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Minireview

Stress on redox

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Abstract **Redox imbalance in the endoplasmic reticulum lumen is the most frequent cause of endoplasmic reticulum stress and consequent apoptosis. The mechanism involves the impairment of oxidative protein folding, the accumulation of unfolded/misfolded proteins in the lumen and the initiation of the unfolded protein response. The participation of several redox systems (glutathione, ascorbate, FAD, tocopherol, vitamin K) has been demonstrated in the process. Recent findings have attracted attention to the possible mechanistic role of luminal pyridine nucleotides in the endoplasmic reticulum stress. The aim of this minireview is to summarize the luminal redox systems and the redox sensing mechanisms of the endoplasmic reticulum.**
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Keywords: Endoplasmic reticulum; Oxidative protein folding; Unfolded protein response; Glutathione; NADPH; Ascorbate

1. Introduction

Decades after its formulation, Hans Selye's stress theory [1] triumphantly descended to the subcellular level. In the original context, animal life exists by maintaining a complex dynamic equilibrium, termed homeostasis that is constantly challenged by intrinsic or extrinsic adverse forces, the stressors. Stress is defined as a state of threat, or perceived threat to homeostasis. Imbalance of homeostasis at the cellular level can be sensed by various organelles. The stress-sensing capability of mitochondria, lysosomes and the constituents of the secretory pathway including the endoplasmic reticulum (ER) and the Golgi-apparatus has been described [2–5]. In particular, ER stress has been in the focus of increased attention in the last years (for recent reviews see [6–9]). The role of the ER as a sensor for electron donors and acceptors, i.e. nutrients and oxygen, is a recent suggestion. Diverse nocuous agents can affect adversely the proper functioning of the ER, causing the typical syndrome of the ER stress, the signs of which are independent of the nature of either the damaging agent or the pharmacological type of the drug employed. Most of these agents disturb the redox homeostasis of the ER lumen, which is subsequently detected by local sensors resulting in the activation of ER-to-nucleus signalling pathways.

2. Redox environment in the ER lumen

The major redox couples participating in the electron transfer processes of the cell are also present in the ER lumen, although concentrations and redox states can differ between the two compartments. Redox state refers to the ratio of the interconvertible oxidized and reduced forms of a specific redox couple. This term is also used in a more general sense not only for defining the state of a particular redox pair, but for describing the entire redox environment of a subcellular compartment. The term redox environment most accurately defines the redox characteristics of a certain organelle, as a sum of redox potentials and reducing capacities of linked redox couples present [10].

However, it is impossible to determine the redox environment by the measurement of all linked redox couples. Instead, the changes in the redox environment are monitored using a representative redox couple as an indicator. In the animal cell, the abundant glutathione disulfide (GSSG) /reduced glutathione (GSH) couple provides a very large pool of redox competent molecules, hence it is considered to be the major cellular redox buffer. Therefore, the redox state of this couple can be used as an indicator for the redox environment of the cell or a subcellular compartment.

Local oxidoreductions, transmembrane transport of extraluminally synthesized redox-active compounds and transmembrane electron fluxes lay down the redox environment of the ER lumen. Similarly to other compartments of the cell, the major redox buffer of the lumen is the GSSG/GSH couple. Although the ER luminal and cytosolic glutathione concentrations are similar (i.e. 1–10 mM), the ratio of [GSSG] to [GSH]

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Abbreviations: ATF6, activating transcription factor 6; BiP, immunoglobulin heavy chain-binding protein; DHAR, dehydroascorbate reductase; ER, endoplasmic reticulum; Ero1, endoplasmic reticulum oxidoreductin 1; ERp44 and 57, endoplasmic reticulum protein of molecular weight 44 or 57 kDa; GRP78, glucose-regulated protein of molecular weight 78 kDa; G6PT, glucose-6-phosphate transporter; H6PDH, hexose-6-phosphate dehydrogenase; 11βHSD1, 11β-hydroxysteroid dehydrogenase type 1; InsP3R, inositol 1,4,5-trisphosphate receptor; IRE1, inositol-requiring enzyme 1; PDI, protein disulfide isomerase; PERK/PEK, PKR-like ER protein kinase/pancreatic eIF2α (eukaryotic translation initiation factor 2, α subunit) kinase; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; UPR, unfolded protein response

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is higher in the ER as compared to the cytosol, falling in the ranges of approximately 1:1–3 versus 1:30–100, respectively [11,12]. These ratios result in redox potentials of about −180 mV and −230 mV, respectively, i.e. the ER lumen can be regarded as a more oxidizing environment. Moreover, high percentage of luminal glutathione was shown to form mixed disulfides with proteins, which may play a role as a GSH reserve and also as a component of the thiol-disulfide redox buffering system [13].

