Reduction of the endoplasmic reticulum accompanies the oxidative damage of diabetes mellitus[1]

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Abstract. The endoplasmic reticulum (ER), similarly to other subcompartments of the eukaryotic cell possesses a relatively oxidizing environment. The special milieu of ER lumen is important for many ER-specific processes (redox protein folding, glycoprotein synthesis, quality control of secreted proteins, antigen presentation, etc.). Despite of the vital importance of redox regulation in the ER, we have a surprisingly fragmented knowledge about the mechanisms responsible for the ER redox balance. Moreover, new observations on disulfide bridge synthesis and on glutathione functions urge us to revise our recent theories based on many indirect and *in vitro* results. We have also very little information about the effects of different pathological conditions on the thiol metabolism and redox folding in the ER. Examining the role of molecular chaperones in the cellular pathology of diabetes mellitus we found that the ER redox environment shifted to a more reducing state, which was followed by changes of the thiol metabolism and structural-functional changes of the protein machinery involved in the redox folding process in diabetes. The possible consequences of these unexpected changes are also discussed.

1. Thiol metabolism in the endoplasmic reticulum

The average redox potential of the endoplasmic reticulum (ER) is about -160 mV, but theoretical calculations and some experimental results suggest that redox potential gradients and redox potential inhomogenities are typical of the subcompartment. The ER redox potential was thought to be maintained mostly by the glutathione/glutathione-disulfide redox buffer (GSH:GSSG=1 trough 3:1), and can be described by the thiol/disulfide ratio [1]. However, there are many other systems, which are able to influence ER thiol metabolism. The *in vitro* mechanisms to alter the redox state include a NADPH-dependent oxigenase and the vitamin-K redox cycle. There is an increasing number of evidence about the participation of different sulfhydryl-oxidases in the regulation of ER redox homeostasis of eukaryotic organisms [2]. In yeast models the direct role of hem, ubiquinone, Fe-S clusters and the exclusive role of molecular oxygen were recently excluded [3]. The possible involvement of flavin adenine dinucleotide (FAD) in the electron transport necessary to maintain ER redox homeostasis was also documented on yeast, where the addition of FAD accelerated the disulfide bridge formation by the ER-resident enzyme, ERO1p [3]. The yeast lumenal ER protein, ERV2 was also described as a flavoenzyme. ERV2 is able to accelerate O_2-dependent disulfide bridge formation under aerobic conditions [4]. Besides flavin
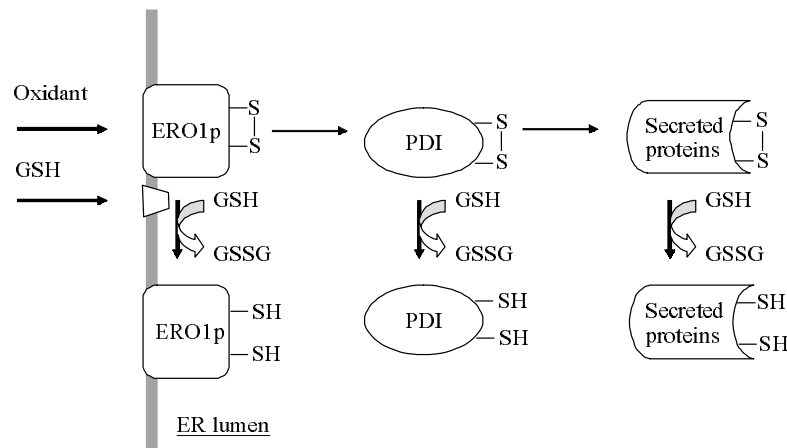

Fig. 1. Redox balance in the ER. Adapted from ref. [10].

adenine dinucleotide, an increasing number of evidence supports the involvement of an other, well known redox system on ER redox state. Ascorbate/dehydroascorbate concentration is in milimolar range in the ER lumen, and asorbate is a very important cofactor of the enzymes catalyzing prolyl- and lysyl-hydroxylation. The characteristics of their transport was described by G. Bánhegyi and co-workers [5]. Cytoplasmic ascorbic acid is first oxidized and dehydroascorbic acid is transported to the lumen by facilitated diffusion. Inside, an increased concentration of dehydroascorbic acid helps to mantain the transitional redox state, and takes part in the oxidation of GSH and protein thiols [6]. Moreover, protein disulfide isomerase itself has dehydroascorbate reductase activity [7]. The membrane-bound antioxidant agent, tocopherol was also mentioned as a possible contributor of the electron transport by ascorbic acid [8].

