ANTIOXIDANTS & REDOX SIGNALING
Volume 13, Number 1, 2010

DOI: 10.1089/ars.2009.2529

COMPREHENSIVE INVITED REVIEW

Redox Control of Endoplasmic Reticulum Function

Miklós Csala, Éva Margittai, and Gábor Bánhegyi

Abstract

The lumen of the endoplasmic reticulum constitutes a separate intracellular compartment with a special proteome and metabolome. The redox conditions of the organelle are also characteristically different from those of the other subcellular compartments. The luminal environment has been considered more oxidizing than the cytosol due to the presence of oxidative protein folding. However, recent observations suggest that redox systems in reduced and oxidized states are present simultaneously. The concerted action of membrane transporters and oxidoreductase enzymes maintains the oxidized state of the thiol–disulfide and the reduced state of the pyridine nucleotide redox systems, which are prerequisites for the normal redox reactions localized in the organelle. The powerful thiol-oxidizing machinery of oxidative protein folding continuously challenges the local antioxidant defense. Alterations of the luminal redox conditions, either in oxidizing or reducing direction, affect protein processing, are sensed by the accumulation of misfolded/unfolded proteins, and may induce endoplasmic reticulum stress and unfolded protein response. The activated signaling pathways attempt to restore the balance between protein loading and processing and induce programmed cell death if these attempts fail. Recent findings strongly support the involvement of redox-based endoplasmic reticulum stress in a plethora of human diseases, either as causative agents or as complications. *Antioxid. Redox Signal.* 13, 77–108

Reviewing Editors: *Adam Benham, Masanori Kitamura, Rammohan Rao, Lloyd Ruddock, Wiep Scheper, Martin Schroeder, Roberto Sitia, and Geoffrey Werstuck*

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

I. Introduction

Redox conditions are tightly associated with the redox reactions in the intermediary metabolism, therefore they represent a base of cellular regulation, sensing, and signaling. Each subcellular compartment can be characterized by a typical redox environment. The redox conditions within a compartment are mainly defined by the quantitatively most significant local redox reactions, but they can be influenced also by the transmembrane transport of redox-active compounds and the transmembrane electron fluxes. Redox-active compounds are usually small electron carriers and function as cofactors/substrates of oxidoreductases. The redox state of these couples can be regarded as an indicator of the redox environment of the cell or an organelle. Moreover, major redox pairs can constitute redox buffers that, on one hand soothe sudden changes in redox conditions, on the other hand define the oxidative or reductive character of the compartment.

Similarly to other subcellular organelles, the major redox buffers in the endoplasmic reticulum (ER) lumen are the thiol/disulfide and the reduced/oxidized pyridine nucleotide couples. The most striking feature of the ER lumen is the oxidized state of the thiol/disulfide system, which results in a redox potential of approximately −180 mV, much higher than that of the cytosol (−230 mV) (128). Consequently, the ER lumen has been regarded as an oxidizing environment, in agreement with the fact that the oxidative protein folding—a major pathway of the compartment—requires oxidizing power. Recent observations point out that the findings related to the thiol/disulfide system should not be generalized. Several, although indirect, results indicate that pyridine nucleotides are overwhelmingly present in a reduced form in the ER lumen.

Luminal redox systems can also act as sensors for electron donors and acceptors (*i.e.*, nutrients and oxygen); this way, the ER has a central role in the fine tuning of environmental and internal stimuli. Redox imbalance affecting any redox system finally leads to ER stress and initiates ER-dependent signaling pathways to restore the physiological conditions. Exhaustion of the protective mechanisms results in various ER-dependent forms of programmed cell death.

Despite the remarkable interest in the field and the experimental efforts, the present knowledge on the ER redox conditions is largely deductive. With the exception of the thiol/disulfide redox system, there are no data on the luminal composition and concentration of redox-active compounds. Appropriately targetable fluorescent redox indicators for the real-time redox measurements in the ER lumen have been developed only for thiols and hydrogen peroxide (28, 44, 207, 278). Moreover, the mapping of the luminal proteome has just started, including the local oxidoreductases (167, 213).

The aim of this review is to summarize the main pathways of intermediary metabolism localized in the ER lumen and connected to the redox homeostasis in the organelle. It gives an overview of the major redox systems of the ER, highlighting the connections and interplay between them. The role of the ER in redox sensing is also outlined, together with the underlying mechanisms. Finally, the pathological consequences of ER redox imbalance are discussed, including human diseases such as neurodegenerative and cardiovascular diseases, metabolic diseases, and cancer.

II. The Endoplasmic Reticulum as a Metabolic Compartment

The membrane network, which envelops the nucleus in all eukaryotic cells, also forms naked tubules and ribosome-covered sheets of various sizes referred to as smooth and rough ER, respectively. This inhomogeneous, yet continuous, membrane provides a platform for a variety of enzymatic reactions and completely separates the ER lumen from the cytosol. Although some nonspecific permeability could be observed and mainly attributed to the translocon peptide channel (107, 183, 184, 333), it is now evident that the majority of transmembrane traffic is controlled by selective transporters in the ER membrane (58, 61). These transport activities link cytosolic and luminal metabolic processes. The membrane barrier allows the maintenance of characteristic differences between the compositions of the two compartments. For example, the luminal Ca^{2+} concentration is orders of magnitude higher than the cytosolic one, which is due to a continuous inward ion pumping. It is especially relevant to the subject of this review that the main redox systems are separated and the redox conditions are remarkably different on the two sides of the ER membrane. However, the relatively oxidized state of the luminal thiol–disulfide system is generated by local oxidation rather than active transport activities (see next section).

The rough ER is specialized in protein synthesis and processing. The proteins containing ER targeting signals are translated by ribosomes docked to translocon (Sec61) peptide channels. The translocation of the nascent polypeptides into the membrane or lumen of the ER occurs simultaneously with the elongation, and chemical modifications also begin as soon as the N-terminal amino acid residues appear in the lumen. Cleavage of the signal peptide, formation of intrachain and interchain disulfide bridges, N-glycosylation and γ-carboxylation of glutamyl side chains are the main co- and post-translational modifications of proteins in the ER. Protein maturation in the ER is assisted by chaperones. The main ER chaperones, 78 kDa glucose-regulated protein or immunoglobulin heavy chain-binding protein (GRP78/BiP) and 94 kDa glucose-regulated protein (GRP94), attach to the hydrophobic patches of unfolded or misfolded polypeptides and help them achieve their native conformation (149, 180, 228). Some ER chaperones, such as certain members of the protein disulfide isomerase (PDI) family (74) within the thioredoxin superfamily have thiol–disulfide oxidoreductase activities and catalyze disulfide formation and rearrangement (*i.e.*, oxidative folding); and are therefore often referred to as foldases (7, 31, 74). Calnexin and calreticulin are lectin chaperones with specificity for the glycoproteins containing an asparagine-linked $GlcMan_9GlcNAc_2$ oligosaccharide moiety (25, 209, 275, 331). In addition to preventing the aggregation of the immature proteins, they recruit ERp57, one of the foldase enzymes that catalyze disulfide formation/isomerization (25).

The ER employs careful quality control systems in order to prevent the export of immature, misfolded, or proteins damaged in other ways. The best known quality control system uses the asparagine-linked oligosaccharide group as a label to indicate the actual state of the N-glycoprotein. Therefore, the main roles in this system are played by glucosidase, mannosidase, and glucosyl transferase enzymes, as well as lectins (6, 270). A monoglucosylated protein is considered immature and retained by calnexin and calreticulin.

An attempt is made to complete the folding of the polypeptide and then the glucose unit is removed by glucosidase II. If the protein has achieved its native conformation, it can bind to the cargo receptors (11) and leave the ER on the secretory pathway by vesicular transport. Otherwise, the UDP-glucose:glycoprotein glucosyltransferase reglucosylates the glycoprotein and the maturation cycle is repeated. Once a protein is incapable of folding properly and is sentenced to degradation, the distal mannose unit of branch B is chopped off by ER mannosidase I. ER degradation enhancing α-mannosidase-like proteins (EDEMs), the lectins specific to $Man_8GlcNAc_2$ (isomer B) oligosaccharide flag, directs these aberrant proteins for retrotranslocation into the cytosol through Sec61 or other putative peptide channels composed by derlin-1 and other subunits (323). Recently, the α1,2-specific exomannosidase function of the EDEM protein Htm1p has been reported (49). Htm1p generates the $Man_7GlcNAc_2$ oligosaccharide with a terminal α1,6-linked mannosyl residue on degradation substrates, which is recognized by the ER-localized lectin Yos9p (258).

Since ERAD substrates usually contain disulfide bonds, they must be reduced before their retrotranslocation. The ER-resident protein ERdj5 has been postulated as a luminal reductase, which can accelerate ERAD by forming a complex with EDEMs and BiP (316). As soon as the proteins appear at the cytosolic side of the ER membrane, they get ubiquitinated and are degraded by the 26S proteasome in a process called ER-associated degradation (ERAD) (72, 104, 139, 215, 321). The proteins synthesized in the ER have various destinations. They are targeted to the plasma membrane, stay on the cell surface, or get secreted from the cell unless they contain signals that control their retention in the ER or Golgi or lead to their diversion to the lysosomes. The membrane orientation is retained by vesicular transport; therefore, the luminal proteins and luminal domains of membrane proteins remain luminal or extracellular.

The vesicles budding from the ER are not only vehicles for protein sorting but also sources of lipids for the target membranes. Synthesis of membrane lipids, including cholesterol, indeed takes place in the ER. Moreover, triglycerides to be either secreted in lipoproteins (chylomicrons and VLDL in enterocytes and hepatocytes, respectively) or deposited in fat droplets (typically in adipocytes) are also assembled in this organelle (81, 235, 236, 335).

Besides its prominent role in protein and lipid synthesis, the ER also significantly contributes to carbohydrate metabolism (Fig. 1). The maintenance of blood glucose level in starvation depends on the hydrolysis of glucose-6-phosphate mostly in liver and kidney. The hydrolytic enzyme, glucose 6-phosphatase, is an integral protein of the ER membrane in these cells (318). The substrate, glucose-6-phosphate derived either from glycogen breakdown or gluconeogenesis, is generated in the cytosol, while the active site of glucose-6-phosphatase faces the luminal compartment of the ER. Therefore, the enzyme requires the cooperation of three transporters for the entry of glucose-6-phosphate (G6P) and for the exit of glucose and phosphate. The putative glucose and phosphate transporters of the ER still remain to be identified. The human glucose-6-phosphate transporter (G6PT) protein is encoded by a single copy gene at chromosome 11q23 (86, 320). As it could be expected, G6PT deficiency (glycogen storage disease type 1b; GSD1b) impaires hepatic glucose production similarly to the primary glucose-6-phosphatase deficiency (GSD1a). However, GSD1b patients suffer from additional disorders, such as neutropenia, which suggests that G6PT has additional functions, which are independent of glucose-6-phosphatase activity (47). Indeed, the transporter is expressed in nonglucogenic cells (*e.g.*, granulocytes and adipocytes), where glucose-6-phosphatase is absent (171, 194). It turned out that glucose-6-phosphate is also utilized by hexose-6-phosphate dehydrogenase (H6PD; Fig. 1), another luminal enzyme in the ER, which catalyzes the first two steps of the pentose phosphate pathway (*i.e.*, glucose-6-phosphate dehydrogenation and 6-phosphoglucono δ-lactone hydrolysis), and converts glucose-6-phosphate to 6-phosphogluconate (48, 109, 202, 242, 299, 330). This $NADP^+$-dependent process is considered as the main source of ER luminal NADPH, which drives local glucocorticoid activation (Fig. 1), seems to provide fatty acid desaturation (163) with electrons and probably contributes to antioxidant defense in the compartment as well (see more details in the next section).