It was supposed that this peculiar intraluminal ER milieu ensures disulfide bond formation in secretory proteins by maintaining an oxidizing power. However, it has been shown that protein disulfide formation is independent of glutathione, both under *in vivo* and *in vitro* conditions [14,15]. The process is catalyzed by a protein-mediated electron relay system, in which protein disulfide isomerase [16] and Ero1p [17–20] are the main players and oxygen is the ultimate electron acceptor. These studies demonstrated that, contrary to previous assumptions, glutathione is not the source of oxidizing equivalents for oxidative protein folding within the ER. Instead, it functions as a reductant for both protein disulfide isomerase (PDI) and secretory proteins, allowing the isomerization of disulfide bonds, until the correct conformation is achieved. Another important conclusion of these recent findings is that the prooxidant environment within the ER is not the actual cause, but rather the consequence of the oxidative protein folding. Nevertheless, the possible interferences between the GSSG/GSH system and both oxidation and reduction of protein thiols underscores the importance of a tight regulation of the ER redox milieu, since changes in *either* direction drastically interfere with ER functions.

Beside glutathione, the other antioxidant components of the Halliwell-Asada cycle are also present in the ER. The cycle may represent an alternative (minor?) pathway for the transfer of electrons from protein thiols to oxygen. Ascorbate acts not only as an antioxidant in the lumen, but also as a cofactor for various luminal enzymes (e.g. prolyl- and lysyl-hydroxylases) and its oxidized form, dehydroascorbate can accept electrons from protein disulfide isomerase [21–23]. Ascorbate, in turn, can regenerate the oxidized form of tocopherol, a hydrophobic antioxidant in the ER membrane, which can mediate a transmembrane electron transfer [24]. Ascorbate is formed in the ER of ascorbate-synthesizing animals, while in species unable to produce this vitamin it is transported through the membrane as dehydroascorbic acid [22]. This circumstance might cause – presently unexplored – differences in the ascorbate-dependent processes of the ER.

The presence of other redox-active compounds has also been demonstrated. γ-Carboxylation of vitamin K-dependent proteins in the ER involves the formation of reduced vitamin K, an essential cofactor for γ-carboxylase catalyzing this post-translational protein modification. By accepting electrons from protein disulfide isomerase, vitamin K can be regenerated during the protein γ-carboxylation-linked redox cycle [25]. FAD is known to act both as a tightly associated prosthetic group and as a relatively free cofactor of Ero1, therefore making this enzyme highly responsive to small changes in the physiological levels of free FAD [15,26]. Although FAD transport has been demonstrated in yeast and rat liver microsomes, the concentration and redox state of luminal FAD are unknown.

Most of these redox couples are linked to each other to form complex redox systems (Fig. 1). In some cases, couples are independent from each other if they are not linked by appropriate enzyme systems and the activation energies of the electron transfer reactions are high. For example, the luminal pyridine nucleotide pool and the GSH/GSSG system seem to be uncoupled. Although their *in vivo* redox state and concentrations have not been elucidated, pyridine nucleotides must be present in the ER lumen, since several oxidoreductases with intraluminal active site require them as coenzymes. In fact, ER-derived microsomal vesicles contain a significant amount of pyridine nucleotides [27]. This pool is overwhelmingly

Fig. 1. The best known redox systems in the ER. For simplicity, only one member of each redox couple is indicated. Redox potentials are increasing from the left to the right. The grey bar symbolizes the redox potential of the ER lumen calculated on the basis of glutathione and glutathione disulfide concentrations: −0.18 V. *Abbreviations*: DHAR, dehydroascorbate reductase; H6PDH, hexose-6-phosphate dehydrogenase; 11βHSD1, 11β-hydroxysteroid dehydrogenase type 1; PDI, protein disulfide isomerase.

composed of reduced NAD(P)H, which presumably mirrors the *in vivo* situation. This assumption is supported by observations concerning the functioning of the ER enzyme 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1). 11βHSD1 catalyzes the reversible interconversion of cortisone and cortisol in the lumen of the ER [28]. Under *in vivo* conditions this reaction is predominantly shifted towards cortisone reduction, indicating a high luminal [NADPH]/[$NADP^+$] ratio. The high ratio can be maintained by luminal dehydrogenases, such as hexose-6-phosphate dehydrogenase (H6PDH), because the permeability of the ER membrane to pyridine nucleotides is insignificant [29]. H6PDH knockout mice lack 11βHSD1 mediated glucocorticoid generation [30], which proves that the enzyme cannot rely on cytosolic NADPH resources and that a separate luminal pyridine nucleotide pool must exist [31,32]. It also clearly shows that the high luminal [NADPH]/[$NADP^+$] ratio is dependent on the H6PDH activity and – consequently – on the transport of the substrate glucose-6-phosphate across the ER membrane [33].

In the cytosol, both NADPH and glutathione are present predominantly in the reduced state due to the enzymatic coupling of the two redox couples, glutathione disulfide being reduced at the expense of NADPH in a reaction catalyzed by glutathione reductase. In spite of the oxidizing power of the GSSH/GSH system, pyridine nucleotides remain reduced in the ER lumen. The peaceful coexistence of the two redox systems is ensured by their uncoupling, since glutathione reductase is hardly detectable in the lumen [32].