The importance of the glutathione/glutathione-disulfide redox buffer was recently assessed. The estimated redox potential of the ER (-160 mV) correlates with the current GSH/GSSG ratio, and its total concentration (1 to 2 mM) is high enough to affect redox environment and protein redox states [9]. However, the processes setting the balance between GSH and GSSG have not yet been clearly identified. Glutathione synthetase, an enzyme responsible for the *de novo* GSH generation, is located only in the cytoplasm, so GSH must enter to the ER lumen through transporters. A much faster GSSG transport was hypothesized to sustain the oxidative environment, but recent data are quite controversial on this topic [5]. Another possible way for the increase of GSSG concentration is described by some publications involving specific enzymatic GSSG generation by intraluminal redox enzymes [10].

The next chapter of glutathione research, its role on the protein folding process, is also under re-evaluation today. The increasing importance of GSH is underlined as a counterbalance for the ERO1p-mediated oxidation in yeast. According to this model, the oxidizing equivalents (whose precise nature is still unidentified) come from the cytosol, and are transmitted through the ER membrane by the ERO1p protein. Oxidation proceeds via the protein disulfide isomerases and finally reaches the secreted proteins. To avoid hyperoxidizing conditions a part of these proteins (and perhaps ERO1p itself) are reduced by GSH. This process is probably responsible for the relatively low ER GSH/GSSG ratio [11]. The final step of ER glutathione metabolism is the secretion of GSH, which occurs by the vesicular transport system. The concentration of the glutathione in the vesicles targeting secreted and membrane surface proteins to the cell membrane is about 1 mM [12].
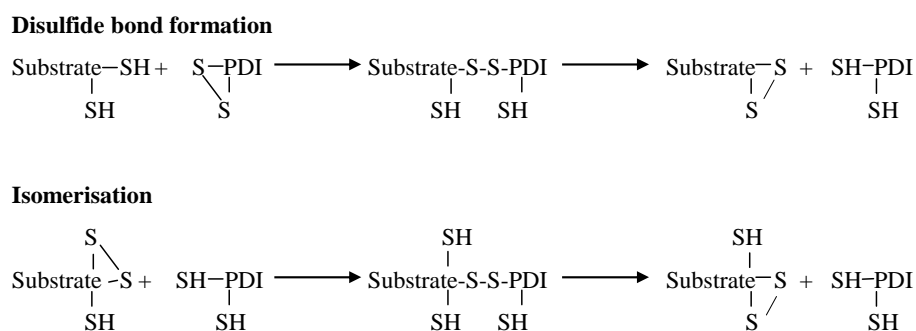
Disulfide bond formation

Isomerisation

Fig. 2. The mechanism of thiol-disulfide exchange.

2. Protein disulfide bond formation in the ER

The cytoplasmic redox potential (-230 mV) and the high GSH/GSSG ratio (30 through 100:1) is not favorable to the formation and existence of disulfide bridges. Therefore, only 0.1% of the total protein thiol groups is bound in intramolecular or mixed disulfides permanently. Transitional disulfide bridges can be observed in case of many enzymes. This function is connected to a thiol-disulfide catalytic cycle [13,14]. Therefore, folding of the secreted and integral membrane proteins, which usually have many disulfide bridges to stabilize their tertiary and quaternary structure, must occur in the more oxidizing environment of the ER. Only a few viral proteins were identified as disulfide bridge-forming proteins in the cytoplasm [15].