The major NADPH-consuming enzyme system in the ER is that of the cytochrome P450 (CYP450) monooxygenases. A wide array of CYP450 enzymes are localized in the ER membrane (51, 131). They consume oxygen and receive electrons from NADPH through the cytochrome P450 reductase flavoprotein on the cytosolic side of the membrane (Fig. 1). CYP450s catalyze the main reactions in the first phase of biotransformation, including the oxygenation of drugs and other xenobiotics, as well as the metabolism of endobiotics, such as heme, cholesterol, and fatty acyl-CoAs. The CYP450-dependent (and cytochrome b_5-dependent) hydroxylation of fatty acyl-CoAs is part of the microsomal desaturation process, which is crucial for the maintenance of optimal membrane fluidity, as well as for the synthesis of arachidonic acid from linoleic acid. Recent findings indicate that the Δ9 fatty acid desaturase system receives electrons from the luminal NADPH pool of the ER through NADPH cytochrome b_5 oxidoreductase (Ncb5or). This unique soluble enzyme (341) contains two domains: one homologous to cytochrome b_5 and the other one homologous to classic microsomal cytochrome b_5 reductase flavoprotein. Pancreatic β-cells and white adipose tissue are progressively lost in Ncb5or knock-out mice, and a defective synthesis of palmitoleic and oleic acids was observed in the islet-transplanted knock-out animals (163, 164).

Steroid oxidoreductases associated to the ER membrane catalyze the conversion of oxo and hydroxyl groups in steroids, and therefore activate or inactivate the hormones. These reactions, therefore, also belong to biotransformation (113, 114, 212). The best characterized steroid oxidoreductases, the 11β-hydroxysteroid dehydrogenases (11βHSD) are responsible for the interconversion of inert glucocorticoids (cortisone, 11-dehydrocorticosterone) and their active forms (cortisol, corticosterone). The two types of the enzyme are localized in two different compartments, use different cofactors, and catalyze the reaction in opposite directions. 11βHSD1 uses NADPH in the ER lumen and activates glucocorticoids by reduction (234, 282, 308). 11βHSD2 uses NAD^+ in the cytosol and inactivates glucocorticoids by oxidation (114, 250). The intracellular level of the active receptor ligands largely depends on the balance between the two activities. Altered expression and activity of 11βHSD1 have been observed in various human pathological conditions, such as hypertension, atherosclerosis, metabolic syndrome and related diseases

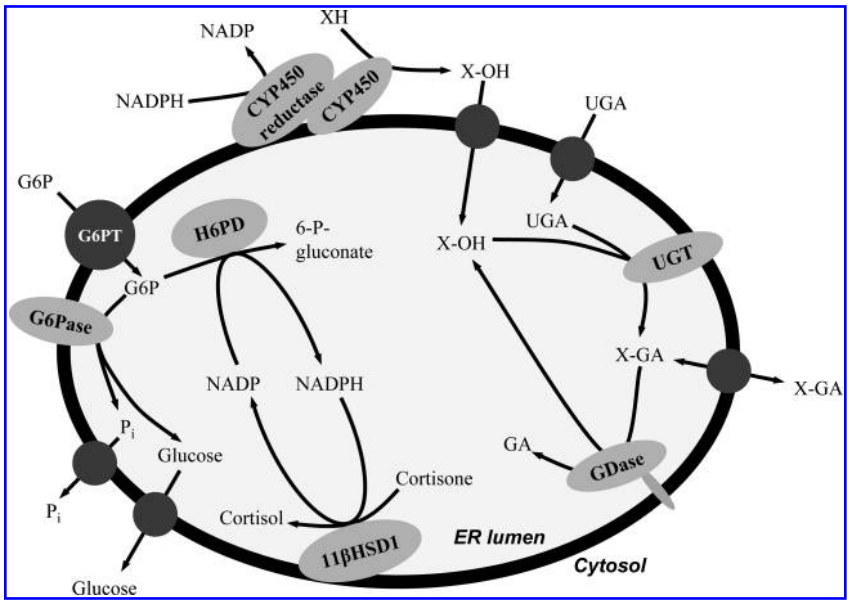

FIG. 1. The ER as a metabolic compartment. The lumen of the ER is separated from the cytosol by a continuous membrane. Some typical luminal reactions are shown, which are supported by membrane transporters for the delivery of substrates and removal of products. The traffic of metabolic intermediates across the ER membrane is mediated by transporter proteins (*darker circles*), most of which have not been identified yet. G6P is transported into the lumen by G6PT and utilized by two enzymes in this compartment. G6Pase removes the phosphate and produces glucose in the glucogenic tissues. Its function is assisted by putative glucose and phosphate transporters. H6PD converts G6P to 6-phosphogluconate and provides the luminal reductase enzymes with NADPH. 11βHSD1 catalyzes the NADPH-dependent reduction of cortisone to active glucocorticoid cortisol at the inner surface of the ER membrane. Endo/xeno-biotics (XH) are monooxygenated by CYP450 enzymes on the outer surface of the membrane. CYP450s receive two electrons from the cytosolic NADPH through the CYP450 reductase flavoenzyme. The product (X-OH) is often conjugated with GA in the ER lumen by UGTs. The import of X-OH and UGA, as well as the export of the glucuronide (X-GA) are catalyzed by unknown transporters. X-GA can also be deconjugated by GDase that hydrolyzes the bond between the aglycone and GA in the lumen.

(obesity, type 2 diabetes), age-related cognitive dysfunction, osteoporosis, arthritis, which indicates the importance of prereceptorial cortisol activation (220, 281, 282, 307, 308). The exact subcellular localization of other steroid oxidoreductases has not been revealed. Nevertheless, the preferred reductase or dehydrogenase activity of these isoenzymes that has been observed [*e.g.*, in case of 17β-hydroxysteroid dehydrogenase family (186)] is presumably due to different orientations in the ER membrane, similarly to 11βHSDs.

Both conjugation and deconjugation reactions of biotransformation occur in the ER (Fig. 1). The active center of membrane-embedded UDP-glucuronosyltransferases faces the inner side of the membrane, so glucurono-conjugates are produced in the lumen (35, 259). Interestingly, the same compartment also contains β-glucuronidase, the enzyme that cleaves glucuronides by hydrolysis (59, 263). Its physiological role and the significance of the colocalization of the two antagonistic enzymes are to be elucidated. The transporters mediating the traffic of UDP-glucuronate and of the glucuronides have not been identified. Similarly to the UDP-glucuronosyltransferases, steroid sulfatase the enzyme deconjugating various steroid and thyroid sulfo-conjugates is an integral ER membrane protein with the active center localized in the lumen (88).

III. Redox Conditions in the Endoplasmic Reticulum

The redox systems of the ER lumen are separated from those of the cytosol by the membrane barrier. Basically, the two compartments contain independent pools of the main electron carriers; and local redox reactions can maintain characteristically different redox potentials, *i.e.* different ratios of the oxidized and reduced forms of redox-competent compounds, on the two sides of the ER membrane. The most relevant redox systems in the ER lumen, similarly to the cytosol, are the thiol-disulfide couple and the reduced and oxidized pyridine nucleotides.

A. The thiol/disulfide system

The proteins synthesized and processed in the ER have remarkably more disulfide bridges and less free cysteinyl thiols than the cytosolic ones. This difference is also mirrored by the different ratios of glutathione (GSH) and glutathione disulfide (GSSG) (*i.e.*, the luminal [GSH]/[GSSG] ratio is nearly 20 times lower compared to the cytosol) (23, 69, 128). Since GSH and GSSG are potential substrates for protein disulfide isomerase, it was hypothesized that imported GSSG oxidizes the active thiols of protein disulfide isomerase and hence provides the oxidizing power for the generation of disulfides in the nascent proteins. This model is strongly opposed by the results of transport measurements, which revealed that GSSG cannot pass the ER membrane, while GSH is slowly transported (20; Fig. 2). Therefore, the disulfide bonds, either glutathione or protein cysteinyl disulfides, must be generated in the lumen by local redox reactions. Indeed, an electron transfer chain conducting electrons from nascent proteins to oxygen has been elucidated (Fig. 2). Endoplasmic reticulum oxidoreductase 1 (Ero1) is a thiol oxidase flavoprotein that oxidizes the active cysteinyl thiols of protein disulfide isomerase and delivers the electrons to oxygen converting it to hydrogen peroxide (42, 80, 254). Since FAD is efficiently transported across the ER membrane, it is possible that FAD contributes to the generation of the luminal thiol oxidizing environment as a mobile electron carrier (310, 319). In addition, other small redox active compounds (*e.g.*, dehydroascorbic acid and vitamin K epoxide) can receive electrons from protein disulfide isomerase and hence provide alternative means of electron transfer to oxygen (see below). Moreover, quiescin sulfhydryl oxidase (QSOX) isoforms have been

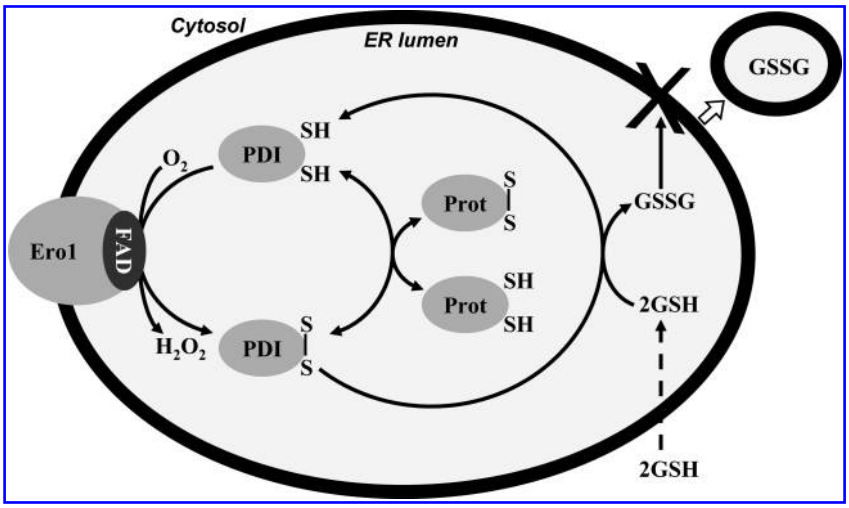

FIG. 2. Oxidative protein folding in the ER. The active thiol groups of PDI can dynamically transfer electrons to/from a variety of reaction partners. The oxidized form of PDI (disulfide; S–S) is capable of creating disulfides, while the reduced form (thiols; -SH) can break and rearrange disulfides in the nascent proteins (Prot). The thiols of PDI are mostly oxidized by an ER oxidase flavoprotein (Ero1) producing hydrogen peroxide. GSH seems to be necessary for the disulfide isomerization by reducing the PDI disulfides to thiols, which at the same time, generates GSSG and maintains the oxidized state of ER luminal glutathione.

found along the secretory pathway. They are capable of directly oxidizing cysteinyl thiol groups of nascent proteins; however, their physiological role in protein folding remains to be investigated (105). GSH in the ER appears to be required for reduction of non-native disulfide bonds and to maintain a pool of reduced PDI for catalysis of disulfide bond isomerization reactions (53, 216). Therefore, the relatively low luminal [GSH]/[GSSG] ratio is the consequence, rather than the cause, of oxidative protein folding; GSSG is generated by protein disulfide isomerase as a by-product of isomerization (74) or *via* the action of hydrogen peroxide (143), produced by Ero1. In other terms, intensive thiol oxidation is necessary for appropriate protein processing but might come at the price of decreased antioxidant capacity of the ER lumen.