3. Transport of redox-active compounds in the ER

The ER is an island surrounded by a sea of cytosol. Not surprisingly, redox-active compounds coming from or leaving towards the cytosol can profoundly affect the environment in the ER lumen. Although the permeation of several redox-active compounds has been explored in the ER membrane (Fig. 2), identification of the proteins participating in these transport processes lags behind. In comparison with other organelles, limited knowledge is available with regard to ER transporters, due to the technical difficulty of ER transport measurements and of the purification and reconstruction approach [34].

The majority of reducing equivalents are transported into the lumen through the translocon protein channel as cysteinyl thiols of the newly synthesized secretory proteins. An open translocon can mediate the transport of small molecules; its participation in the permeation of redox-active compounds cannot be excluded [35]. GSH has a slow protein-mediated transport, while the membrane is impermeable to GSSG [36]. This observation rules out the previous hypothesis [12] that the high [GSSG]/[GSH] ratio can be obtained through preferential import of GSSG from the cytosol. On the contrary, the locally oxidized GSH is entrapped in the form of GSSG in the lumen, contributing to the maintenance of the oxidizing environment in the compartment.

Reducing equivalents are transported into the lumen also in the form of glucose-6-phosphate. The corresponding transporter (G6PT) is one of the few ER transporters characterized at the molecular level [33]. The transport and the coupled H6PDH activity seem to be mainly responsible for the maintenance of the reduced state of the luminal pyridine nucleotide pool [29,32].

Although luminal oxidoreductases contribute dominantly to the oxidative environment, transport of oxidants is also important. The transport of dehydroascorbate and FAD has been evidenced in the ER [26,37,38]. Both compounds facilitate luminal thiol oxidation [21,22,26,38], therefore the physiological role of their transport has been proposed. The ER membrane is selectively permeable to dehydroascorbate, the oxidized form of ascorbate [37]. Luminal reduction of dehydroascorbate to ascorbate is associated with thiol oxidation and leads to an ascorbate entrapment [22]. FAD uptake and consequent thiol oxidation have also been found in yeast and liver microsomes [26,38]. In contrast to FAD, pyridine nucleo-

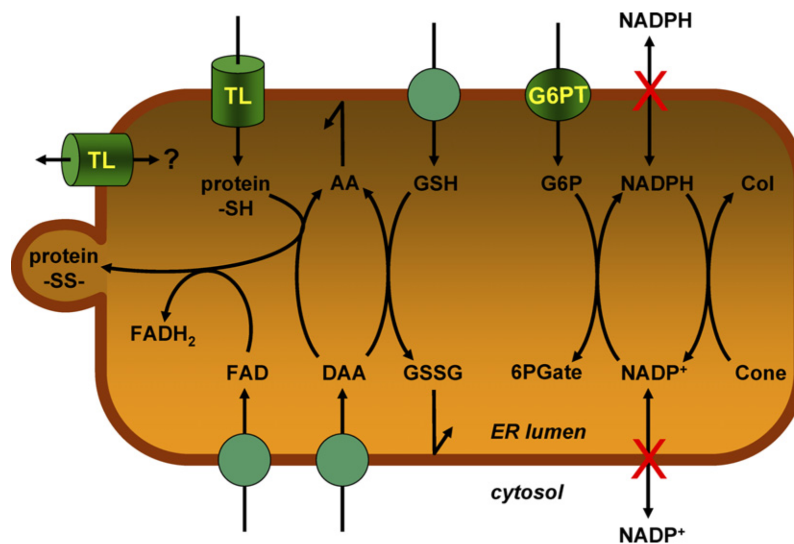

Fig. 2. Permeability and entrapment: determinants of luminal redox. Upper side: transport of electron donors; lower side: transport of electron acceptors. Translocon channel (and presumably other less identified pores in the ER membrane) can be responsible for the non-specific, low-affinity low-capacity transport of various small molecules. With the exception of translocon and G6PT, other redox-competent transport processes have only been characterized functionally. *Abbreviations*: AA, ascorbate; DAA, dehydroascorbate; Col, cortisol; Cone, cortisone, TL, translocon channel; G6P, glucose-6-phosphate; 6PGate, 6-phosphogluconate; G6PT, glucose-6-phosphate translocase.

tides of similar size and structure cannot enter the ER lumen at a significant rate, which is indicated by the high latency of luminal H6PDH and 11βHSD1 [31] and by direct transport measurements [32].

4. BiP-mediated redox sensing

Formation of disulfide bonds in proper positions is required for the folding of the majority of secretory and membrane proteins. Redox imbalance caused by either experimental agents or pathophysiological conditions leads to the accumulation of unfolded/misfolded proteins in the ER lumen. Either oxidative or reductive stress can pose a similar danger as implicated by the ER stress component involved in the putative pathomechanism of ageing, Alzheimer, diabetes, etc. (for review see [8,9]). The accumulation of defective proteins can lead to the activation of a coordinated adaptive program through ER-to-nucleus signalling, called the unfolded protein response (UPR) [6]. In response to the accumulation of unfolded proteins in the ER, the rate of general translation initiation is attenuated, ER resident chaperones and foldases are induced and the ER membrane proliferates to enlarge the luminal compartment, also ER-associated degradation is activated to eliminate the misfolded proteins. Finally, if prosurvival efforts are exhausted, ER-stress related apoptosis is activated. The present theory of the UPR premises that the various misfolded proteins are recognized and the diverse signalling pathways are initiated by a single master regulator, BiP.

