*Endoplasmic Reticulum: A Metabolic Compartment*
*A. Benedetti et al. (Eds.)*
*IOS Press, 2005*



# Possible Links between Metabolism and Oxidative Protein Folding. Consequences of a Diabetes Study

Gábor NARDAI, Eszter PAPP, Tamás KORCSMÁROS, Krisztián STADLER*, Judit JAKUS* and Péter CSERMELY
*Department of Medical Chemistry, Semmelweis University, P.O. Box 260, H-1444 Budapest 8, Hungary*
*[2]Chemical Research Center of the Hungarian Academy of Sciences, P.O. Box 17, H-1525 Budapest, Hungary*

**Abstract.** The endoplasmic reticulum is a preferred compartment for several cellular processes (e.g.: glucose-6-phosphatase system, triglyceride and cholesterol synthesis, different steps of drug metabolism), its metabolic status and regulatory mechanisms are well characterized both in health and in several metabolic disorders. However, the regulation of oxidative protein folding, the chaperone-catalyzed formation and isomerization of protein disulfide bridges is rather poorly characterized. The influence of different metabolic pathways in disulfide bond formation and their changes under metabolic disorders are practically unknown in mammals. Recent studies uncovered a few connections between intermediate metabolism and oxidative protein folding. Here we summarize these data and our recent findings on changes of oxidative protein folding in diabetes. Our results suggest a possible role of small molecular redox systems in the regulation of oxidative protein folding, and can help us to map the metabolic links of the folding process.

## Introduction

Subcellular compartmentalization was undoubtedly one of the major steps of evolution. Separation, besides many other consequences (e.g. enhanced effectiveness of enzyme reactions), helped the development of the more sophisticated regulation characteristic to complex organisms. Beyond the intra-compartmental regulation, the communication between the lumen of the compartment and the cytoplasm is also under tight control. In recent years a growing number of studies proved that there are direct structural and functional connections between the different subcellular compartments (e.g. between mitochondria and the endoplasmic reticulum (ER), phagosomes and the ER, as well as the nuclear membrane and the ER; see refs. 1-3). These interactions can also affect the different intra-compartmental pathways resulting in a more combined regulatory network of the metabolism and cellular homeostasis.

Taken into consideration the above complexity of regulatory pathways, it is not surprising that the regulation and the metabolic integration of some ER-specific processes are ill defined. One of them is protein disulfide bond formation, which is a redox process and requires the contribution of several proteins and small molecules [4]. Disulfide bond formation is integrated to other metabolic pathways, but the precise manner of these regulatory links is not yet uncovered.

Diabetes mellitus is a severe metabolic disorder causing significant changes not only in the carbohydrate balance, but in many other pathways. In our recent experiments we identified the changes of the endoplasmic reticulum redox folding in diabetes [5, 6; G. Nardai, K. Stadler, E. Papp, T. Korcsmáros, J. Jakus and P. Csermely, submitted for publication) indicating a possible interaction between intracellular metabolism and disulfide bond formation. These results, as well as some hypotheses based on them are summarized below.

## 1. ER: its Metabolic Links

Many cellular functions are associated to the ER (Table 1.). Compartment-associated events are not independent from other cellular functions, but they probably need special pathways. E.g.: transport systems have to be integrated to the overall cellular homeostasis. Here we briefly summarize the metabolic relations of the most important ER functions in mammalian cells.

**Table 1.** Cellular Events Associated to the Endoplasmic Reticulum

| | |
|---|---|
| Glucose-6-phosphatase system<br>Termination of the hexuronic acid cycle<br>Gulonolactone oxidase | Phospholipid synthesis<br>Sphingolipid synthesis<br>Triglyceride synthesis<br>Fatty acid elongation<br>Fatty acid desaturation<br>Cholesterol synthesis<br>Cholesterol conjugation |
| Glycoprotein synthesis<br>Disulfide bond formation<br>Protein quality control<br>Protein complex assembly<br>Peptide loading and assembly<br>of MHC-complexes | Microsomal drug metabolism |
| | $Ca^{++}$-storage |

*$Ca^{++}$-storage*: the ER is the most important compartment of intracellular calcium storage and release. Intracellular calcium level has a well defined role in the regulation of numerous metabolic pathways (e.g. *via* the $Ca^{++}$-calmodulin complex; 7). It is well-known that the calcium storage is ATP-dependent and mediated by a family of $Ca^{++}$-ATPases. However, it was also demonstrated that the luminal, $Ca^{++}$-binding proteins of the ER can also modulate the $Ca^{++}$-transport [8].