The newly synthetized polypeptide chain labeled by the N-terminal, ER-targeting signal peptide, enters to the ER lumen, where molecular chaperones and folding catalysts help to get its final structure. Molecular chaperones, like BiP and Grp94 trap the unfolded protein and stabilize it against aggregation, incorrect folding. However, these chaperones do not participate in the disulfide bond synthesis directly [16]. The formation of native disulfide bridges may occur without enzymatic catalysis under highly diluted and mild oxidative conditions as it has been demonstrated almost 40 years ago [17,18], but the redox folding assistance, such as protein disulfide isomerases can accelerate and make the thiol-disulfide exchange more efficient in the crowded and dynamically changing environment of the ER. These proteins have some common caracteristics. They are intraluminal, mostly soluble enzymes containing the ER retention signal, and at least one thioredoxin-domain and a special amino acid sequence (CXXC) in their active site.

The major steps of the enzymatic catalysis are the formation of a transitional enzyme-substrate complex, connected by intermolecular disulfide bridges and then the rearrangement of these bonds. Protein disulfide isomerases can create, cleave and isomerize the disulfide bonds depending on the state of the substrate and the redox equilibrium [17,18].

3. Members of the protein disulfide isomerase protein family

The best known member of the PDI family is the 58 kDa protein disulfide isomerase, which is usually called as *ERp58*, or simply as *protein disulfide isomerase* (*PDI*) was described forty years ago [17, 18]. This PDI contains two thioredoxin domains, and its local concentration in the ER is about 200 to 300 M [19,20]. It has both "molecular chaperone" and "antichaperone" activities on different substrates. As a chaperone it prevents protein aggregation. Just conversely, as an antichaperone PDI mediates

aggregate formation, when the amount of unfolded or aggregation-prone proteins is far greater than that of PDI [21]. PDI seems to be a redox-regulated chaperone: binding of the cholera-toxin substrate to PDI is stronger, when the protein is reduced and cholera toxin dissociates under oxidizing conditions [22]. PDI is also a constant part of various enzyme complexes assisiting in quite different processes (e.g. prolyl-4-hydroxilase, tryacylglycerol transfer protein, etc.) and can bind the hormone, estrogen [23]. Some extraluminal localizations and specific actions of the protein were also demonstrated.

The most important PDI function is to form native disulfide bridges on newly syntethized and reversibly denatured proteins. The reduced PDI is reoxidized by the ER membrane protein ERO1p [24]. *ERO1p* was discovered a couple of years ago in yeast, but later mammalian isoforms were also identified. ERO1p contains a CXXCXXC motif in its active site, and is able to oxidize both GSH and PDI under aerobic and anaerobic conditions. PDI was found to bind directly to the ERO1p. Neither structural, nor functional interaction between the ERO1p and other members of the protein disulfide isomerase family (ERp72, ERp57) were detectable so far. In mammals, two ERO1p isoforms exist, ERO1-L and ERO1-Lβ. However, only the expression of form is induced by the unfolded protein response. This is a flavoprotein, which is able to accept electrons coming from the secretory and other substrate proteins through protein disulfide isomerase and donate them to unknown participants in the cytosol [10].

ERp72 is a protein disulfide isomerase containing three thioredoxin domains. By its active redox and disulfide isomerase activity can replace PDI in PDI-deficient cells. *In vivo* it binds calcium, and the *in vitro* binding of different peptides could be also demonstrated together with some chaperone-like activities [20,25].

ERp57 is a protein disulfide isomerase specialized to glycoproteins. It has two thioredoxin domains, and forms complexes by calnexin and calreticulin the two ER lectin-like chaperones. The isomerase activity of the calreticulin/ or calnexin/ERp57 complex is higher than that of ERp57 alone. ERp57 interacts specifically with N-glycosylated polypeptides and its function is strongly determined by the lectin-like chaperones and the glycosylation-state of the substrate [26,27]. ERp57 takes part in the maturation of MHC-immunocomplexes, has carnitine-palmitoyltransferase activity and its cell membrane localization was also identified.

ERp44 is the newest member of the thioredoxin domain-containing ER luminal proteins, but the primary structure of its active site (CRFS) is different from that of other PDI-s in the ER. ERp44 helps immunoglobulin folding, binds covalently to ERO1p and can influence its redox state [28].