Nascent unfolded polypeptides are maintained in soluble form by interactions with ER luminal chaperones in stress-free conditions. BiP (immunoglobulin heavy chain-binding protein or GRP78, glucose-regulated protein of molecular weight 78 kDa) is one of the most abundant ER resident chaperones. To date there are three identified proximal sensors of the UPR: the PKR-like ER protein kinase/pancreatic eIF2α (eukaryotic translation initiation factor 2, α subunit) kinase (PERK/PEK); the activating transcription factor 6 (ATF6); and the inositol-requiring enzyme 1 (IRE1). These sensors are integral proteins of the ER membrane; the association of their luminal domain with BiP keeps them in inactive state. According to the current model of UPR, the perturbed ER homeostasis leads to the accumulation of unfolded/misfolded proteins in the ER lumen, which can preferentially bind BiP sequestering the chaperon in the lumen. The consequent dissociation of BiP from the transmembrane sensors permits their signalling [6]. This hypothesis is supported by the fact that BiP is found in association with the stress transducers under stress-free conditions and is released upon accumulation of unfolded proteins. Moreover, overexpression of BiP attenuates UPR through all the three branches of signalling. In conclusion, BiP functions as an unfolded protein sensor to mechanistically link luminal redox imbalance with UPR activation. This attractive model, though valid in case of experimental, acute and severe ER stress, cannot account for the selective activation of ER stress sub-pathways in pathophysiological conditions. Therefore, the *in vivo* mechanism seems much more complicated than previously thought. The passive competition model for BiP between unfolded proteins and transmembrane signal transducers has been challenged by recent observations. A relatively stable binding between ATF6 and BiP was observed, and a region within the ATF6 luminal domain was identified as a specific ER stress-responsive sequence required for ER stress-triggered BiP release [39]. Furthermore, deletion of the BiP-binding site of IRE1 failed to alter the inducibility of ER stress, showing that BiP is not the principal determinant of IRE1 activity, but an adjustor for sensitivity to various stresses [40]. On the basis of these findings it can be supposed that other regulators are also involved in the response initiation, or that other tissue-specific adaptor proteins can ensure the pleiotropic signalling and biological responses upon the activation of UPR in response to different physiological stimuli.

5. Direct redox sensing

Changes in the luminal redox state can be sensed not only by secretory proteins that await folding, but also directly by transmembrane proteins involved in signalling. For example, in case of ATF6 the complex formation/dissociation with BiP is not the only regulatory mechanism. It has been recently shown that owing to the presence of intra- and intermolecular disulfide bridges, ATF6 is present in unstressed ER in monomer, dimer or oligomer form. The disulfide bonds of ATF6 are reduced upon treatment of cells with various experimental ER stress inducers, which increase the amount of reduced ATF6 monomers that are active in the UPR signalling. ER stress evoked by more physiological mechanism, such as glucose starvation, also activates ATF6. Beside an enhanced ATF6 synthesis likely due to transcriptional induction, reduction of disulfide bridges and transport of reduced monomers to Golgi occurred in response to glucose starvation. The results show that at least two events are necessary for ATF6 activation, namely the dissociation of BiP and the reduction of disulfide bridges [41,42]. Although the mechanism of ATF6 reduction is still enigmatic, it was supposed that ER luminal oxidoreductases, activated upon glucose starvation, may participate in the process. The enzymes responsible and the source of luminal reducing power remain to be clarified.

Ca^{2+} release from the luminal store is an important event of ER stress and ER-dependent apoptosis. Several observations stress the role of luminal redox changes in cellular calcium homeostasis. Both ER/SR calcium channels and calcium pumps can serve as subjects for intraluminal redox regulation. Redox-sensitive thiols in the ryanodine receptor calcium channel are targets for oxidoreduction; the reactions modulate the open probability of the channel. On this ground, the ryanodine receptor calcium channel has been postulated as a transmembrane redox sensor in the SR [43,44]. Recent findings demonstrate that the function of the InsP3 receptor (InsP3R) is also governed by ER luminal redox status. It was found that ERp44, an ER luminal protein belonging to the thioredoxin family, directly interacts with the third luminal loop of InsP3R type 1 (InsP3R1) and directly inhibits the receptor. This interaction is dependent on the redox state (as well as on luminal pH and Ca^{2+} concentration). The presence of reduced cysteinyl thiols in the third loop is required for the interaction. Thus, ERp44 senses the environment in the ER lumen and modulates the calcium homeostasis through IP3R1 activity [45].

