2006. The Diversity of Oxidative Protein Folding. *Antioxidants & Redox Signaling* **8**:3-4, 271-273. [Citation] [Full Text PDF] [Full Text PDF with Links]