B. Pyridine nucleotides

NAD(H) and its phosphorylated derivative, NADP(H) are the major water-soluble electron carriers in the metabolism (253). NAD^+ is loaded with electrons by the majority of dehydrogenase enzymes participating in the central catabolic pathways of carbohydrate and lipid metabolism (*e.g.*, glycolysis, pyruvate dehydrogenase complex, citrate cycle, fatty acid β-oxidation), while a few cytosolic dehydrogenases (*i.e.*, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme, cytosolic isocitrate dehydrogenase) use $NADP^+$ specifically as electron acceptor. NADH principally delivers electrons to the mitochondrial respiratory chain, while NADPH provides reducing power for biosynthesis, biotransformation and antioxidant defense (Fig. 3A). Most of the biosynthetic and biotransforming enzymes embedded in the ER membrane (*e.g.*, CYP450 reductase, 3-hydroxy-3-methyl-glutaryl-CoA reductase, squalene synthase, enoyl-CoA reductase, 3-ketoacyl-CoA reductase, biliverdin reductase) catalyze the reaction on the outer surface of the lipid bilayer, and hence utilize cytosolic NADPH. The lumen of the ER also contains NADPH-consuming reductase enzymes (Fig. 3B), such as Ncb5or (341) and 11βHSD1 (243). Since the ER membrane is not permeable to pyridine nucleotides, these enzymes are using a separate NADP(H) pool (40). Ncb5or transfers electrons to the Δ9 fatty acid desaturase system (163). The importance and consequences of its compartmentation have not been investigated yet due to its recent discovery. In contrast, the functional relationships of 11βHSD1 with other ER proteins have been more extensively studied in the last few years. The significance of this enzyme from the aspect of human health is obvious as it activates glucocorticoid precursors by reduction, and hence its activity is a major determinant of the local glucocorticoid hormone levels (71, 234, 308). The reaction catalyzed by 11βHSD1 is reversible and its actual direction largely depends on the redox state of the pyridine nucleotides. In fact, the physiological direction of the process is the strongest indirect evidence for a high ER luminal [NADPH]/[$NADP^+$] ratio, which has not yet been directly determined. Since NADPH cannot enter the lumen at sufficient rate, the maintenance of the high [NADPH]/[$NADP^+$] ratio (*i.e.*, the NADPH supply for 11βHSD1) relies on luminal NADPH production. Although an isocitrate dehydrogenase activity capable of reducing $NADP^+$ has been found in the ER lumen (196), several observations support that H6PD is the major NADPH generating enzyme in the compartment (Fig. 3B) and its activity is linked to that of 11βHSD1 (109, 165, 330). The substrate supply for H6PD, in turn, depends on the efficient import of glucose-6-phosphate across the ER membrane, which is mediated by G6PT. The presence and cooperation of G6PT, H6PD, and 11βHSD1 have been demonstrated in the ER of hepatocyte (16), adipocyte (194), and neutrophil granulocyte (144) microsomes, and can be assumed to exist in various other cells. The functioning of this triad is ultimately determined by the availability of glucose-6-phosphate, which reflects the nutritional and hormonal conditions in the organism; therefore the G6PT-H6PD-11βHSD1 system is a perfect candidate for a metabolic sensor connecting intermediary metabolism and hormone action (see below).

C. Other redox systems

Besides glutathione and pyridine nucleotides, the ER is rich in other electron transfer compounds such as ascorbate, tocopherol, FAD, FMN, vitamin K, and ubiquinone. Although their presence in the ER is evident since they are required for various reticular reactions, little is known about their concentration, membrane transport and redox connections.

1. Ascorbic acid/dehydroascorbic acid. Ascorbate is synthesized in the hepatocytes of most animals (17). The synthesis starts from UDP-glucose primarily derived from

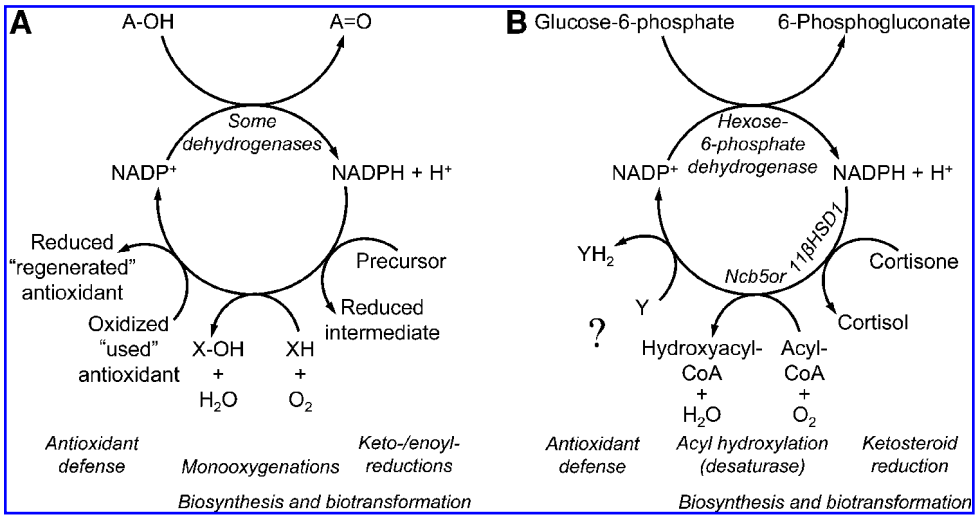

FIG. 3. The redox cycle of NADP(H). (**A**) In general, most dehydrogenases use NAD^+ and hence conduct electrons towards the mitochondrial respiratory chain. The reducing power of NADPH is also maintained by a few enzymes typically creating oxo (=O) groups in the substrates (A-OH). The electrons carried by NADPH are utilized for the reduction of C=O or C=C double bonds, for the generation of water in monooxygenations and for the regeneration of oxidized antioxidant molecules; therefore, they contribute to biosynthesis, biotransformation and antioxidant defense, rather than ATP generation. (**B**) The redox cycle of NADP(H) is similar in the ER too. The main NADPH-producing enzyme is hexose-6-phosphate dehydrogenase, which oxidizes glucose-6-phosphate to 6-phosphogluconate. 11βHSD1 uses NADPH to reduce a keto group in cortisone and form the active hormone cortisol. Recent results indicate that Ncb5or feeds the monooxygenase component of the acyl-CoA desaturase system with electrons from the luminal side of the membrane. The role of luminal NADPH in the antioxidant defense of the compartment has also been suggested, although the reactions involved remain to be elucidated.

glycogenolysis (178). The last step of the synthesis is catalyzed by the ER membrane-bound flavoprotein gulonolactone oxidase (GLO) (229). Interestingly, in humans and in a few animal species GLO is inactive due to a mutation in its active site; hence these species need to ingest vitamin C with the diet (230).

Indirect experimental evidence shows that the active site of GLO is located in the ER lumen; consequently, ascorbate synthesized *de novo* first appears in the lumen of the hepatic ER according with the high local ascorbate concentration (257). Moreover, hydrogen peroxide—the byproduct of the reaction (151)—is also produced in the lumen, contributing to the oxidative character of the compartment (19, 257). A microsomal transport activity specific to dehydroascorbic acid has been described (22); in cells devoid of GLO this transporter can ensure the ascorbate (and dehydroascorbic acid) supply for the ER lumen.

Ascorbate utilizing reactions—either the detoxification of reactive oxygen species or redox reactions using ascorbate as a cofactor—take advantage of the susceptibility of ascorbate to oxidation. The reaction starts with the one-electron oxidation of ascorbate to semidehydroascorbic acid radical, which can be further oxidized to dehydroascorbic acid or rapidly disproportionated to dehydroascorbic acid and ascorbate. Since dehydroascorbic acid is an unstable compound at physiological pH, effective recycling mechanisms are needed to prevent the loss of ascorbate. From an economic point of view, it is more advantageous for the cell to regenerate ascorbate, than to synthesize it *de novo*. The recycling is even more important for the species unable to produce ascorbate (17).

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) (123, 152). Moreover, its oxidized form, dehydroascorbic acid can accept electrons from protein disulfide isomerase (227, 328). The latter reaction suggested that dehydroascorbic acid might be involved in the electron transfer chain of the oxidative protein folding. In intact cells, the oxidative environment in the ER allows disulfide bond formation in newly synthesized proteins. However, disulfide bonds are not formed in ER-derived microsomal vesicles (60), which suggest that the process needs a cytosolic factor or a membrane-permeable compound that is lost during the preparation of microsomes. GSSG was long considered to have a key role in the process, but this assumption was opposed by the observations that the disulfide bond formation in GSH-deficient yeast is intact (53), and the GSSG transport through the ER membrane is negligible. GSSG is produced in the ER lumen parallelly with protein disulfides, as GSH is used to maintain the reducing (isomerizing) capacity of protein disulfide isomerase (see section III, A). The next candidate to fulfill this role was ascorbate, the most abundant water-soluble antioxidant in the ER. To execute its role as a pro-oxidant, ascorbate has to be present in its fully oxidized form, dehydroascorbic acid. While in plants the enzymes producing ascorbyl free radical are well-known (120), in animal tissues the oxidation of ascorbate was considered to be a nonenzymatic process catalyzed by metal ions, free radicals, etc. However, an ascorbate oxidizing activity was detected at the surface of rat liver microsomes (296), by which ascorbate is continuously transformed to ascorbyl free radical and then dehydroascorbic acid (Fig. 4). Ascorbate oxidation is a heat- and protease-sensitive reaction showing that it is indeed a protein-mediated process. It can be inhibited by chelators, especially the copper-specific neocuproine (296), which is remarkable considering the fact that the ascorbate oxidase in plants contains copper.