Calcium reuptake into the ER is also regulated by the redox state and calcium. The luminal protein ERp57 was shown to regulate SERCA2b activity [46]. ERp57 overexpression

reduces the frequency of SERCA 2b-dependent Ca^{2+} oscillations; the effect is dependent on the presence of cysteinyl residues located in intraluminal loop 4. Store depletion results in ERp57 dissociation and a relief of SERCA2b inhibition. The results suggest that ERp57 modulates the redox state of luminal thiols in SERCA 2b in a Ca^{2+}-dependent manner, providing dynamic control of ER Ca^{2+} homeostasis.

These interactions between luminal redox and Ca^{2+} signalling may also be significant in the cellular response to stress, serving to protect the cell from apoptosis. Indeed, expression of both ERp57 and ERp44 is increased by cellular stress. ERp44 overexpression was shown to inhibit apoptosis [45]. In conclusion, these studies underline the interdependence of luminal redox state, oxidative protein folding and calcium signalling. InsP3R-induced calcium release may be an important link between luminal redox imbalance and apoptosis.

6. Beyond thiols

One can suppose that the imbalance of luminal redox systems other than the GSH/GSSG couple also initiates ER stress response. Since the ascorbate/dehydroascorbate and FAD/$FADH_2$ couples are in intimate connection with the thiol/disulfide systems, it is not surprising that riboflavin deficiency in Jurkat or HepG2 cells [47,48] or ascorbate deficiency (scurvy) in guinea pigs [49] triggers ER stress. These findings underline the possible role of the corresponding transporters in ER pathology.

However, the $NAD(P)H/NAD(P)^+$ system is not connected by a direct enzymatic route to the thiol/disulfide systems. Consequently, alterations of the redox state of pyridine nucleotides are not mirrored in the redox state of glutathione or secretory proteins, therefore the "classic" UPR signalling pathways are presumably not activated. However, several observations indicate that conditions which can be linked to an altered redox state of luminal pyridine nucleotides are associated with ER stress.

The reduced state of luminal NADPH is maintained by the concerted action of G6PT and of the luminal H6PDH [28,29]. A high $[NADPH]/[NADP^+]$ ratio is important not only for the substrate supply of luminal reductases (including 11βHSD1), but also because of the role NADPH plays as an antiapoptotic factor. Indeed, inhibition or downregulation of G6PT results in increased apoptosis in glioma cells [50,51] or neutrophil granulocytes [52]. In line with these observations, inborn deficiency of G6PT in glycogen storage disease type 1b is characterized by severe neutropenia and neutrophil dysfunction [53].

Although the *in vivo* redox state of the luminal NADPH/$NADP^+$ system is still unknown and there is no direct evidence of it being changed under pathological conditions, it can be hypothesized that the altered expression/activity of luminal NADP(H)-dependent oxidoreductases is reflected by a redox shift. For example, 11βHSD1 and H6PDH expression and activity are changed in the experimental animal models of obesity, metabolic syndrome and type 2 diabetes. These enzymes are mainly expressed in the liver and in adipose tissue.

In accordance with this assumption, obesity causes ER stress in adipose and liver tissues of mouse models with either dietary or genetic obesity. The stress, in turn, leads to the IRE1-dependent activation of c-Jun N-terminal kinase and subsequent serine phosphorylation of insulin receptor substrate-1. These events suppress insulin receptor signalling. Deficiency of the Xbox-binding protein-1, a transcription factor that modulates the ER stress response, leads to insulin resistance in mice [54]. These findings demonstrate that ER might be a sensor organelle for metabolic stress in obesity and the ER stress response is an important component in the development of further metabolic consequences, such as insulin resistance and type 2 diabetes [55]. These observations are confirmed by other independent studies that have demonstrated the close connection between ER function and insulin sensitivity [56,57]. If we accept that ER stress has an important role in mediating insulin resistance not only in animal models of obesity but also in humans, pharmacological stimulation of folding capacity might be a promising therapeutic approach. Chemical chaperones – i.e. small molecules that can facilitate protein folding and attenuate unfolded protein response activation – are promising candidates in the treatment of insulin resistance [58]. The effectiveness of these agents has been already proved in a mouse model of type 2 diabetes [59].

Since the ER is very sensitive to glucose, lipid, oxygen and energy availability, it could be regarded as an essential and ancient site of integration between nutrients. However, the mechanism of this integration is poorly understood. The cytosolic NADPH supply as an integrative tool of carbohydrate and lipid metabolism has been known for a long while; it is tempting to suppose that similar mechanism can be operative in the ER lumen. High glucose-6-phosphate supply through the activation of H6PDH and 11βHSD1 leads to increased cortisol production. This event, prereceptorial glucocorticoid activation is supposed to be responsible for the majority of metabolic changes seen in obesity, metabolic syndrome and type 2 diabetes. In conclusion, sensing of metabolic alterations through the altered activity of certain luminal oxidoreductases could change the luminal redox state which leads to glucocorticoid activation and autocrine signalling. This putative novel mechanism of ER stress response echoes Selye's stress theory on a subcellular level.