*Carbohydrate metabolism*: glucose-6-phosphatase is located into the ER in the liver, so the final step of the blood glucose generation is associated to this compartment. In spite of the separation of glucose-dephosphorylation, it is under strict metabolic control [9]. The activity of the glucose-6-phosphatase itself is increased by its substrates. Adrenergic hormones and glucagon also stimulate the enzyme. Conversely, glucose-6-phosphatase is inhibited by amphiphilic compounds, such as fatty acids and acyl-CoA-s. The expression of glucose-6-phosphatase is increased by glucocorticoids, starvation and diabetes [9]. The synthesis of amino-sugars is also connected to the metabolism by its substrate and energy supply.

*Lipid metabolism*: numerous important, rate limiting steps of lipid metabolism are also confined to the ER. These are usually classical, integrated parts of the metabolism and their regulation is well-understood. Fatty acid chain elongation is affected by the malonyl-CoA concentration and the NADPH level. Desaturation of the fatty acid chain is $NADP^+$-dependent and the metabolic state of the cell itself defines the activity of the desaturating enzyme. Triglycerol synthesis requires ATP and is linked to the glycolysis by glycerol-3-phosphate. The key enzyme of the cholesterol synthesis, HMG-CoA reductase is also an ER protein [10]. Besides many well-known regulatory mechanisms, HMG-CoA reductase activity is also regulated by the modulation of the enzyme level, thus ERAD (endoplasmic reticulum associated degradation) is also involved in the cholesterol metabolism by degrading this unstable protein [11]. Generation of phospholipids occurs in the ER and is under metabolic control [12].



*Drug metabolism*: each of the three phases of the microsomal drug elimination process is somehow linked to the intermediate metabolism. During the first phase, substrates are prepared for conjugation by introducing highly reactive groups mainly by oxidative reactions. Enzymes are involved in this process are oxido-reductases (NADPH-cytochrome P450-reductase, cytochrome P450s) using cofactors shared by other metabolic pathways (NADPH, $FADH_2$). Induction of the first phase results in the acceleration of NADPH production by e.g. the pentose-phosphate pathway and heme-synthesis [13]. The latter is required for the cytochrome P450 holoenzyme assembly. In the second phase the substrates become conjugated, where the synthesis of the conjugating molecules (e.g.: glutathione, 3-phospho-adenosine-5-phosphosulphate, UDP-glucuronic acid) is an ATP-requiring process. The third phase is the transport phase, when the water-soluble products are delivered from the ER. Non-specific transport systems and ABC transporters are also involved in this process, thus the competition of different metabolic and drug particles and the energy supply of the cell can all influence the procedure.

*Posttranslational changes of the protein structure*: numerous posttranslational modifications happen in the ER. One of the most important among them is the formation and isomerization of disulfide bonds, we discuss later. Glycoprotein and lipoprotein synthesis are both ATP-dependent processes linked to the carbohydrate, amino sugar and lipid metabolism but the details of their regulation are not clearly resolved yet [14, 15]. Protein transport through the ER membrane, posttranslational modifications, quaternary structure assembly and quality control are all molecular chaperone-dependent processes. This is a general and essential cellular function, but its connections with the intermediate metabolism are poorly understood.