Ascorbate addition induces protein thiol oxidation in hepatic microsomes, and this can be prevented by the inhibitors of ascorbate oxidase. Dehydroascorbate is efficiently transported across the ER membrane (Fig. 4), while the transport of ascorbate is negligible (22). Accordingly, inhibition of the ascorbate oxidase enzyme hinders inward ascorbate transport

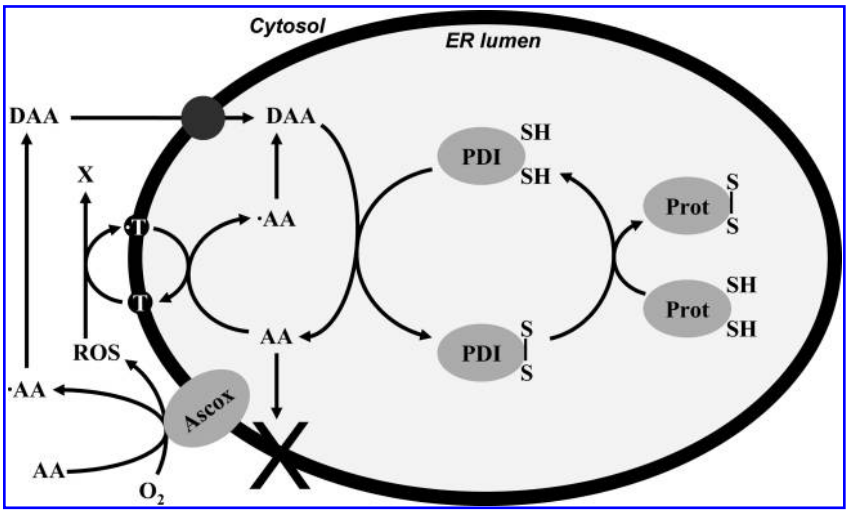

FIG. 4. The role of ascorbate and tocopherol in oxidative protein folding. Ascorbate (AA) is oxidized to ascorbyl radical (·AA) by an unidentified ascorbate oxidase enzyme (Ascox) on the outer surface of the ER membrane. Further oxidation or dismutation yields dehydroascorbate (DAA), which can enter the ER lumen and oxidize the active thiols of PDI and hence contribute to the generation of disulfide bonds in the nascent proteins (Prot). The ROS produced by Ascox drives the luminal oxidation of AA and consequently further disulfide formation. Tocopherol (T) has been suggested as a putative transmembrane electron carrier in this process.

in the microsomes (62). Dehydroascorbic acid, as a small molecular weight electron acceptor can participate in the machinery of oxidative folding of proteins in the ER lumen (60) as a substrate for the protein disulfide isomerase (Fig. 4). As a result of this enzymatic function, ascorbate is generated (and accumulated) in the ER lumen. Recent observations suggest that the dehydroascorbate reductase activity of protein disulfide isomerase is relatively weak; however, dehydroascorbate is able to oxidize thiols rapidly in folding proteins such as bovine pancreatic trypsin inhibitor (273). In certain pathological states, such as diabetes mellitus, unfolded nascent proteins accumulate in the ER lumen. The increased protein thiol availability under such circumstances results in an enhanced accumulation of luminal ascorbate, providing a further evidence for the process (227).

The physiological role of ascorbate in oxidative protein folding is supported by *in vivo* observations. Ascorbate deficiency (scurvy) causes ER stress and apoptosis in the liver of guinea pigs (197). ER stress was attributable to the missing pro-oxidant rather than antioxidant effect of ascorbate. Nevertheless, the indirect effects of scurvy on ER function or defects in collagen hydroxylation contributing to the development of ER stress cannot be ruled out either. On the basis of existing data the relative contribution of the ascorbate/dehydroascorbate redox system to oxidative folding cannot be estimated.

2. Tocopherol. The membrane of the ER contains a high amount of tocopherol (vitamin E), the most important and abundant lipophilic antioxidant (34). Similarly to ascorbate, tocopherol has two transferable electrons, but is also able to donate one electron. Vitamin E (tocopheryl) radical is generated, for example, when a reactive oxygen species (ROS) oxidizes tocopherol. The redox connection between vitamin C and E is well-known (Fig. 4); the tocopheryl radical can be re-reduced to tocopherol by ascorbate, while ascorbyl radical is produced (41, 288). The observation that ascorbate addition to microsomes promoted protein thiol oxidation more effectively than dehydroascorbic acid, indicates that the formation of dehydroascorbic acid from ascorbate contributes to disulfide bond generation also in an indirect way. This also suggests the penetration of ROS and/or the involvement of an ER-membrane located lipid-soluble electron carrier molecule, which connects the two processes: ascorbate oxidation on the outer surface, and protein thiol oxidation in the lumen. According to a theoretical model, reactive oxygen species produced in the intimate neighborhood of the ER membrane during ascorbate oxidation generates tocopheryl radicals that can be reduced again to tocopherol by luminal ascorbate. This permits tocopherol to function as a transmembrane electron carrier, and contribute to the electron transfer between protein thiols and oxygen (63).

3. FAD. FAD plays a central role in the machinery of oxidative protein folding. It acts 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 (otherwise unknown) physiological levels of free FAD (309, 310). Although FAD transport has been demonstrated in yeast (310) and rat liver (319) microsomes, the transport mechanism as well as the concentration and redox state of luminal FAD remain to be elucidated. Since the addition of FAD facilitates luminal thiol oxidation both in yeast and liver microsomes, a physiological role of FAD transport in disulfide formation has been proposed. It should be noticed that riboflavin deficiency has been shown to trigger ER stress in Jurkat (43) or HepG2 (190, 329) cells.

4. Vitamin K. Vitamin K is a lipophilic electron carrier that is known to function in the ER. Post-translational modification of glutamate to γ-carboxyl glutamate is required for the activity of vitamin K-dependent proteins. Carboxylation is accomplished by the vitamin K cycle located in the ER lumen (83). Substrate proteins are γ-carboxylated by the enzyme γ-glutamyl carboxylase which requires the propeptide-containing substrate and three co-substrates: reduced vitamin K, CO_2, and O_2. Since vitamin K is epoxidated in the reaction, ancillary reactions are needed to regenerate the reduced form of vitamin K. Vitamin K epoxide reductase (VKORC1) plays a central role in the recycling. The enzyme contains a thioredoxin-like CXXC center involved in the reduction, but the system providing electrons to the center was unknown. Recent data demonstrates that the reduction is linked to the oxidative folding of proteins in the ER by protein disulfide isomerase. A stable complex of protein disulfide isomerase and VKORC1 has also been suggested (324). It can be

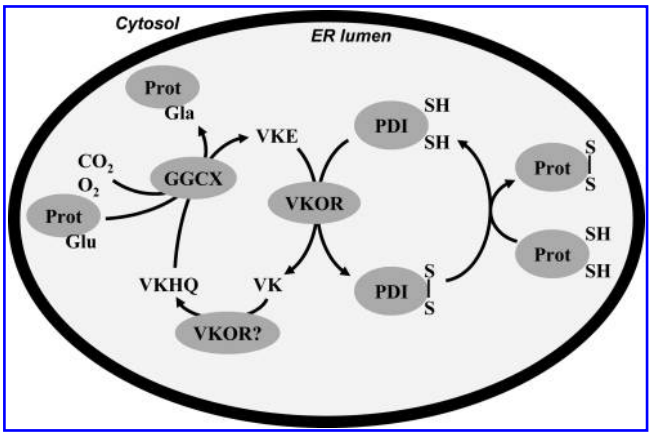

FIG. 5. The vitamin K cycle. Formation of γ-carboxyglutamate (Gla) residues catalyzed by the glutamate γ-carboxylase (GGCX) is one of the post-translational modifications in the hepatic ER. The reaction is coupled to the oxidation and oxygenation of vitamin K hydroquinone (VKHQ) to vitamin K epoxide (VKE). The regeneration of VKHQ for further carboxylations requires electrons from NADPH and/or from PDI in a reaction mediated by the vitamin K oxidoreductase (VKOR). By this means, the vitamin K (VK) cycle provides oxidizing power for the generation of disulfide bonds in the nascent proteins (Prot) (*i.e.*, for oxidative protein folding).

concluded that electrons required for γ-carboxylation of proteins are provided by the cysteinyl thiols of cargo proteins *via* protein disulfide isomerase (Fig. 5), therefore various post-translational modifications of secretory proteins are linked to each other in an economical way.

5. Reactive oxygen species in the endoplasmic reticulum. Although reactive oxygen species are formed in all cellular compartments, the ER seems to be a major place of production. This organelle is particularly rich in oxygenases and oxidases (*e.g.*, cytochrome P450s, flavin-containing monooxygenases, prolyl and lysyl hydroxylases), which often produce ROS as a byproduct. Many of these enzymes are located in the ER lumen; their activities greatly determine the luminal redox environment. Oxidative protein folding, a representative process in the ER, is responsible for about one-fourth of the reactive oxygen species produced in a professional secretory cell (311). Reactive oxygen species (presumably hydrogen peroxide) generated by Ero1 are putative end products of the electron transfer. The reduction of molecular oxygen by recombinant yeast Ero1p yields stoichiometric hydrogen peroxide production under aerobic conditions (93). Since ROS generated during oxidative protein folding can compromise the redox and antioxidant homeostasis of ER lumen, it has been postulated as a causative factor of ER stress in conditions characterized by the overproduction of secretory proteins (189). However, recent studies explored intriguing mechanisms to prevent hyperoxidizing conditions during intensive oxidative folding in the ER lumen. It has been demonstrated in yeast that noncatalytic cysteine pairs of Ero1p act as a substrate sensor modulating the catalytic activity of the enzyme. Disulfide formation between these thiols (Cys^{90}–Cys^{349} and Cys^{150}–Cys^{295}) inhibits, while their reduction restores Ero1p activity (Fig. 6A). Redox changes in the ER correlate with the thiol–disulfide conversion of the regulatory cysteinyl moieties. As a result of this regulatory mechanism, Ero1p activity is attenuated by the hyperoxidation of the ER (287).