7. Concluding remarks

ER stress is an important tool of the cell to cope with the changes of both intra- and extracellular environment. ER "eustress" (i.e. a positive, desirable form of stress) and UPR are indispensable for professional secretory cells, such as antibody-secreting plasma cells, collagen-secreting osteoblasts, cells of endocrine and exocrine organs. In these cells physiological ER stress serves as an accommodation process to the increased demand to ensure proper function and survival. On the other hand, chronic activation of the ER signalling pathways in ER "distress" is an emerging candidate as a crucial factor in the pathomechanism of numerous civilization diseases caused by longevity, lifestyle factors, high input of and low demand for energy. Understanding the luminal redox and mapping the pathways involved is essential for the development of successful therapeutic strategies for the treatment of ER-stress-related diseases.

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References

[1] Selye, H. (1950) Stress and the general adaptation syndrome. Br. Med. J. 1, 1383–1392.
[2] Lin, M.T. and Beal, M.F. (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443, 787–795.
[3] Hicks, S.W. and Machamer, C.E. (2005) Golgi structure in stress sensing and apoptosis. Biochim. Biophys. Acta 1744, 406–414.
[4] Terman, A., Kurz, T., Lin, M.T., Beal, M.F., Gustafsson, B. and Brunk, U.T. (2006) Lysosomal labilization. IUBMB Life 58, 531–539.
[5] Maag, R.S., Hicks, S.W. and Machamer, C.E. (2003) Death from within: apoptosis and the secretory pathway. Curr. Opin. Cell. Biol. 15, 456–461.
[6] Schroder, M. and Kaufman, R.J. (2005) ER stress and the unfolded protein response. Mutat. Res. 569, 29–63.
[7] Sitia, R. and Molteni, S.N. (2004) Stress, protein (mis)folding, and signaling: the redox connection. Sci. STKE 239, 27.
[8] Yoshida, H. (2007) ER stress and diseases. FEBS J. 274, 630–658.
[9] Marciniak, S.J. and Ron, D. (2006) Endoplasmic reticulum stress signaling in disease. Physiol. Rev. 86, 1133–1149.
[10] Schafer, F.Q. and Buettner, G.R. (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic. Biol. Med. 30, 1191–1212.
[11] Braakman, I., Helenius, J. and Helenius, A. (1992) Manipulating disulfide bond formation and protein folding in the endoplasmic reticulum. EMBO J. 11, 1717–1722.
[12] Hwang, C., Sinskey, A.J. and Lodish, H.F. (1992) Oxidized redox state of glutathione in the endoplasmic reticulum. Science 257, 1496–1502.
[13] Bass, R., Ruddock, L.W., Klappa, P. and Freedman, R.B. (2004) A major fraction of endoplasmic reticulum-located glutathione is present as mixed disulfides with protein. J. Biol. Chem. 279, 5257–5262.
[14] Cuozzo, J.W. and Kaiser, C.A. (1999) Competition between glutathione and protein thiols for disulphide-bond formation. Nat. Cell Biol. 1, 130–135.
[15] Tu, B.P., Ho-Schleyer, S.C., Travers, K.J. and Weissman, J.S. (2000) Biochemical basis of oxidative protein folding in the endoplasmic reticulum. Science 290, 1571–1574.
[16] Kersteen, E.A. and Raines, R.T. (2003) Catalysis of protein folding by protein disulfide isomerase and small-molecule mimics. Antioxid. Redox Signal. 5, 413–424.
[17] Frand, A.R. and Kaiser, C.A. (1998) The ERO1 gene of yeast is required for oxidation of protein dithiols in the endoplasmic reticulum. Mol. Cell 1, 161–170.
[18] Frand, A.R., Cuozzo, J.W. and Kaiser, C.A. (2000) Pathways for protein disulphide bond formation. Trends Cell Biol. 10, 203–210.
[19] Mezghrani, A., Fassio, A., Benham, A., Simmen, T., Braakman, I. and Sitia, R. (2001) Manipulation of oxidative protein folding and PDI redox state in mammalian cells. EMBO J. 20, 6288–6296.
[20] Pollard, M.G., Travers, K.J. and Weissman, J.S. (1998) Ero1p: a novel and ubiquitous protein with an essential role in oxidative protein folding in the endoplasmic reticulum. Mol. Cell 1, 171–182.
[21] Bánhegyi, G., Csala, M., Szarka, A., Varsányi, M., Benedetti, A. and Mandl, J. (2003) Role of ascorbate in oxidative protein folding. Biofactors 17, 37–46.
[22] Nardai, G., Braun, L., Csala, M., Mile, V., Csermely, P., Benedetti, A., Mandl, J. and Bánhegyi, G. (2001) Protein-disulfide isomerase- and protein thiol-dependent dehydroascorbate reduction and ascorbate accumulation in the lumen of the endoplasmic reticulum. J. Biol. Chem. 276, 8825–8828.
[23] Wells, W.W., Xu, D.P., Yang, Y.F. and Rocque, P.A. (1990) Mammalian thioltransferase (glutaredoxin) and protein disulfide isomerase have dehydroascorbate reductase activity. J. Biol. Chem. 265, 15361–15364.