*Oxidative folding*: as one of the most important posttranslational modifications, the formation and isomerization of disulfide bridges also occurs in the ER [4]. Secreted and plasma membrane proteins require disulfide bonds to stabilize their tertiary-quaternary structure. The luminal environment seems to be optimal for the oxidative protein folding pathway: it has a unique and strictly regulated oxidizing milieu (the redox potential is about -160 mV), a high chaperone concentration (stabilizing non-native, reversibly unfolded structures), a quality control and a degradative machinery to eliminate misfolded polypeptides. The first step of the procedure is the import of the nascent polypeptide chain from the ribosomes to the ER lumen. Leaving the transmembrane channel the polypeptide chain becomes associated with molecular chaperones (e.g. Grp78/BiP, Grp94, protein disulfide isomerase, etc.), which keep the nascent chain in a folding-competent state and prevent its aggregation [16]. Some of these chaperones are not involved directly in disulfide bond formation, but others, such as protein disulfide isomerase (PDI) and related enzymes (e.g. ERp57, ERp72, etc.) are themselves protein-disulfide oxido-reductases catalyzing redox folding reactions [17]. During the reaction the protein substrate binds to the oxidized PDI. This event stabilizes the polypeptide chain. Then a mixed disulfide is formed between PDI and the substrate as a next step the new disulfide bond of the substrate protein is generated [18]. Finally, the oxidized substrate either binds to PDI starting a new redox cycle or dissociates from the chaperone in its native conformation and transported to the Golgi system.

One of the most interesting questions is the redox regulation of oxidative protein folding. Disulfide bond formation is coupled to chain of redox reactions, which have not

been elucidated yet in details. During disulfide bridge synthesis PDI gets reduced and its re-oxidation is carried out by a luminal oxido-reductase, Ero1-L *in vivo* [19] and small molecular oxidizing agents (e.g. oxidized glutathione, dehydroascorbate, hydrogen peroxide, etc.) *in vitro*. Ero1-L is a 56 kDa glycoprotein containing a FAD prosthetic group, which is involved in the redox reactions. However, the final, potentially cytoplasmic electron acceptor has not been identified yet. In yeast molecular oxygen is able to support oxidative folding directly, but in mammalian cells $O_2$ seems to be inefficient as an oxidative source [20].

The role of glutathione is under re-evaluation. Reduced and oxidized glutathione form an important redox buffer of the ER. Contrary to recent theories, glutathione is not involved in the PDI-catalyzed disulfide bond formation directly. However, its high luminal concentration, the glutathione/protein mixed disulfides, and the glutathione-specific transport systems all suggest that glutathione must have an important role in disulfide bond formation. It is hypothesized that reduced glutathione works as a counterbalance of the oxidative pressure caused by Ero1-L [21], or glutathione participates in the redox cycle of Ero1-L-independent protein disulfide oxido-reductases (most probably ERp57, ERp72, etc.).

A further possible contributor of the luminal redox cycle is the ascorbate/dehydroascorbate redox pair. Its microsomal concentration is in the millimolar range. Ascorbate is a key co-factor of several synthetic pathways as well as the luminal antioxidant defense [22]. Dehydroascorbate is transported into the ER lumen and its involvement in the protein thiol modification has been substantiated by some published [23] as well as our latest (see below) results.

## 2. Diabetic Changes of the Oxidative Folding

Diabetes mellitus is described as a complex metabolic disease characterized by the absolute or relative shortage of insulin. In diabetes the integration of the cellular metabolism is disrupted causing the disturbances of not only the carbohydrate, but the lipid, amino acid and other metabolic pathways as well. In general, catabolic pathways are preferred and anabolic-synthetic pathways are inhibited in diabetes. One of the consequences of the metabolic disorganization is the increased generation of reactive oxygen species, ROS [24]. The oxidative stress is initially prevented by various antioxidative defense systems, but later on these mechanisms become exhausted, and oxidative damage develops. The accelerated production of ROS is the result of the disorganized function of the mitochondria, glucose autooxidation, and the free radicals generated by the non-enzymatically glycated proteins [25]. These changes are typical of the extracellular space, but the signs of the oxidative stress are also detectable in intracellular compartments. Parallel to these changes there is an altered level and function of antioxidant redox buffers, such as glutathione and ascorbate. Both the level and the regeneration of reduced glutathione are decreased [26]. In diabetes the total cellular level and the ratio of ascorbate/dehydroascorbate are lowered [27, 28] and the luminal transport of dehydroascorbate can be also compromised by the high glucose level in the liver [29]. Beyond oxidative stress the cellular disorders mentioned above can lead to an enhanced protein damage and consequently to an increased demand of chaperone function.