There are many other ER-resident proteins (ERp28, P5, ERp55) associated to these folding catalysts, but their function and structure are poorly described. Some of them contain the thioredoxin domain, but lack the active site sequence, so they do not likely play a direct role in redox folding. However, they were suggested as possible co-chaperones of the redox folding process [29,30].

According to our current knowledge and theories three different pathways of disulfide bond formation are present in the ER. In the first pathway, the electron transport occurs from the reduced substrate through protein disulfide isomerase to the ERO1p. The ERO1p flavin adenine dinucleotide group gives it to unidentified participants, which can integrate the thiol metabolism to the general cellular redox metabolic pathways. The second pathway (performed mostly by ERp72, and ERp57) seems to be independent from ERO1p, and requires other, probable small molecular mediators [31]. The third pathway, discovered only in yeast uses ERV2 protein and molecular oxygen as the final electron acceptor.

4. Disturbances of redox protein folding

As we discussed above, the redox regulation of the ER is closely connected to redox protein folding. Therefore, it is not surprising that any major disturbances of the ER milieu, especially that of the thiol

Table 1
Agents that activate UPR or EOR

	UPR	EOR
2-Deoxyglucose	+	+
Tunicamycin	+	+
Brefeldin A	+	+
Buthylhydroperoxide	+	+
Dithiothreitol	+	−
Heavy metal ions	+	−
Calcium ionophores	+	±
Castanospermine	+	−
Cycloheximide	−	+
TNF-alpha	−	+
Overexpression of proteins	+	+

metabolism can lead to inefficient protein folding, and the consequent accumulation of non-native, aggregation-prone polypeptides. These changes induce ER stress, and provoke the unfolded protein response [32].

Unfolded protein response (*UPR*) was first characterized in yeast, but we have an increasing number of data about the mammalian pathway [33]. In this system the increased concentration of the unfolded proteins indicates the danger, and the cell, by a complex signal transduction mechanism, accelerates the expression of different ER proteins, which are necessary to survive and handle non-native proteins. The sensors of the UPR are ER transmembrane proteins, which are able to bind chaperones (BiP) and unfolded proteins with their luminal domain. In case of ER stress, ER chaperones become overloaded by damaged polypeptides, dissociate from sensor domains, and free unfolded proteins emerge occupying the sensor binding sites. *PERK* is a stress-induced protein kinase, it can phosphorylate eiF-2, and thus it prevents any further protein synthesis under ER stress. However, the synthesis of UPR induced proteins must proceed via a different mechanism and is not inhibited by PERK. ATF6 has a cytosolic transactivation domain, which is cleaved from the ER upon stress, goes to the nucleus and helps the transcription of genes containing the ER stress response element (ERSE) [34]. *Ire1 proteins* have a cytoplasmic ribonuclease activity. In ER stress they undergo oligomerization and phosphorylation, and are suspected to splice the XBP-1 transcription factor mRNA resulting in a more efficient XBP-1 translation and a concomitant induction of ERSE-regulated genes [35]. In general, UPR inhibits transcription and translation, but induces the expression of molecular chaperones, members of the redox folding pathway, glycoprotein folding catalysts, enzymes of lipid metabolism, participants of the vesicle transport, protein translocation and ER-associated protein degradation [36] and these proteins can restore the original homeostasis of the ER lumen.

The *ER overload response* (*EOR*) is induced, when misfolded or normal proteins (because of e.g. the accelerated expression of viral proteins) accumulate in the ER lumen, fulfill the space, and disturb the normal function of the ER [37]. In EOR, besides the induction of chaperones, a special pathway is also activated, and generates an immune response against the infected of disorganized cell. ER overload induces a Ca^{++}-efflux to the cytosol, followed by reactive oxigen species generation. The increased concentration of ROS activates the NFκB transcription factor, which is responsible for the induction of several inflammatory genes, such as interferon, interleukines and other cytokines. Besides these changes the expression of the other proteins participating in antigen presentation is also increased [36].