Human Ero1α activity can also be regulated by a similar mechanism which prevents hyperoxidizing conditions: Ero1α is regulated by noncatalytic disulfides with a low midpoint reduction potential (approximately −275 mV). This means that they are stable under the redox conditions of the ER lumen and are only partially reduced by protein disulfide isomerase (12). This mechanism can prevent excessive Ero1α activation and uncontrolled oxidation of protein and glutathione thiols. An additional regulatory mechanism based on intramolecular disulfide switches has also been described in case of human Ero1α. Formation of a disulfide bond between the active-site Cys^{94} (connected to Cys^{99} in the active enzyme) and Cys^{131} results in a decreased activity. Competition between substrate thiols (*i.e.*, the thiols of the reduced protein disulfide isomerase) and Cys^{131} creates a regulatory mechanism, thus the activation of Ero1α is dependent on the availability of its substrate, reduced protein disulfide isomerase (8). These data show that protein disulfide isomerase is not only a center of electron flow in the ER, but also a central regulator of ER redox homeostasis (Fig. 6B).

Oxidative protein folding is not the only source of oxidizing equivalents in the ER lumen. Nitrosative effects can target luminal proteins, for example, the S-nitrosylation of protein disulfide isomerase has been observed (312), which indicates that NO is present in the lumen—either produced locally or entering across the ER membrane. Nevertheless, nitrosylation of ER-resident proteins in the Golgi or recycling compartments cannot be ruled out either. It should be noted that besides enzymatic processes, ER-resident hydroxyl radical generation by an iron-dependent Fenton reaction has also been reported (182).

The fact that the redox state of the luminal glutathione redox buffer is more oxidized than in the cytosol clearly shows that the redox buffers in the organelle are strongly challenged by continuous pro-oxidant effects. Furthermore, the enzymatic antioxidant defense of the ER lumen seems more vulnerable than that of the other subcellular compartments [*e.g.*, there are no reports of catalase or superoxide dismutase isoforms being present; for a review, see (15)]. Hydrogen peroxide produced in the lumen has been thought to be eliminated at the expense of glutathione by glutathione peroxidase, phospholipid hydroperoxide glutathione peroxidase (200), and peroxiredoxins (264). From the latter family of enzymes, the ER is equipped with peroxiredoxin IV, belonging to the class of 2-Cys peroxiredoxins (203). A redox-active cysteine in the active site of these proteins is oxidized to a sulfenic acid by the peroxide substrate. The recycling of sulfenic acid back to a thiol occurs at the expense of glutathione.

Besides the antioxidant-dependent elimination of reactive oxygen species, recent observations suggest alternative mechanisms. *In vivo* generation of hydrogen peroxide in the ER lumen (by the stimulated activity of gulonolactone oxidase) was accompanied by transient liver swelling and reversible dilatation of endoplasmic reticulum cisternae. Moreover, it resulted in an increased permeability of the microsomal membrane to various compounds of low molecular weight (198). Thus, luminal hydrogen peroxide formation in the ER provokes a temporary increase in nonselective mem-

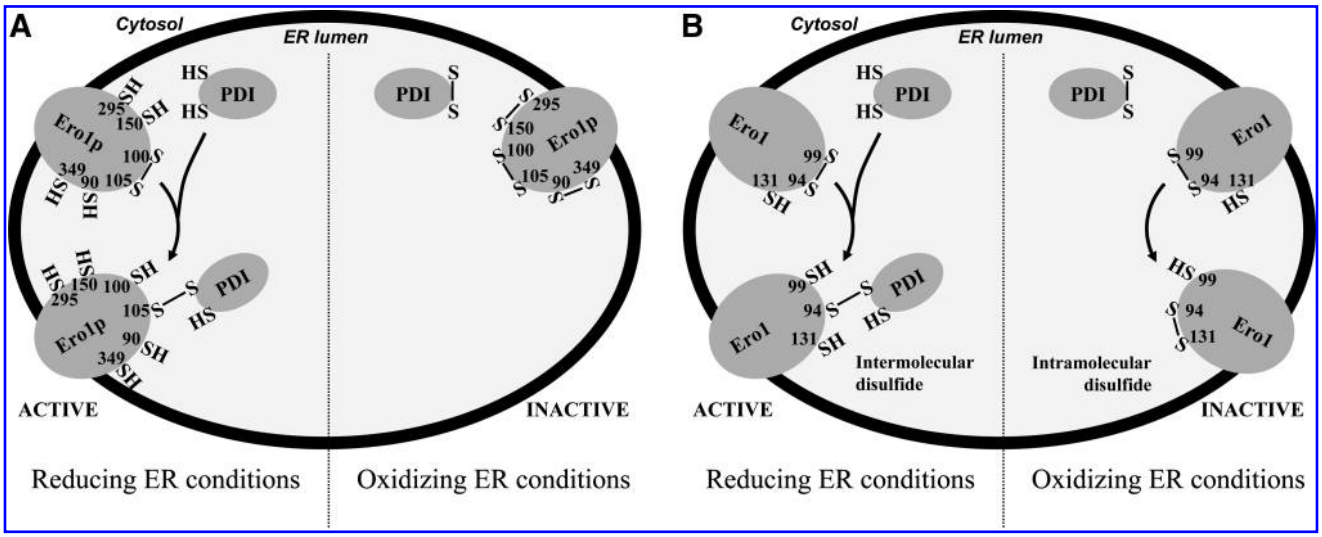

FIG. 6. Regulation of Ero1α activity by intraluminal redox conditions. Redox conditions in the ER lumen affect Ero1α activity, to prevent hyperoxidation. (**A**) In yeast, disulfide bond formation between the regulatory cysteines of Ero1p (Cys^{90}–Cys^{349}; Cys^{150}–Cys^{295}) may restrict the functional dynamics of the loop containing the shuttle cysteines (Cys^{100}–Cys^{105}) and decrease Ero1p activity (*right side*). Upon reduction of the regulatory disulfides Ero1p is reactivated (*left side*). (**B**) In humans, an additional mechanism is also operative. When protein disulfide isomerase is present in reduced form (*left side*), it acts as a substrate for Ero1α. Competition between the thiols of protein disulfide isomerase and the Cys^{131} of Ero1α prevents the formation of the intramolecular disulfide bond within Ero1α. In this case, the thiol–disulfide exchange reaction between Ero1α and protein disulfide isomerase is unhampered, the electron flow from substrate proteins to oxygen is continouos. In the absence of the competition [*i.e.* when protein disulfide isomerase is present in oxidized form (*right side*)], the reaction between the outer active-site disulfide of Ero1α and Cys^{131} will be favored. In this condition Ero1α is inactive due to the covalent blockade of the active site.

brane permeability, which results in the dilation of the organelle and in enhanced transmembrane fluxes of small molecules. It is uncertain whether the phenomenon can be attributed to hydrogen peroxide itself or other ROS, such as hydroxyl radical. Nevertheless, the increased permeability of the ER membrane would allow the escape of oxidizing agents from the lumen and the influx of reducing equivalents (*e.g.* GSH) from the cytosol (Fig. 7).

Another recent study based on *in vitro* findings suggests that hydrogen peroxide is not necessarily a potentially dangerous by-product of disulfide bond formation that results in oxidative stress to cells. Hydrogen peroxide addition to GSH or reduced protein disulfide isomerase resulted in their oxidation to the disulfide state. Hydrogen peroxide added directly or generated enzymatically *in situ* in the neighborhood of a reduced protein resulted in the rapid and efficient refolding of the model protein bovine pancreatic trypsin inhibitor. Furthermore, no other oxidative modifications of the refolding protein were observed (143). Thus, hydrogen peroxide produced by Ero1 and other sulfhydryl oxidases during disulfide bond formation in the ER may be used efficiently and economically to make further disulfide bonds (Fig. 7). However, it should also be taken into account that Fenton decay of H_2O_2 in the presence of metal ions may compete with thiol oxidation and may generate hydroxyl radicals.

D. Connections and interplay between the redox systems of the ER

The major redox pairs participating in the electron transfer processes of the cell are also present in the ER. The ER membrane is rich in lipophilic electron carriers, while the lu-

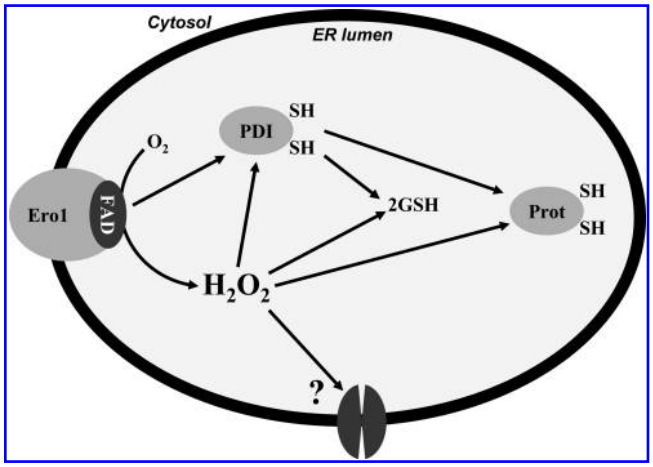

FIG. 7. The effects of hydrogen peroxide produced in the ER. The main electron transfer chain of oxidative protein folding drives the electrons from the thiols of nascent proteins (Prot) or GSH to the active thiols of PDI, then to the FAD cofactor of ER oxidase 1 (Ero1), and finally to molecular oxygen. The partial reduction of oxygen yields hydrogen peroxide, which can oxidize further thiol groups. Accumulation of H_2O_2 is an oxidative stress, which leads to the overproduction of disulfides, disables the disulfide isomerization, and depletes luminal GSH. Significant increase in the nonspecific permeability of the ER membrane in this condition has recently also been observed.

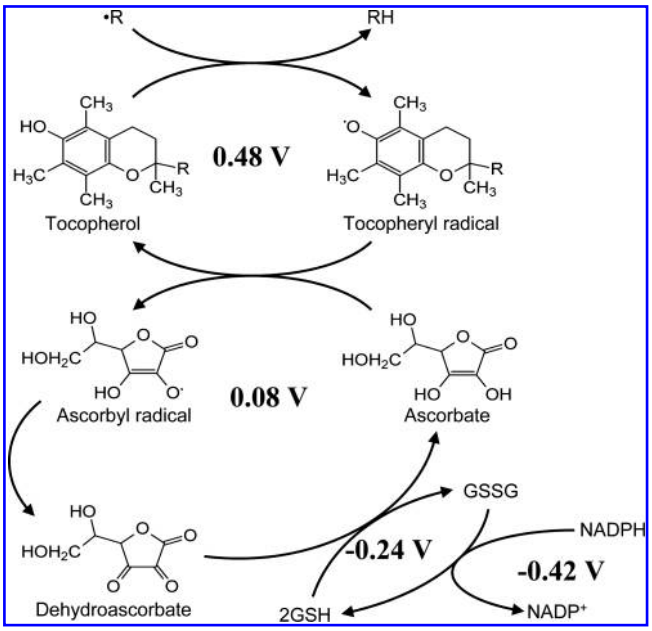

FIG. 8. The Foyer–Halliwell–Asada pathway. An electron transfer chain composed of the main antioxidant compounds can protect the biological membranes from oxidative damages caused by free radicals. The components reduce each other according to their standard redox potentials. The chain is linked to the intermediary metabolism by NADPH, which reduces GSSG to GSH in an enzymatic reaction. Standard redox potentials (ε°) are indicated.