**Table 2.** Diabetic Changes of the ER Redox State
Control and diabetic rat liver microsomal fractions were analyzed before (A) and after (B) the treatment of small molecular oxidants by Ellman's method and by enzymatic determination of GSH/GSSG level. GSH, reduced glutathione; GSSG, oxidized glutathione; DHA, dehydroascorbate; Asc, ascorbate; FAD, flavine adenine dinucleotide.

**A**

| | control | diabetic |
|---|---|---|
| Total –SH content (*nmol -SH/mg protein*) | 139 ± 18 | 187 ± 24 |
| Protein –SH content (*nmol –SH/mg protein*) | 56 ± 5 | 79 ± 8 |
| GSH/GSSG ratio | 1.92 ± 0.06 | 2.25 ± 0.3 |

**B**

| | Total –SH content (*nmol -SH/mg protein*) |
|---|---|
| control | 56 ± 5 |
| diabetic | 79 ± 8 |
| diabetic + GSSG | 48 ± 11 |
| diabetic + DHA | 59 ± 4 |
| diabetic + Asc | 68 ± 4 |
| diabetic + FAD | 70 ± 7 |

Recently, using an animal model (streptozotocin induced diabetes in young, Wistar rats) we studied the diabetic changes of the ER luminal molecular chaperones and oxidative folding. Liver microsomal fraction was isolated and its redox parameters, chaperone levels and chaperone redox states were determined. Surprisingly, we found that in spite of the oxidative changes of the extracellular space, the redox environment of liver microsomal vesicles was shifted to a more reducing state compared to controls (Table 2.A)[5]. Diabetic microsomal redox status was characterized by increased total disulfide content and by and increased protein-thiol: disulfide ratio. The dehydroascorbate reductase activity was also higher in diabetes [5]. Neither the general chaperone levels, nor the level of protein disulfide oxido-reductases showed significant changes.

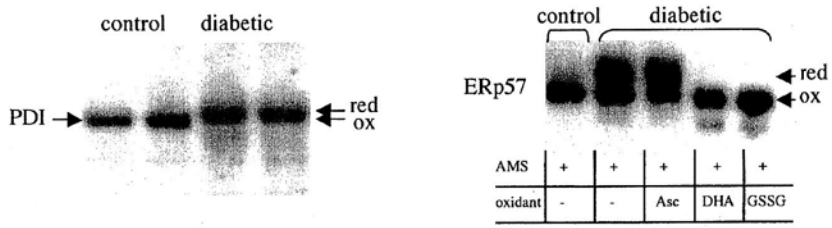


**Figure 1.** Diabetes-Induced Redox Changes of Protein Disulfide Isomerases
Control and diabetic rat liver microsomal fractions were isolated. The redox state of protein disulfide isomerases was determined before and after the *in vitro* treatment with small molecular oxidants by the thiol specific agent, AMS performing a subsequent nonreducing SDS-PAGE.

However, the redox state of the chaperones involved directly in the disulfide bridge synthesis has been changed. Using AMS-(4-acetamido-4'-maleimidylstilbene-2, 2'-disulfonic acid)-labeling and consequent nonreducing SDS-PAGE separation, the appearance of a more reduced fraction of PDI and ERp57 was detectable (Fig. 1.), parallel with a more oxidized status of the Ero1-L. The redox state of other chaperones, which are not involved directly in the disulfide bond formation, was unchanged.

The reduced state of protein disulfide isomerases means that the cysteines in their catalytic site are in a thiol form causing a switch from the disulfide bond formation to the isomerase activity [4]. The presence of the oxidized Ero1-L and the fact that the *in vitro* treatment of diabetic microsomes by FAD was inefficient to reoxidize the luminal protein thiols (Table 2.B) seem to exclude a lowered Ero1-L function or FAD shortage as reasons for the reduced and possibly less functional state of PDI. Further experiments proved that contrary to FAD, the treatment of microsomes by oxidized glutathione and dehydroascorbate could re-oxidize the microsomes (Table 2.B) and restore the original (more oxidized) form of protein disulfide isomerases (Fig. 1.). These data also emphasize the importance of a stable redox environment and the small molecules in redox protein folding. These points also constitute important links, where the cellular metabolism can influence and regulate the ER oxidative protein folding.