5. Changes of the ER redox state in diabetes mellitus and in other pathological states

Several *in vitro* studies prove that the changes of the thiol/disulfide ratio, inhibition of the electron transport disrupts the redox folding process. On the contrary, many other factors, which can inhibit the proper protein folding by different mechanisms, will alter the redox balance. Thus, the redox folding process and thiol metabolism are closely connected in the ER. Although the interrelations of the different processes were recognized, the possible folding consequences of the pathological states, which are suspected to affect the redox state of the cellular compartments have been largely unnoticed.

It was previously shown that the presence of reducing agents (DTT, β-mercaptoethanol) or strong oxidants (butylhydroperoxide, metal ions) in the cell culture medium blocks disulfide bridge formation [38].

Oxidative/reductive changes of the cellular environment were observed in many diseases as the cause or the consequence of the pathological state. The redox potential is hardly mantained upon hypoxic conditions. Acute or chronic hypoxia is general in many common diseases (ischemic injury of the tissues – myocardial infarction, stroke; tumor growth; pulmonary diseases; poisoning). Here, not only the low O_2 concentration, but the ATP depletion and the reperfusion-induced generation of reactive oxygen species (ROS) all disturb the normal redox balance [39]. The oxidative stress is characteristic to inflammations also, where ROS are produced by the phagocytes. Drugs can also influence thiol metabolism. NO-donor chemicals (nitroglycerol, nitroprusside sodium, etc) require free protein thiols to act on the vascular smooth muscles. Besides their direct effect we should keep in mind that many drugs are metabolised by the microsomal oxigenases, the P450 proteins, and that these redox enzymes might be in contact with other redox systems than the thiol/disulfide pathway [40].

The diabetes mellitus is described as a complex metabolic disease characterized by the absolute or relative shortage of insulin. One of the consequences of the metabolic disorganization is the increased generation of ROS [41,42]. The oxidative stress is initially prevented by the different antioxidative defense systems, but later on these mechanisms become exhausted, and oxidative damage develops. The elevated concentration of the oxidative stressors is the result of the disorganized function of the mitochondria, the altered metabolism of the arachidonic acid, protein kinase C activation, glucose autooxidation, and the free radicals generated by the non-enzymatically glycated proteins. These redox changes are typical of the extracellular space, but the signs of the oxidative stress are also detectable in the intracellular space [39].

Besides the oxidative damages of the membranes and enzymes diabetic thiol metabolism might also be affected by other factors. There are several pieces of evidence showing that the intracellular level of ascorbic acid, tocopherol and FAD is lower in both diabetic animal models and diabetic patients [43]. These changes are caused partly because of the accelerated cofactor consumption, and partly by the deficient cofactor-transport. Both the plasma membrane transporter of ascorbic acid and dehydroascorbic acid and their microsomal uptake are blocked by high glucose concentration [5,44]. The activity of some FAD-containing enzymes is significantly lower in experimental diabetes. These small molecules are all suggested to take part in the thiol metabolism of the ER, and more experiments are required to decide if they are responsible for the suspected disturbances of the redox state and protein folding. Decreased PDI amounts and functional changes of the enzyme were also observed in diabetes.

In our recent work examining streptozotocin-induced diabetic rats, we found that inspite of the oxidative changes of the extracellular space, the redox environment of liver microsomal vesicles was shifted to a more reducing state [45]. Diabetic microsomal redox status was characterized by an increased total thiol content and by and increased protein-thiol:disulfide ratio. The dehydroascorbate reductase activity was also higher in diabetes [45]. The cytoplasmic parameters remained to be unchanged. A similar redox

shift was also observed in some ER proteins involved in the disulfide bond formation. Importantly, a significant portion of the protein disulfide isomerase and ERp72 and ERp57 were found to be in reduced form (data not shown). These changes were detected by the verification of the mobility shift resulted by the covalent modifications of SH-groups on the reduced proteins [46]. In diabetic samples we found also a marked decrease in PDI content. The redox state of ERO1-L was also altered compared to the controls.