[24] Csala, M., Szarka, A., Margittai, É., Mile, V., Kardon, T., Braun, L., Mandl, J. and Bánhegyi, G. (2001) Role of vitamin E in ascorbate-dependent protein thiol oxidation in rat liver endoplasmic reticulum. Arch. Biochem. Biophys. 388, 55–59.
[25] Wajih, N., Hutson, S.M. and Wallin, R. (2007) Disulfide-dependent protein folding is linked to operation of the vitamin K cycle in the endoplasmic reticulum. A protein disulfide isomerase-VKORC1 redox enzyme complex appears to be responsible for vitamin K1 2,3-epoxide reduction. J. Biol. Chem. 282, 2626–2635.
[26] Tu, B.P. and Weissman, J.S. (2002) The FAD- and O_2-dependent reaction cycle of Ero1-mediated oxidative protein folding in the endoplasmic reticulum. Mol. Cell 10, 983–994.
[27] Bublitz, C. and Lawler, C.A. (1987) The levels of nicotinamide nucleotides in liver microsomes and their possible significance to the function of hexose phosphate dehydrogenase. Biochem. J. 245, 263–267.
[28] Odermatt, A., Atanasov, A.G., Balazs, Z., Schweizer, R.A., Nashev, L.G., Schuster, D. and Langer, T. (2006) Why is 11β-hydroxysteroid dehydrogenase type 1 facing the endoplasmic reticulum lumen? Physiological relevance of the membrane topology of 11β-HSD1. Mol. Cell. Endocrinol. 248, 15–23.
[29] Hewitt, K.N., Walker, E.A. and Stewart, P.M. (2005) Minireview: hexose-6-phosphate dehydrogenase and redox control of 11β-hydroxysteroid dehydrogenase type 1 activity. Endocrinology 146, 2539–2543.
[30] Lavery, G.G., Walker, E.A., Draper, N., Jeyasuria, P., Marcos, J., Shackleton, C.H., Parker, K.L., White, P.C. and Stewart, P.M. (2006) Hexose-6-phosphate dehydrogenase knockout mice lack 11β hydroxysteroid dehydrogenase type 1-mediated glucocorticoid generation. J. Biol. Chem. 281, 6546–6551.
[31] Bánhegyi, G., Benedetti, A., Fulceri, R. and Senesi, S. (2004) Cooperativity between 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase in the lumen of the endoplasmic reticulum. J. Biol. Chem. 279, 27017–27021.
[32] Piccirella, S., Czegle, I., Lizák, B., Margittai, E., Senesi, S., Papp, E., Csala, M., Fulceri, R., Csermely, P., Mandl, J., Benedetti, A. and Bánhegyi, G. (2006) Uncoupled redox systems in the lumen of the endoplasmic reticulum: pyridine nucleotides stay reduced in an oxidative environment. J. Biol. Chem. 281, 4671–4677.
[33] van Schaftingen, E. and Gerin, I. (2002) The glucose-6-phosphatase system. Biochem. J. 362, 513–532.
[34] Csala, M., Bánhegyi, G. and Benedetti, A. (2006) Endoplasmic reticulum: a metabolic compartment. FEBS Lett. 580, 2160–2165.
[35] Lizák, B., Czegle, I., Csala, M., Benedetti, A., Mandl, J. and Bánhegyi, G. (2006) Translocon pores in the endoplasmic reticulum are permeable to small anions. Am. J. Physiol. Cell Physiol. 291, C511–C517.
[36] Bánhegyi, G., Lusini, L., Puskás, F., Rossi, R., Fulceri, R., Braun, L., Mile, V., di Simplicio, P., Mandl, J. and Benedetti, A. (1999) Preferential transport of glutathione versus glutathione disulfide in rat liver microsomal vesicles. J. Biol. Chem. 274, 12213–12216.
[37] Bánhegyi, G., Marcolongo, P., Puskás, F., Fulceri, R., Mandl, J. and Benedetti, A. (1998) Dehydroascorbate and ascorbate transport in rat liver microsomal vesicles. J. Biol. Chem. 273, 2758–2762.
[38] Varsányi, M., Szarka, A., Papp, E., Makai, D., Nardai, G., Fulceri, R., Csermely, P., Mandl, J., Benedetti, A. and Bánhegyi, G. (2004) FAD transport and FAD-dependent protein thiol oxidation in rat liver microsomes. J. Biol. Chem. 279, 3370–3374.
[39] Shen, J., Snapp, E.L., Lippincott-Schwartz, J. and Prywes, R. (2005) Stable binding of ATF6 to BiP in the endoplasmic reticulum stress response. Mol. Cell Biol. 25, 921–932.
[40] Kimata, Y., Oikawa, D., Shimizu, Y., Ishiwata-Kimata, Y. and Kohno, K. (2004) A role for BiP as an adjustor for the endoplasmic reticulum stress-sensing protein Ire1. J. Cell Biol. 167, 445–456.
[41] Nadanaka, S., Okada, T., Yoshida, H. and Mori, K. (2007) Role of disulfide bridges formed in the luminal domain of ATF6 in sensing endoplasmic reticulum stress. Mol. Cell Biol. 27, 1027–1043.
[42] Nadanaka, S., Yoshida, H. and Mori, K. (2006) Reduction of disulfide bridges in the lumenal domain of ATF6 in response to glucose starvation. Cell. Struct. Funct. 31, 127–134.
[43] Zable, A.C., Favero, T.G. and Abramson, J.J. (1997) Glutathione modulates ryanodine receptor from skeletal muscle sarcoplasmic