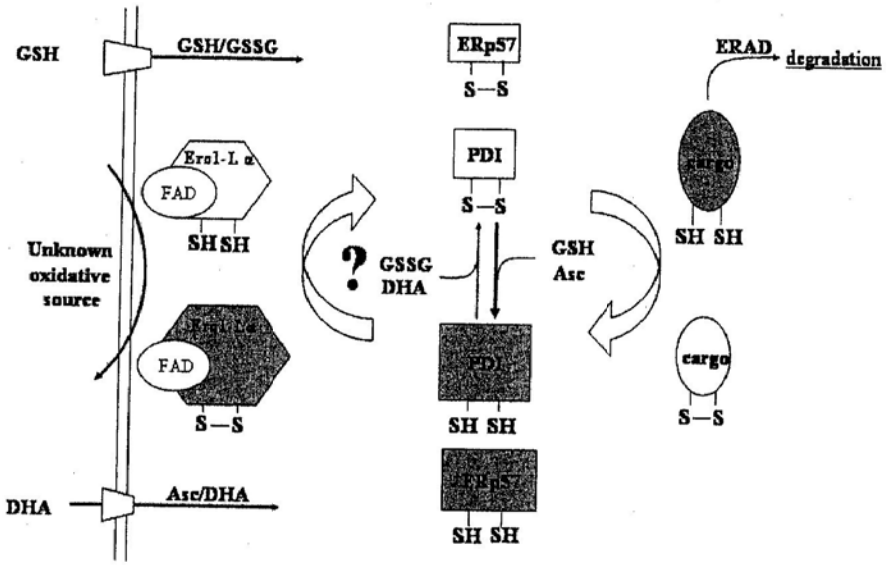


**Figure 2.** Summary of Changes in ER Redox Protein Folding in STZ-Diabetes
Protein disulfide isomerases (PDI-s) become more reduced in the lumen of the endoplasmic reticulum in diabetes, and are unable to assist to secreted proteins for their correct oxidative folding. Both oxidized glutathione as well as dehydroascorbic acid are able to reverse this effect. The protein helping the reoxidation of PDI-s, Ero1-L becomes more oxidized, either as a compensation for the defect in PDI function or reflecting a block in the oxidative chain after Ero1-L. Proteins remain partially unfolded, and most probably leave the ER *via* retrograde transport and proteasomal degradation causing a defect in hepatic protein secretion. Asc, ascorbic acid; DHA, dehydroascorbic acid; ERAD, endoplasmic reticulum associated protein degradation; Ero1-L, endoplasmic reticulum oxidoreductin1-L; ERp57, protein disulfide isomerase; FAD, flavine adenine dinucleotide; GSH, reduced glutathione; GSSG, oxidized glutathione; PDI, protein disulfide isomerase; –SH, sulfhydryl.

## 3. Possible Links between Metabolic Pathways and Oxidative Folding

The chaperone-mediated disulfide bridge formation process is highly redox sensitive. Both reductive and oxidative dominance inhibit the pathway [30, 31]. In spite of their importance, the events sustaining the luminal redox homeostasis have not been clearly



identified yet. NADPH-dependent oxidases, sulfhydryl oxidases and the vitamin K cycle were all suspected to influence the redox potential [32, 33]. In these processes the substrate and cofactor supply integrates these processes to the general metabolism. The reduced and oxidized forms of glutathione are thought to form the most important redox buffer of the ER. However, there are some problems about their role in the formation of the relatively oxidizing environment of the lumen. First, more than 50% of the luminal glutathione content forms mixed protein disulfides [34], second, the import of oxidized glutathione is much less effective than that of the reduced form [35]. Our data from diabetic rats also suggest that a discrepancy can be between the real luminal redox state and the ratio between oxidized and reduced glutathione (data not shown). These observations support the hypothesis mentioned above, that glutathione can be a counterbalance of the oxidative pressure mediated by Ero1-L.

Intriguingly, one of the most important participants of the microsomal redox balance has not yet been discovered in mammals. The final oxidative source of the disulfide bond formation is still unknown. This oxidative agent most probably acts on the FAD prosthetic group of Ero1-L. In some experiments addition of external FAD was able to influence luminal redox state suggesting the existence of a microsomal FAD transport and exchange system [36). These results indicate the possible importance of the cellular FAD level preserved by different metabolic pathways.