6. Discussion and perspectives

Redox changes in diabetes alter the structure and perhaps the function of the ER redox protein folding-machinery in liver causing a folding deficient state. This might be a possible explanation of the decreased hepatic protein secretion observed in some studies [47], and can also contribute to the accelerated protein turnover detected upon oxidative stress conditions [48]. The exact consequences of the more reducing ER environment are not clear yet. However, the regulatory role of the redox state is well known in many processes. PDI chaperone activity was found to be redox sensitive, where the accumulation of reduced PDI can lead the formation of more noncovalently bound complexes and a slowdown in redox-chaperoning [22]. Reducing conditions may also decrease protein stability and increase ER protein degradation. These redox changes in diabetes may influence the transport, presence and redox function of extracellular PDI on the plasma membrane [49]. The origin of the ER redox change is still unknown, but the alteration of the ERO1-L redox state in the process can enforce the role of the cytoplasmic redox changes typical of the disorganized metabolism in diabetes mellitus. The identification of the exact causes of these changes can help us to discover the specific cytosolic link of the ERO1-L mediated disulfide bond formation.

Finally, we do not know, how typical are the changes of the ER metabolism we observed in diabetes mellitus. Are these changes common consequences of the oxidative stress or of any other aspecific events? More experiments and the use of different models is necessary to answer this question, and to understand the mechanisms involved in the pathology of the ER thiol metabolism, and to their evaluation as possible therapeutic targets in various diseases.

References

[1] C. Hwang, A.J. Sinskey and H.F. Lodish, Oxidized redox state of glutathione in the endoplasmic reticulum, *Science* **257** (1992), 1496–1502.

[2] B. Benayoun, A. Esnard-Feye, S. Castella, Y. Courty and F. Esnard, Rat seminal vesicle FAD-dependent sulfhydryl oxidase, Biochemical characterization and molecular cloning of a member of the new sulfhydryl oxidase/quiescin Q6 gene family, *J. Biol. Chem.* **276** (2001), 13830–13837.

[3] B.P. Tu, S.C. Ho-Schleyer, K.J. Travers and J.S Weissman, Biochemical basis of oxidative protein folding in the endoplasmic reticulum, *Science* **290** (2000), 1571–1574.

[4] C.S. Sevier, J.W. Couzzo, A. Vala, F. Aslund and C.A. Kaiser. A flavoprotein oxidase defines a new endoplasmic reticulum pathway for biosynthetic disulfide bond formation, *Nat. Cell Biol.* **3** (2001), 874–882.

[5] G. Bánhegyi, P. Marcolongo, F. Puskás, R. Fulceri, J. Mandl and A. Benedetti, Dehydroascorbate and ascorbate transport in rat liver microsomal vesicles, *J. Biol. Chem.* **273** (1998), 2758–2762.

[6] M. Csala, V. Mile, A. Benedetti, J. Mandl and G. Bánhegyi. Ascorbate oxidation is a prerequisite for its transport into rat liver microsomal vesicles, *Biochem. J.* **349** (2000), 413–415.

[7] W.W. Wells, D. Peng Xu, Y. Tang and P.A. Rocque, Mammalian thiotransferase (glutaredoxin) and protein disulfide isomerase have dehydroascorbate reductase activity, *J. Biol. Chem.* **265** (1990), 15361–15364.

[8] M. Csala, A. Szarka, É. Margittai, V. Mile, T. Kardon, L. Braun, J. Mandl and G. Bánhegyi, Role of vitamin E in ascorbate-dependent protein thiol oxidation in rat liver endoplasmic reticulum, *Arch. Biochem. Biophys.* **388** (2001), 55–59.