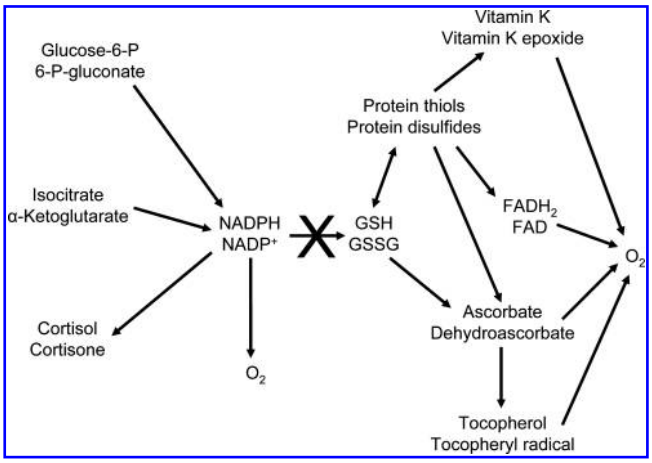

FIG. 9. Redox connections in the ER. Connections between the redox competent couples are shown. The *arrows* point to the oxidizing agent in each relationship. Redox-active compounds of the lumen form a bicentral network around protein disulfide isomerase and pyridine nucleotides, due to the uncoupling between the two systems.

men contains most of the water-soluble electron transfer compounds, although in concentrations and redox states differing from those of the cytosol.

Co-localized redox pairs are often linked to one another by oxidoreductases to form complex redox systems. The direction of the electron flux is determined by the redox potentials of the participating redox pairs. The best known example for such a system is the Halliwell–Asada cycle (Fig. 8). This cycle is operative in the cytosol, ensuring the reduced state of all the compounds in the system. However, if the linking enzymes are missing and the nonenzymatic electron transfer is not feasible, the redox pairs can co-exist independently and have different redox potentials. That is the case in the ER lumen, where in spite of the oxidizing power of the GSSH/GSH system, pyridine nucleotides remain reduced. The different redox potential of the two redox systems is ensured by their uncoupling, since thioredoxin reductase (185) and glutathione reductase (251) are hardly detectable in the lumen. Thus, redox-active compounds in the ER lumen constitute a bicenter network (Fig. 9). One system responsible for biosynthesis, biotransformation, and antioxidant defense is organized around pyridine nucleotides. The second system is principally involved in the post-translational modification of secretory proteins and comprises GSH/GSSG, ascorbate/dehydroascorbic acid, and vitamin K. The central actor of this latter system is protein disulfide isomerase, which participates in oxidative folding, vitamin K cycle, and dehydroascorbic acid reduction. Electrons deriving from the reaction of disulfide bond formation can be used by this mechanism for the regeneration of active vitamin K and ascorbate, for protein γ-carboxylation and hydroxylations, respectively.

The two redox systems of the ER are separated, but not isolated. Although the exact mechanisms have not been elucidated, NADPH contributes somehow to the antioxidant defense of the lumen, which suggests the presence of a NADPH-dependent dehydroascorbic acid reductase. Moreover, NADPH can partially contribute to the regeneration of vitamin K hydroquinone by a luminal diaphorase (325).

Besides the local connections, the redox state of the ER lumen is greatly affected by the influx and efflux of oxidizing and reducing agents. Reducing equivalents are mainly imported in the form of cysteinyl thiols, which enter the lumen with the translocation of nascent polypeptides or with the influx of GSH. Reducing equivalents for the pyridine nucleotide system are also imported in the form of glucose-6-phosphate (85) and probably as fatty acyl-carnitine (61, 91). Although the generation and maintenance of the oxidative luminal environment can be attributed mostly to local oxidations, the transport of electron acceptors can also contribute to it. Dehydroascorbic acid and FAD transport reported in the ER membrane (22, 310, 319) may be relevant from this aspect. On the other hand, pyridine nucleotides, either reduced or oxidized, cannot pass the ER membrane at significant rate (56, 251, 299). The presence of lipophilic electron carriers and transmembrane electron transfer proteins (*e.g.*, a homologue of cytochrome b561 in the ER membrane) can also influence the redox properties of the lumen (214).

There are two important, but presently unresolved questions concerning the redox systems of the ER. First, one may suppose that they are subcompartimentalized within the ER (*i.e.*, the thiol/disulfide system would colocalize with protein synthesis and folding in the rough ER, while pyridine nucleotides would be overrepresented in the smooth ER). Second, the relative contribution of each redox system to the overall luminal redox can be different in various cell types. However, experimental evidence demonstrating redox subcompartimentation and the systematic investigation of ER redox-active compounds in different tissues is still lacking.

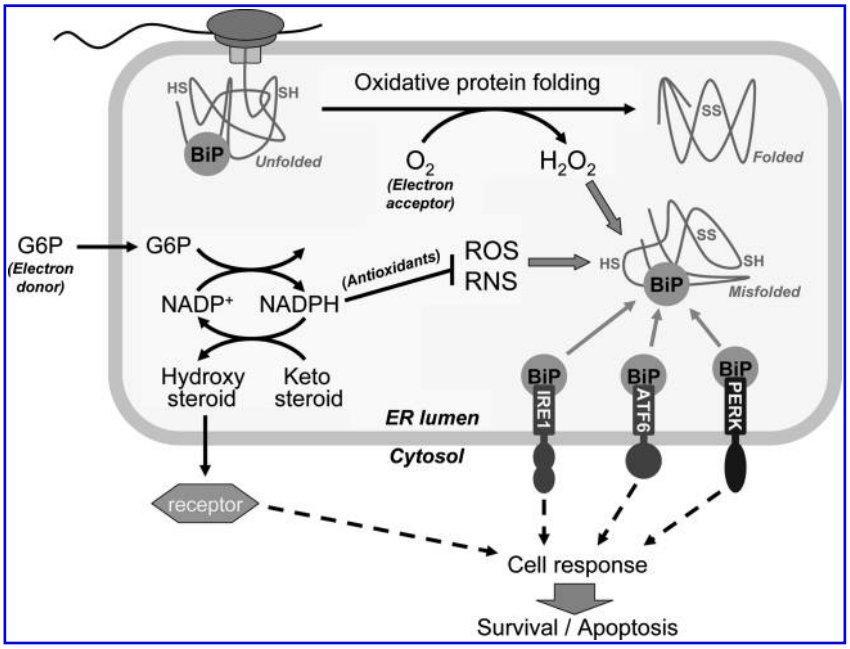

FIG. 10. Luminal redox conditions and the unfolded protein response. Electron donors enter the ER lumen as the cysteinyl thiol groups of native proteins and as intermediates of nutrient metabolism (*e.g.*, G6P). The electrons are accepted either by molecular oxygen in the oxidative protein folding or by carriers (*e.g.*, $NADP^+$). The antioxidant defense of the compartment is likely based on local NADPH production and counterbalances the generation of reactive species (ROS and RNS) by means of the oxidative protein folding or by other redox reactions. NADPH-dependent reductase activities also participate in the prereceptorial steroid hormone activation. Reductive ER stress can be caused by the shortage of electron acceptor (hypoxia) or by the excess of electron donors (overfeeding). Oxidative ER stress is usually part of a cellular oxidative stress caused by a variety of pathological conditions. Both redox imbalances can affect the *cell* functions *via* altered steroid metabolism and/or the accumulation of unfolded/misfolded proteins, which triggers the UPR. The signaling events can ultimately protect or even kill the cell.

IV. Redox-Based Sensing

Physiological external (*e.g.*, nutrients, xenobiotics) or internal (*e.g.*, production of secretory proteins) stimuli can profoundly affect the redox systems of the ER. The alterations of the redox conditions in the luminal compartment of the ER serve as important signals for the cell and require mechanisms for restoring them. Thus, redox imbalance initiates signaling pathways and cellular responses *via* molecular events that are referred to as redox sensing. Since redox alterations adversely influence the folding of secretory proteins, the luminal chaperone BiP detecting unfolded proteins can be regarded as the major indirect redox sensor in the ER. Recently the direct redox sensor function of the ER stress receptor membrane proteins has been revealed and signaling pathways with the contribution of pyridine nucleotides have emerged. These direct and indirect redox sensing mechanisms associated to the ER are discussed in this section.

A. The unfolded protein response—thiol/disulfide redox sensors

Since one of the most important functions of the ER is the synthesis and post-translational modification of secretory and membrane proteins, the lumen of the organelle is equipped with a powerful protein-folding machine composed of chaperones, foldases, and also with sensors that detect the presence of misfolded or unfolded proteins. Physiological and pathological effects or experimental agents that disturb the normal folding process provoke the unfolded protein response (UPR), an intracellular signaling pathway that coordinates ER protein-folding demand with protein-folding capacity and is essential to adapt to homeostatic alterations (collectively named as ER stress) that cause protein misfolding. These include changes in intraluminal calcium ion concentration, altered glycosylation, nutrient deprivation, pathogen infection, expression of folding-defective proteins, and changes in the redox status. Excellent recent reviews summarize the principal events of ER stress and the UPR (189, 269, 276, 277, 294, 297), therefore, the present review emphasizes the redox-based mechanisms only.

The main proximal sensors of the UPR are the PKR-like ER protein kinase/pancreatic eukaryotic translation initiation factor 2, α subunit (eIF2α) 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 (Fig. 10); according to the present model of UPR, the association of their luminal domain with one of the most abundant ER resident chaperones BiP keeps them in inactive state. Perturbed ER homeostasis leads to the accumulation of unfolded/misfolded proteins in the ER lumen. Their preferential binding with BiP sequesters the chaperone in the lumen. The consequent dissociation of BiP from the transmembrane sensors permits their signaling, which is principally based on dimerization–phosphorylation (in case of IRE1 and PERK) or translocation–proteolysis (ATF6). The luminal domain of IRE1 and PERK regulates the protein kinase activity of these proteins by ER stress-regulated di- and oligomerization. The luminal domain of the transmembrane basic leucine zipper (bZIP) transcription factor ATF6 functions as a retention motif under normal conditions, but in ER stress, the protein is translocated to the Golgi complex, where it undergoes limited proteolysis. The cytosolic bZIP transcription factor domain is released from the membrane to allow its translocation to the nucleus.