Results of the diabetes study summarized above raise also the possible involvement of the ascorbate/dehydroascorbate redox pair in oxidative protein folding and luminal redox state. Ascorbate and dehydroascorbate are essential for human organisms. Ascorbate is an essential cofactor of the collagen, carnitine, catecholamine and peptide hormone syntheses [22]. Ascorbate is a potent antioxidant; its regeneration from dehydroascorbate requires NADPH or glutathione. By this effect dehydroascorbate stimulates the pentose phosphate pathway and glutathione synthesis. In higher concentration it inhibits facilitative glucose transport, hexokinase, glyceraldehyde-3-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase [37 and refs. inside]. Although the metabolic relations of ascorbate are quite well mapped we do not know how it participates in redox protein folding. Its concentration in the ER lumen is in the low milimolar range. The import of dehydroascorbate is preferred and the transporter is blocked by high concentration of glucose [28]. Inside, dehydroascorbate reductases, such as PDI or glutathione (or protein thiols?) can reduce dehydroascorbate back to ascorbate [38]. We hypothesize that ascorbate can be an alternative counterbalance of the oxidative effects and dehydroascorbate can participate in the redox cycle of other, Ero1-L-independent protein disulfide oxido-reductases, such as ERp57 (Fig. 2.).

## 4. Conclusions

The metabolic links and regulation of disulfide bond formation are still rather unexplored, while other relations of redox protein folding were intensively studied. Recently the importance of several small molecular redox systems in the thiol-disulfide exchange was uncovered, but the most important question - the final oxidative source of the process - is still unanswered. Nevertheless, these small molecular redox systems (such as oxidized/reduced glutathione, ascorbate/dehydroascorbate, FAD/$FADH_2$) give us already important clues, how the oxidative protein folding is integrated to the cellular metabolism.

## Acknowledgements

The authors would like to acknowledge the advice and help of Csaba S_ti and Katalin Mihály (Department of Medical Chemistry, Semmelweis University, Budapest, Hungary) in the isolation of microsomes and Western blots. We are thankful to Ineke Braakman (Department of Bio-Organic Chemistry, Bijvoet Center, University of Utrecht, Utrecht, The Netherlands) for the anti-Ero1-L and to David Ferrari (Department of Neurobiology, Max Planck Institute of Biophysical Chemistry, Göttingen, Germany) for the anti-ERp28 antibodies as well as for members of the COST B17 network for advice. This work was supported by research grants from the EU 6th Framework (FP6-506850), ICGEB (CRP/HUN99-02), Hungarian Science Foundation (OTKA-T37357), Hungarian R&D Foundation (NKFP 1/047/2001 MediChem) and the Hungarian Ministry of Social Welfare (ETT-32/03).

### References

[1] T. Andrienko, A.V. Kuznetsov, T. Kaambre, Y. Usson, A. Orosco, F. Appaix, T. Tiivel, P. Sikk, M. Vendelin, R. Margreiter and V.A. Saks. Metabolic consequences of functional complexes of mitochondria, myofibrils and sarcoplasmic reticulum in muscle cells. *J. Exp. Biol.* **206** (2003) 2059-2072.

[2] P. Guermonprez, L. Saveanu, M. Kleijmeer, J. Davoust, P. van Endert and S. Amigorena. ER-phagosome fusion defines an MHC class I cross-presentation compartment in dendritic cells. *Nature* **425** (2003) 397-402.

[3] G. Perkins, C. Renken, M.E. Martone, S.J. Young, M. Ellisman and T. Frey. Electron tomography of neuronal mitochondria: three-dimensional structure and organization of cristae and membrane contacts. *J. Struct. Biol.* **119** (1997) 260-272.

[4] C.S. Servier and C.A. Kaiser. Formation and transfer of disulphide bonds in living cells. *Nat. Rev. Mol. Cell Biol.* **3** (2002) 836-847.

[5] 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.

[6] G. Nardai, T. Korcsmaros, E. Papp and P. Csermely. Reduction of the endoplasmic reticulum accompanies the oxidative damage of diabetes mellitus. *Biofactors* **17** (2003) 259-267.