reticulum. Evidence for redox regulation of the Ca^{2+} release mechanism. J. Biol. Chem. 272, 7069–7077.
[44] Feng, W., Liu, G., Allen, P.D. and Pessah, I.N. (2000) Transmembrane redox sensor of ryanodine receptor complex. J. Biol. Chem. 275, 35902–35907.
[45] Higo, T., Hattori, M., Nakamura, T., Natsume, T., Michikawa, T. and Mikoshiba, K. (2005) Subtype-specific and ER lumenal environment-dependent regulation of inositol 1,4,5-trisphosphate receptor type 1 by ERp44. Cell 120, 85–98.
[46] Li, Y. and Camacho, P. (2004) Ca^{2+}-dependent redox modulation of SERCA 2b by ERp57. J. Cell Biol. 164, 35–46.
[47] Camporeale, G. and Zempleni, J. (2003) Oxidative folding of interleukin-2 is impaired in flavin-deficient jurkat cells, causing intracellular accumulation of interleukin-2 and increased expression of stress response genes. J. Nutr. 133, 668–672.
[48] Manthey, K.C., Chew, Y.C. and Zempleni, J. (2005) Riboflavin deficiency impairs oxidative folding and secretion of apolipoprotein B-100 in HepG2 cells, triggering stress response systems. J. Nutr. 135, 978–982.
[49] Margittai, É., Bánhegyi, G., Kiss, A., Nagy, G., Mandl, J., Schaff, Z. and Csala, M. (2005) Scurvy leads to endoplasmic reticulum stress and apoptosis in the liver of guinea pigs. J. Nutr. 135, 2530–2534.
[50] Belkaid, A., Copland, I.B., Massillon, D. and Annabi, B. (2006) Silencing of the human microsomal glucose-6-phosphate translocase induces glioma cell death: potential new anticancer target for curcumin. FEBS Lett. 580, 3746–3752.
[51] Belkaid, A., Currie, J.C., Desgagnes, J. and Annabi, B. (2006) The chemopreventive properties of chlorogenic acid reveal a potential new role for the microsomal glucose-6-phosphate translocase in brain tumor progression. Cancer Cell Int. 6, 7.
[52] Leuzzi, R., Bánhegyi, G., Kardon, T., Marcolongo, P., Capecchi, P.L., Burger, H.J., Benedetti, A. and Fulceri, R. (2003) Inhibition of microsomal glucose-6-phosphate transport in human neutrophils results in apoptosis: a potential explanation for neutrophil dysfunction in glycogen storage disease type 1b. Blood 101, 2381–2387.
[53] Kuijpers, T.W. (2002) Clinical symptoms and neutropenia: the balance of neutrophil development, functional activity, and cell death. Eur. J. Pediatr. 161 (Suppl. 1), S75–S82.
[54] Özcan, U., Cao, Q., Yilmaz, E., Lee, A.H., Iwakoshi, N.N., Özdelen, E., Tuncman, G., Görgün, C., Glimcher, L.H. and Hotamisligil, G.S. (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 306, 457–461.
[55] Hotamisligil, G.S. (2006) Inflammation and metabolic disorders. Nature 444, 860–867.
[56] Nakatani, Y., Kaneto, H., Kawamori, D., Yoshiuchi, K., Hatazaki, M., Matsuoka, T.A., Ozawa, K., Ogawa, S., Hori, M., Yamasaki, Y. and Matsuhisa, M. (2005) Involvement of endoplasmic reticulum stress in insulin resistance and diabetes. J. Biol. Chem. 280, 847–851.
[57] Ozawa, K., Miyazaki, M., Matsuhisa, M., Takano, K., Nakatani, Y., Hatazaki, M., Tamatani, T., Yamagata, K., Miyagawa, J., Kitao, Y., Hori, O., Yamasaki, Y. and Ogawa, S. (2005) The endoplasmic reticulum chaperone improves insulin resistance in type 2 diabetes. Diabetes 54, 657–663.
[58] Hansen, P.A., Waheed, A. and Corbett, J.A. (2007) Chemically chaperoning the actions of insulin. Trends Endocrinol. Metab. 18, 1–3.
[59] Özcan, U., Yilmaz, E., Özcan, L., Furuhashi, M., Vaillancourt, E., Smith, R.O., Görgün, C.Z. and Hotamisligil, G.S. (2006) Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science 313, 1137–1140.