These three mechanistically distinct arms of the UPR induce expression of chaperones, attenuate protein translation, promote the proliferation of ER membrane to enlarge the luminal compartment and activate ERAD by regulating the expression of numerous genes related to protein folding but also affect the metabolism of proteins, amino acids, and lipids

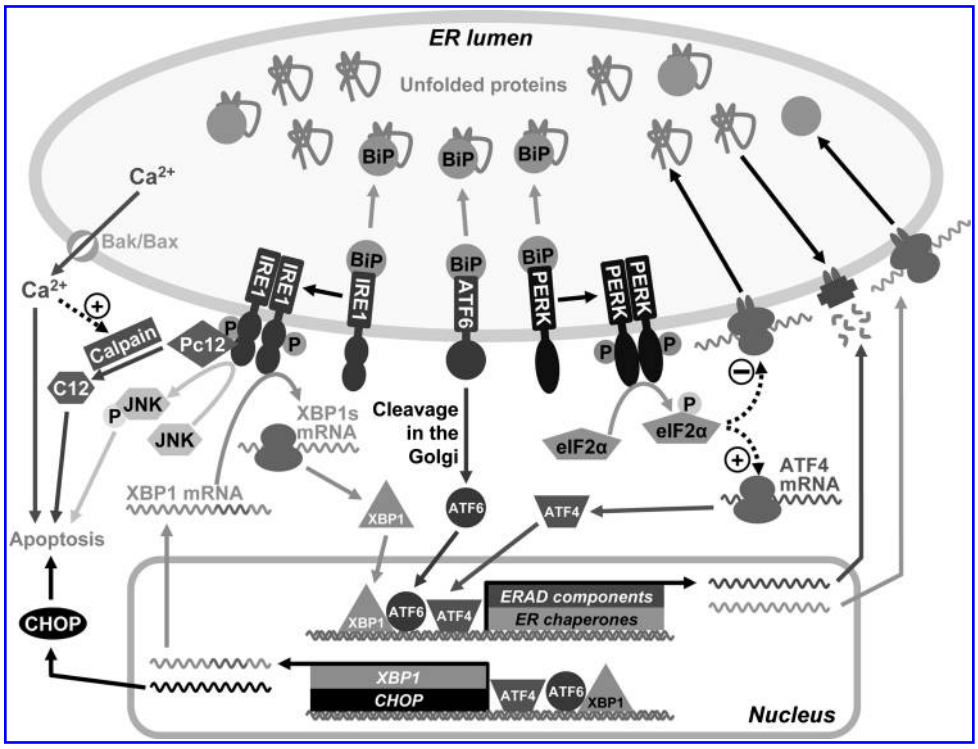

FIG. 11. The unfolded protein response. Upon accumulation of unfolded/misfolded proteins in the ER lumen, BiP chaperone dissociates from the luminal domain of the three ER transmembrane stress receptors, PERK, ATF6, and IRE1, allowing their activation. ATF6 transits to the Golgi where it is cleaved and its cytosolic fragment migrates to the nucleus. Both IRE1 and PERK are oligomerized and autophosphorylated. Phosphorylation of eIF2α by PERK blocks protein synthesis and allows translation of ATF4 mRNA, which induces the transcription of genes required to restore ER homeostasis. Active ATF6 regulates the expression of ER chaperones and XBP1. XBP1 mRNA spliced by IRE1 codes for XBP1s, which controls the transcription of chaperones, ERAD components, and also phospholipid biosynthetic enzymes. This concerted action of the UPR pathways restores the folding capacity of the ER by blocking further build-up of client proteins, enhancing its folding capacity and eliminating terminally misfolded proteins. Unresolved ER stress activates apoptotic mechanisms. Bcl-2 family member proteins are involved in the modulation of UPR by participating in IRE1 signaling. Under the conditions of prolonged stress a shift towards proapoptotic effects occurs: Bak and Bax, ER membrane resident proteins, undergo conformational alteration to permit Ca^{2+} efflux. The elevated cytoplasmic Ca^{2+} activates calpain, which cleaves and activates procaspase-12. The Ca^{2+} efflux and Bak and Bax release also favors activation of mitochondria-dependent apoptosis. CHOP, one of the UPR downstream effectors, inhibits the expression of Bcl-2 to promote apoptosis. IRE1 activation also leads to the activation of JNK, a mediator of inflammation and apoptosis.

(Fig. 11). In addition, PERK activates NF-E2-related factor 2 (Nrf2) transcription factor (54), a key player in the oxidative stress response, which interacts with activating transcription factor 4 (ATF4) (102) and contributes to redox homeostasis and cell survival following endoplasmic reticulum stress (55). ER stress has also been shown to activate the transcription factor nuclear factor-κB (NFκB) by calcium- and ROS-dependent mechanisms (244). The cytoplasmic domain of IRE1 interacts with the adaptor protein tumor necrosis factor receptor-associated factor 2 (TRAF2). The IRE1–TRAF2 complex can recruit IκB kinase (IKK), which phosphorylates IκB, leading to the degradation of IκB and the nuclear translocation of NFκB (127). PERK-mediated attenuation of translation also favors NFκB activation due to the relatively short half-life of inhibitor of NFκB (IκB) (67). Since NFκB has antioxidant functions, its activation might influence the redox conditions in the ER but this potential feedback remains to be investigated. Besides the UPR, ER stress can also induce macroautophagy, a process whereby the cell recycles and remodels its macromolecules and organelles. Autophagy counterbalances ER stress-induced ER expansion, enhances cell survival or commits the cell to nonapoptotic death (126). The UPR provides a tool to remodel the secretory apparatus and aligns cellular physiology to the demands imposed by ER stress. If the efforts of the UPR are insufficient, prolonged ER stress can trigger mitochondria-dependent and -independent forms of apoptosis. Caspase-12 (or caspase-4 in humans) and C/EBP homologous protein (CHOP), also known as growth arrest- and DNA damage-inducible gene 153 (GADD153) are the two major ER-specific mediators of apoptosis (Fig. 11).

Besides the ubiquitous sensors and signaling pathways of UPR, several—sometimes cell specific—other mechanisms have been described. For instance, two additional ER stress sensors have originally been reported in the nervous system. Old astrocyte specifically induced substance (OASIS) and BBF2 human homolog on chromosome 7 (BBF2H7) are ER-resident transmembrane proteins in astrocytes (156) and neurons (157), respectively. Both proteins are bZIP transcription factors activated during ER stress. Upon proteolytic activation, their cleaved cytoplasmic portions translocate into the nucleus and activate the transcription of target genes. Hypo/hyperoxidation of ER proteins due to luminal or cellular redox imbalance affects both the secretory (cargo) proteins and the elements of the UPR signaling pathway. Consequently, redox alterations can be sensed indirectly (*i.e.*, by recognizing the erroneously oxidized, unfolded/misfolded cargo proteins, or directly, through the redox changes of UPR receptors).

1. Indirect redox sensing by BiP. Redox imbalance caused by either experimental agents or pathophysiological conditions leads to the accumulation of unfolded/misfolded

proteins in the ER lumen. As described above, according to the current theory of the ER stress response, the various misfolded proteins are recognized and diverse signaling pathways are initiated by a single sensor, BiP. Transmembrane stress transducer proteins are associated with BiP under stress-free conditions and are released upon accumulation of unfolded proteins. BiP overexpression attenuates the activation of all the three main signaling branches of UPR. In conclusion, BiP functions as a sensor of unfolded proteins, which mechanistically links luminal redox imbalance with UPR activation (Fig. 10). This attractive hypothesis, 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—the mechanism can be much more complicated *in vivo* than previously thought. More or less specific activation of PERK has been found in an animal model of hepatic steatosis (218, 240), while the activation of the IRE1 arm is present in myeloma cells (45). The selective activation might be due to the different cell types or disease models. However, divergent effects of PERK and IRE1 signaling could be produced by a chemical–genetic strategy on cell viability: PERK signaling proved to be pro-apoptotic, while IRE1 signaling promoted the survival of the cell (175). Moreover, 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 luminal domain of ATF6 was identified as a specific ER stress-responsive sequence required for ER stress-triggered BiP release (291). Furthermore, deletion of the BiP-binding site of IRE1 failed to alter the inducibility of ER stress, showing that BiP binding is not the sole or principal determinant of IRE1 activity, but an adjustor for sensitivity to various stresses (150). On the basis of these findings it can be supposed that additional regulators are involved in the initiation of the UPR, or that other tissue-specific adaptor proteins can moderate the signaling events and biological responses once the UPR is initiated by various physiological stimuli.

2. Direct redox sensing. Alterations in the luminal redox state can also be detected by the ER stress receptors directly (*i.e.*, independently of the accumulation of misfolded/unfolded secretory proteins). For example, association/dissociation of BiP is not the sole regulatory mechanism in case of ATF6. It has been recently shown, that owing to the presence of intra- and intermolecular disulfide bridges, ATF6 mono-, di-, and oligomers are formed in the unstressed ER. Various experimental ER stress inducers cause the reduction of these disulfide bonds, which increases the amount of active reduced ATF6 monomers. ER stress evoked by a more physiological mechanism, such as glucose starvation, also activates ATF6. Besides the transcriptional induction of ATF6 synthesis, reduction of disulfide bridges and transport of reduced monomers to Golgi also occurs 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 certain disulfide bridges (222, 223). 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.

3. ERp44. ERp44 has been recognized as an ER resident member of the PDI protein family (74) containing a thioredoxin domain with a CRFS motif (5). ERp44 can bind to both resident and secretory proteins either by forming mixed disulfides or by noncovalent protein–protein interactions within the ER lumen. The binding has important regulatory implications in both cases. ERp44 interacts with the third luminal loop of inositol 1,4,5-trisphosphate receptor type 1 (InsP3R1) and directly inhibits the receptor. The interaction is influenced by the redox state, as well as by luminal pH and Ca^{2+} concentration (Fig. 12). The presence of reduced cysteinyl thiols in the third loop is required for the interaction. Thus, the ERp44/InsP3R1 system may act as a molecular sensor monitoring the redox milieu in the ER lumen and transmitting signals from the lumen of the ER to the cytosolic space for tuning calcium ion homeostasis through InsP3R1 activity (110, 210, 211). ERp44 also interacts with human Ero1 isoforms. Ero1α, an ER oxidoreductin that lacks known ER retention motifs, is retained in the lumen through the formation of reversible mixed disulfides with ERp44 (4). By this mediation, redox mechanisms may be involved in the control of oxidative protein folding. Moreover, these studies underline the interdependence of oxidative protein folding, calcium signaling, ER stress, and apoptosis. It should be noted that Ero1α can also be bound to protein disulfide isomerase (238) or to ER membrane components that could prevent its transport to the Golgi.