[7] H. Schulman. The multifunctional Ca2+/calmodulin-dependent protein kinases. *Curr. Opin. Cell Biol.* **5** (1993) 247-253.

[8] L.M. John, J.D. Lechleiter and P. Camacho. Differential modulation of SERCA2 isoforms by calreticulin. *J. Cell Biol.* **142** (1998) 963-973.

[9] E. van Schaftingen and I. Gerin. The glucose-6-phosphatase system. *Biochem. J.* **362** (2002) 513-532.

[10] G.C. Ness and C.M. Chambers. Feedback and hormonal regulation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase: the concept of cholesterol buffering capacity. *Proc. Soc. Exp. Biol. Med.* **224** (2000) 8-19.

[11] R.Y. Hampton. ER-associated degradation in protein quality control and cellular regulation. *Curr. Op. Cell Biol.* **14** (2002) 476-482.

[12] K. Hanada. Serine palmitoyltransferase, a key enzyme of sphingolipid metabolism. *Biochim. Biophys. Acta* **1632** (2003) 16-30.

[13] M.D. Maines. New developments in the regulation of heme metabolism and their implications. *Crit. Rev. Toxicol.* **12** (1984) 241-314.

[14] AJ. Parodi. Protein glucosylation and its role in protein folding. *Annu. Rev. Biochem.* **69** (2000) 69-93.

[15] J.L. Dixon and H.N. Ginsberg. Hepatic synthesis of lipoproteins and apolipoproteins. *Semin. Liver Dis.* **12** (1992) 364-372.

[16] L. Meunier, Y. Usmerwood, K.T. Chung and L. Hendershot. A sub-set of chaperones and folding enzymes form multi-protein complexes in the ER to bind nascent proteins. *Mol. Cell Biol.* **13** (2002) 4456-4469.

[17] D.M. Ferrari and H.D. Söling. The protein disulphide-isomerase family: unraveling a string of folds. *Biochem. J.* **339** (1999) 1-10.

[18] R.B. Freedman, T.R. Hirst and M.F. Tuite. Protein disulphide isomerase: building bridges in protein folding. *Trends Biochem. Sci.* **19** (1994) 331-336.



[19] 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.

[20] 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.

[21] J.W. Couzzo and C.A. Kaiser. Competition between glutathione and protein thiols for disulphide-bond formation. *Nat. Cell Biol.* **1** (1999) 130-135.

[22] G. Bánhegyi, L. Braun, M. Csala, F. Puskás and J. Mandl. Ascorbate metabolism and its regulation in animals. *Free Radic. Biol. Med.* **23** (1997) 793-803.

[23] M. Csala, L. Braun, V. Mile, T. Kardon, A. Szarka, P. Kupcsulik, J. Mandl and G. Bánhegyi. Ascorbate-mediated electron transfer in protein thiol oxidation in the endoplasmic reticulum. *FEBS Lett.* **460** (1999) 539-543.

[24] J.W. Baynes and S.R. Thorpe. Role of oxidative stress in diabetic complications. A new perspective of an old paradigm. *Diabetes* **48** (1999) 1-9.

[25] P. Martin-Gallan, A. Carrascosa, M. Gussinye and C. Dominguez. Biomarkers of diabetes-associated oxidative stress and antioxidant status in young diabetic patients with or without subclinical complications. *Free Radic. Biol. Med.* **34** (2003) 1563-1574.

[26] A.Y. Lee and S.S. Chung. Contributions of polyol pathway to oxidative stress in diabetic cataract. *FASEB J.* **13** (1999) 23-30.

[27] M. Kashiba, J. Oka, R. Ichikawa, E. Kasahara, T. Inayama, H. Kageyama, T. Osaka, K. Umegaki, A. Matsumo, T. Ishikawa, M. Nishikimi, M. Inoue and S. Inoue. Impaired ascorbic acid metabolism in streptozotocin-induced diabetic rats. *Free Radic. Biol. Med.* **33** (2002) 1221-1230.

[28] A.M. Bode, C.R. Yavarow, D.A. Fry and T. Vargas. Enzymatic basis for altered ascorbic acid and dehydroascorbic acid levels in diabetes. *Biochem. Biophys. Res. Commun.* **191** (1993) 1347-1353.