[9]   S. Raina and D. Missiakas, Making and breaking disulfide bonds, *Annu. Rev. Microbiol.* **51** (1997), 179–202.
[10] A.R. Frand, J.W. Couzo and C.A. Kaiser, Pathways for protein disulfide bond formation, *Trends Cell. Biol.* **10** (2000), 203–210.
[11] J.W. Couzzo and C.A. Kaiser, Competition between glutathione and protein thiols for disulphide-bond formation, *Nat. Cell Biol.* **1** (1999), 130–135.
[12] S. Carelli, A. Ceriotti, A. Cabibbo, G. Fassina, M. Ruvo and R. Sitia, Cysteine and glutathione secretion in response to protein disulfide bond formation in the ER, *Science* **277** (1997), 1681–1684.
[13] V.P. Pigiet and B.J. Schuster, Thioredoxin-catalyzed refolding of disulfide containing proteins, *Proc. Natl. Acad. Sci. USA* **83** (1986), 7643–7647.
[14] E.S.J. Arnér and A. Holmgren, Physiological functions thioredoxin and thioredoxin reductase, *Eur. J. Biochem.* **267** (2000), 6102–6109.
[15] T.G. Senkevich, C.L. White, E.V. Koonin and B. Moss, A viral member of the ERV1/ALR protein family participates in a cytoplasmic pathway of disulfide bond formation, *Proc. Natl. Acad. Sci.* USA **97** (2000), 12068–12073.
[16] A.S. Lee, The glucose-regulated proteins: stress induction and clinical application, *Trends Biochem. Sci.* **26** (2001), 504–510.
[17] R.F. Goldbeger, C.J. Epstein and C.B. Anfinsen, Acceleration of reactivation of reduced bovine pancreatic ribonuclease by a microsomal system from rat liver, *J. Biol. Chem.* **238** (1963), 628–635.
[18] P. Venetianer and F.B. Straub, The enzymic reactivation of reduced ribonuclease, *Biochim. Biophys. Acta* **67** (1963), 166–167.
[19] M. Narayan, E. Welker, W.J. Wedemeyer and H.A. Scheraga, Oxidative folding of proteins, *Acc. Chem. Res.* **33** (2000), 805–812.
[20] D.M. Ferrari and H.D. Söling, The protein disulphide-isomerase family: unravelling a string of folds, *Biochem. J.* **339** (1999), 1–10.
[21] A. Puig and H.F Gilbert, Protein disulfide isomerase exhibits chaperone and anti-chaperone activity in the oxidative refolding of lysozyme, *J. Biol. Chem.* **269** (1994), 7764–7771.
[22] B. Tsai, C. Rodighiero, W.I. Lencer and T.A. Rapoport, Protein disulfide isomerase acts as a redox-dependent chaperone to ubfold cholera toxin, *Cell* **104** (2001), 937–948.
[23] T.P. Primm and H.F. Gilbert, Hormone binding by protein disulfide isomerase, a high capacity hormone reservoir of the endoplasmic reticulum, *J. Biol. Chem.* **276** (2001), 281–286.
[24] A.R. Frand and C.A. Kaiser, The ERO1 gene of yeast is required for oxidation of protein dithiols in the endoplasmic reticulum, *Mol. Cell* **1** (1998), 161–170.
[25] R.A. Mazarella, M Srinivasan, S.M. Haugejorden and M. Green, Erp72, an abundant luminal endoplasmic reticulum protein, contains three copies of the active site sequences of protein disulfide isomerase, *J. Biol. Chem.* **265** (1990), 1094–1101.
[26] S. High, F.J.L. Lecomte, S.J. Russel, B.M. Abell and J.D. Oliver, Glycoprotein folding in the endoplasmic reticulum: a tale of three chaperones, *FEBS Lett* **476** (2000), 38–41.
[27] M. Molinari and A. Helenius, Glycoproteins form mixed disulfides with oxidoreductases during folding in living cells, *Nature* **402** (1999), 90–93.
[28] T. Anelli, M. Alessio, A. Mezghrani, T. Simmen, F. Talamo, A. Bachi and R. Sitia, Erp44, a novel endoplasmic reticulum folding assistant of the thioredoxin family, *EMBO J.* **21** (2002), 835–844.
[29] K. Weis, G. Griffiths and A.I. Lamond, The endoplasmic reticulum calcium-binding protein of 55 kDa is a novel EF-hand protein retained in the ER by a carboxyl-terminal His-Asp-Glu-Leu motif, *J. Biol. Chem.* **269** (1994), 19142–19150.
[30] D.M. Ferrari, P. Nguyen Van, H.D. Kratzin and H.D. S?ling, 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** (1998), 570–579.
[31] A. Mezghrani, A. Fassio, A. Benham, T. Simmen, I. Braakman and R. Sitia, Manipulation of oxidative protein folding and PDI redox state in mammalian cells, *EMBO J.* **20** (2001), 6288–6296.
[32] R.J. Kaufman, Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls, *Genes. Dev.* **13** (1999), 1211–1233.
[33] Y. Ma and L.M. Hendershot, The unfolding tale of the unfolded protein response, *Cell* **107** (2001), 827–830.
[34] H. Yoshida, Y. Haze, H. Yanagi, T. Yura and K. Mori, Identification of the cis-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors, *J. Biol. Chem.* **273** (1998), 33741–33779.
[35] H. Yoshida, T. Matsui, A. Yamamoto, T. Okada and K. Mori, XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor, *Cell* **107** (2001), 881–891.
[36] K.J. Travers, C.K. Patil, L. Wodicka, D.J. Lockhart, J.S. Weisman and P. Walter, Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation, *Cell* **101** (2000), 249–258.