[29] 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.

[30] 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.

[31] M.M. Lyles and H.F. Gilbert. Catalysis of the oxidative folding of ribonuclease A by protein disulfide isomerase: dependence of the rate on the composition of the redox buffer. *Biochemistry* **30** (1991) 613-619.

[32] L.L. Poulsen and D.M. Ziegler. Microsomal mixed-function oxidase-dependent renaturation of reduced ribonuclease. *Arch. Biochem. Biophys.* **183** (1977) 563-570.

[33] B. Benayoun, A. Esnard-Feve, 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.

[34] R. Bass, L.W. Ruddock, P. Klappa and R.B. Freedman. A major fraction of ER-located glutathione is present as mixed disulphides with protein. *J. Biol. Chem.* **279** (2004) in press (M304951200).

[35] G. Bánhegyi, L. Lusini, F. Puskás, R. Rossi, R. Fulceri, L. Braun, V. Mile, P. di Simplicio, J. Mandl and A. Benedetti. Preferential transport of glutathione versus glutathione disulfide in rat liver microsomal vesicles. *J. Biol. Chem.* **274** (1999) 12213-12216.

[36] M. Varsányi, A. Szarka, E. Papp, D. Makai, G. Nardai, R. Fulceri, P. Csermely, J. Mandl, A. Benedetti and G. Bánhegyi. FAD transport and FAD-dependent protein thiol oxidation in rat liver microsomes. *J. Biol. Chem.* **279** (2004) 3370-3374

[37] J.X. Wilson. The physiological role of dehydroascorbic acid. *FEBS Lett.* **527** (2002) 5-9.

[38] W.W. Wells, D.P. Xu, Y.F. Yang and P.A. Rocque. Mammalian thioltransferase (glutaredoxin) and protein disulfide isomerase have dehydroascorbate reductase activity. *J. Biol. Chem.* **265** (1990) 15361-15364.

**NATO Science Series**

A series presenting the results of scientific meetings supported under the NATO Science Programme.

The series is published by IOS Press and Springer Science and Business Media in conjunction with the NATO Public Diplomacy Division.

*Sub-Series*

| | | |
|---|---|---|
| I. | Life and Behavioural Sciences | IOS Press |
| II. | Mathematics, Physics and Chemistry | Springer Science and Business Media |
| III. | Computer and Systems Sciences | IOS Press |
| IV. | Earth and Environmental Sciences | Springer Science and Business Media |
| V. | Science and Technology Policy | IOS Press |

The NATO Science Series continues the series of books published formerly as the NATO ASI Series.

The NATO Science Programme offers support for collaboration in civil science between scientists of countries of the Euro-Atlantic Partnership Council. The types of scientific meeting generally supported are "Advanced Study Institutes" and "Advanced Research Workshops", although other types of meeting are supported from time to time. The NATO Science Series collects together the results of these meetings. The meetings are co-organized by scientists from NATO countries and scientists from NATO's Partner countries – countries of the CIS and Central and Eastern Europe.

**Advanced Study Institutes** are high-level tutorial courses offering in-depth study of latest advances in a field.
**Advanced Research Workshops** are expert meetings aimed at critical assessment of a field, and identification of directions for future action.

As a consequence of the restructuring of the NATO Science Programme in 1999, the NATO Science Series has been re-organized and there are currently five sub-series as noted above. Please consult the following web sites for information on previous volumes published in the series, as well as details of earlier sub-series:

http://www.nato.int/science
http://www.springeronline.nl
http://www.iospress.nl
http://www.wtv-books.de/nato_pco.htm

Series I: Life and Behavioural Sciences - Vol. 363

ISSN: 1566-7693

# Endoplasmic Reticulum: A Metabolic Compartment

Edited by

Angelo Benedetti

*Dipartimento di Fisiopatologia e Medicina Sperimental, Università di Siena, Siena, Italy*

Gábor Bánhegyi

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

and

Ann Burchell

*Division of Maternal and Child Health Sciences, Ninewells Hospital and Medical School, University of Dundee, Dundee, United Kingdom*

IOS Press

Amsterdam • Berlin • Oxford • Tokyo • Washington, DC

Published in cooperation with NATO Public Diplomacy Division