[37] H.L. Pahl, Signal transduction from the endoplasmic reticulum to the cell nucleus, *Physiol. Rev.* **79** (1999), 683–698.

[38] I. Braakman, J. Helenius and A. Helenius, Manipulating disulfide bond formation and protein folding in the endoplasmic reticulum, *EMBO J.* **11** (1992), 1717–1722.

[39] H.F. Gilbert, The thiol-disulfide exchange, *Adv. Enzymol. Relat. Area Mol. Biol.* **63** (1990), 69–172.

[40] M. Bader, W. Muse, D.P. Ballou. C. Gassner and J.C.A. Bardwell, Oxidative protein folding is driven by the electron transport system, *Cell* **98** (1999), 217–227.

[41] T. Nishikawa, D. Edelstein, X. Liang Du, S. Yamagishi, T. Matsumura, Y. Kaneda, M.A. Yorek, D. Beebe, P.J. Oates, H.P. Hammes, I. Giardino and M Brownlee, Normalizing mitochondrial superoxide production blocks three pathways of hyperglycemic damage, *Nature* **404** (2000), 787–790.

[42] B. Lipinski, Pathophysiology of oxidative stress in diabetes mellitus, *J. Diab. Complic.* **15** (2001), 203–210.

[43] A.S. Reddi, Riboflavin nutritional status and flavoprotein enzymes in streptozotocin diabetic rats, *Biochim. Biophys. Acta* **882** (1986), 71–76.

[44] F.C. Ngkeekwong and L.N.G. Leong, Two distinct uptake mechanisms for ascorbate and dehydroascorbate in human lymphoblasts and their interaction with glucose, *Biochem. J.* **324** (1997), 225–230.

[45] G. Nardai, L. Braun, M. Csala, V. Mile, P. Csermely, A. Benedetti, J. Mandl and G. Bánhegyi, Protein disulfide isomerase– and protein thiol-dependent dehydroascorbate reduction and ascorbate accumulation in the lumen of the endoplasmic reticulum, *J. Biol. Chem.* **276** (2001), 8825–8828.

[46] A.R. Frand and C.A. Kaiser, Ero1p oxidizes protein disulfide isomerase in a pathway for disulfide bond formation in the endoplasmic reticulum, *Mol. Cell* **4** (1999), 469–477.

[47] E.M. Berry, E. Ziv and H. Bar-on, Protein and glycoprotein synthesis and secretion by the diabetic liver, *Diabetologia* **19** (1980), 535–540.

[48] J. Young, L.P. Kane, M. Exley and T. Wileman, Regulation of selective protein degradation in the endoplasmic reticulum by the redox potential, *J. Biol. Chem.* **268** (1993), 19810–19818.

[49] R. Mandel, H.J.P. Ryser, F. Ghani, M. Wu and D. Peak, Inhibition of a reductive function of the plasma membrane by bacitracin and antibodies against protein disulfide-isomerase, *Proc. Natl. Acad. Sci.* USA **90** (1993), 4112–4116.