ERp44 is also able to form mixed disulfides with cargo proteins, for example, with the unassembled IgM subunits (4, 5, 75). Recently, an important interaction between ERp44 and the adipocyte hormone adiponectin has been revealed. Adiponectin is an insulin-sensitizing adipokine with anti-diabetic, anti-atherogenic, anti-inflammatory, and cardioprotective properties (94). Maturation and release of adiponectin from adipocytes are subject to thiol-mediated retention. ERp44 plays an important role in this process as well. It forms a mixed disulfide bond with adiponectin oligomers, which allows extensive

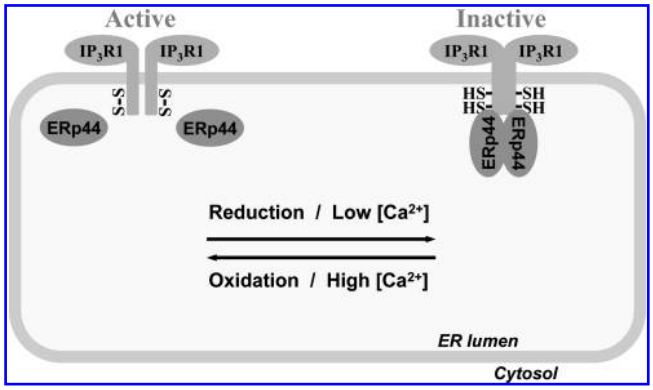

FIG. 12. Redox control of InsP3R1. ERp44, an ER luminal protein belonging to the thioredoxin family, directly interacts with the luminal region of InsP3R1; this interaction is dependent on the pH, the Ca^{2+} concentration, and the redox state. The binding of ERp44 inhibits the Ca^{2+}-release activity of InsP3R1 in vivo. Cysteine residues in the luminal region are required for the inhibition of InsP3R1 by ERp44, as revealed by mutagenesis experiments. Thus, the ERp44/InsP3R1 system may act as a molecular sensor monitoring the (redox) environment in the ER lumen and generating signals in the cytosol.

post-translational modifications preceding the formation of the matured high-molecular-mass oligomeric complex consisting of at least 18 protomers. Ero1α—a privileged partner of ERp44 with the ability to replace the ERp44-retained cargo protein (4)—can release adiponectin from the binding (326, 327). Since both ERp44 and Ero1α are differentially regulated in adipocytes under different metabolic conditions, they can represent major sites of regulation for the release of adiponectin from adipocytes (332). ERp44 as a chaperone participates in the retention of other luminal proteins too, such as the formylglycine-generating enzyme (79, 199). Thus, ERp44-mediated retention of cargo proteins in the ER represents a conditional, redox-dependent mechanism, which is only indirectly dependent on the KDEL receptor-mediated, constitutive retrieval.

4. Redox sensing by Ca^{2+} signaling. Ca^{2+} release from the luminal store is an important event of ER stress and ER-dependent programmed cell death. Luminal redox imbalance is a major cause of the remarkable alteration of cellular calcium homeostasis in ER stress. Luminal redox changes greatly affect both ER calcium ion channels and calcium ion pumps. Besides the ERp44-mediated regulation of InsP3R described above, dynamic redox-sensitive thiols in the ryanodine receptor calcium ion channel are subject to reversible redox conversions, which, in turn, modulate the open probability of the channel (338). Interestingly, the type 1 ryanodine receptor calcium ion channel activity closely follows the transmembrane redox potential difference. As the redox potential difference across the membrane increases, the open probability of the isolated channels increases, irrespectively of which side's redox potential has been modified (76). Therefore, the Ca^{2+} flux through the ryanodine receptor calcium ion channel is controlled by the thiol/disulfide redox state on the two sides of the SR membrane. Hence the channel has been postulated as a transmembrane redox sensor in the sarcoplasmic reticulum (76).

Calcium ion reuptake into the ER is also regulated by the redox state and calcium ion concentrations. The luminal 57 kDa ER protein (ERp57) was shown to regulate sarco/endoplasmic reticulum calcium ATPase type 2b (SERCA2b) activity (172). ERp57 overexpression reduces the frequency of SERCA2b-dependent Ca^{2+} oscillations; an effect 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 SERCA2b in a Ca^{2+}-dependent manner, providing a dynamic control of ER Ca^{2+} homeostasis.

These interactions between luminal redox and Ca^{2+} signaling 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 (110). In conclusion, these studies underline the mutual dependence of luminal redox state, oxidative protein folding, and calcium signaling. InsP3R-induced calcium ion release may be an important link between luminal redox imbalance and apoptosis.

B. Nutrient sensing—redox sensing by pyridine nucleotides

Alterations in the redox state of luminal pyridine nucleotides result in ER stress in various experimental models. However, in contrast to the relatively well-known signaling mechanisms of the thiol/disulfide redox system, practically nothing is known about the sensing and signaling of the redox state of luminal pyridine nucleotides. The uncoupling of the thiol/disulfide and pyridine nucleotide redox systems in the lumen (251) suggests that an—at least partially—separate sensing and signaling system must exist. ER chaperones are possible candidates for sensing the redox state of luminal pyridine nucleotides. The binding of adenine nucleotides by ER chaperones is well known (70, 161); it can be supposed that the structurally similar pyridine nucleotides are also potential ligands, with presumably different affinities towards the reduced and oxidized forms. The functioning of ER chaperones is indeed affected by redox conditions (245, 246). Furthermore, the luminal [NADPH]/[$NADP^+$] ratio defines the direction and rate of the prereceptorial metabolism of several steroids, as it has been demonstrated in case of glucocorticoids (165, 234). High ratio results in prereceptorial activation of glucocorticoids exerting autocrine and paracrine effects *via* the activation of the glucocorticoid receptor.

Hexose-6-phosphate dehydrogenase attracts growing interest due to its major role in the maintenance of the high [NADPH]/[$NADP^+$] ratio in the lumen. The action of the enzyme is regarded as a prerequisite for the prereceptorial activation of glucocorticoids, which has been implicated in the pathomechanism of metabolic syndrome and related diseases (obesity, type 2 diabetes, polycystic ovary syndrome, apparent cortisone reductase deficiency). Genetic observations, results gained in hexose-6-phosphate dehydrogenase knock-out mice and studies on differentiating adipocytes demonstrated the importance of the enzyme in metabolic regulation. A nutrient sensing function of the enzyme, linking metabolism to endocrinology in the ER, has been recently proposed (18). The activity of the enzyme depends on the luminal glucose-6-phosphate concentration, which is presumably similar to the cytosolic one, due to the facilitated diffusion of glucose-6-phosphate through the ER membrane mediated by G6PT (Fig. 13). Intracellular glucose-6-phosphate concentration is a function of blood glucose and insulin levels in insulin-sensitive nongluconeogenic tissues such as adipose tissue and skeletal muscle (68, 283). Moreover, elevation in the level of fatty acids can be sensed by the same system. According to Randle's hypothesis (261), increased free fatty acid (FFA) concentrations lead to elevated mitochondrial acetyl-CoA/CoA and [NADH]/[NAD^+] ratios which, in turn, inhibit pyruvate dehydrogenase activity and lead to an increase in citrate levels that inhibits glycolysis *via* decreased phosphofructokinase activity (Fig. 13). Consequently, the process induces an increase in glucose-6-phosphate concentration (260, 261). Thus, overfeeding either with carbohydrates or lipids results in elevated glucose-6-phosphate levels in insulin-dependent tissues such as adipose tissue and skeletal muscle. In agreement with this assumption, it has been reported recently that luminal NADPH concentration in the ER is highly sensitive to extracellular glucose levels in HEK-293 cells expressing 11βHSD1. Lowering glucose in the culture medium dose-dependently decreased cortisol production by 11βHSD1 and diminished the cortisol/cortisone ratio. Coexpression with H6PH potentiated the reductase activity of 11βHSD1 at high glucose concentration (73). In conclusion, overfeeding (excess of reducing power) *via* the elevation of cytosolic glucose-6-phosphate level and by the mediation of G6PT

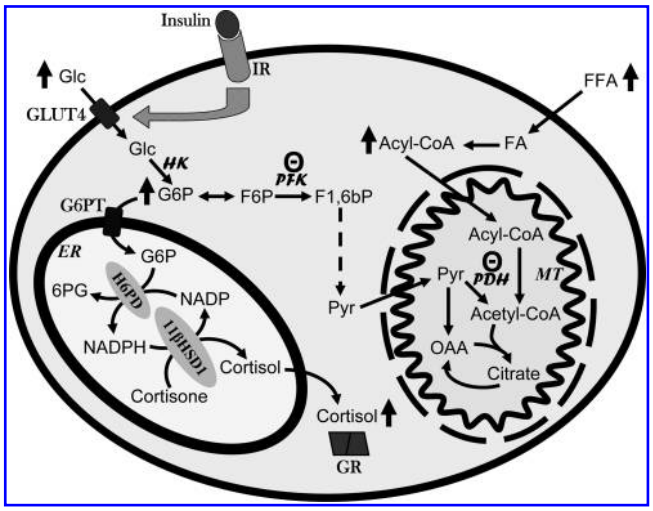

FIG. 13. The G6PT–H6PD–11βHSD1 triad in nutrient sensing of insulin-sensitive cells. Insulin stimulates the translocation of GLUT4 into the plasma membrane and hence enhances glucose uptake in muscle and adipose tissue, which results in elevated intracellular G6P level. Accumulation of G6P, in turn, fuels the G6PT–H6PD–11βHSD1 system, which leads to increased prereceptorial activation of glucocorticoids. On the other hand, high level of FFA—according to Randle's "glucose-fatty acid cycle"—inhibits glucose catabolism at several key steps, most importantly at the level of the pyruvate dehydrogenase (PDH) reaction. As a consequence, G6P accumulates within the cell. Thus, oversupply of reducing equivalents—in the form of either carbohydrates or lipids—leads to the enhancement of glucocorticoid activation.

activates H6PD. The generated and maintained high [NADPH]/[$NADP^+$] ratio in the ER lumen supports glucocorticoid activation by 11βHSD1. High local glucocorticoid levels, on one hand counter-regulate insulin action leading to insulin resistance; on the other hand promote nutrient storage, producing the most characteristic metabolic features of the metabolic syndrome (Fig. 13).

C. Endoplasmic reticulum-dependent programmed cell death

If the ER-to-nucleus signaling pathways, including the UPR, fail to resolve ER stress, various forms of the programmed cell death can be activated. The most common outcome of the prolonged ER stress is the activation of apoptosis (Fig. 11). Apoptosis can be signaled through both mitochondrial-dependent and -independent pathways. Redox imbalance of the thiol/disulfide system in the ER lumen activates the BiP-mediated redox sensing and might generate apoptotic signals. Apoptosis can be induced *via* either the PERK/ATF6-dependent transcriptional induction of the proapoptotic transcription factor CHOP or the IRE1-mediated activation of apoptosis signal-regulating kinase 1 (ASK1)/c-Jun amino terminal kinase (JNK). Recently an alternative mechanism has been proposed: the ribonuclease activity of IRE1, besides processing XBP1 mRNA also causes endonucleolytic cleavage of several other ER-localized mRNAs, including those encoding pro-survival factors such as chaperones. These events ultimately promote apoptosis (98). Calcium ion efflux generated by luminal redox imbalance can result in the cleavage and activation of procaspase-12 (or procaspase-4 in humans) with the subsequent activation of the apoptotic caspase cascade.

The activation of ATF6 and PERK leads to the induction of C/EBP homologous protein (CHOP), a negative regulator of transcription with a bZIP-family domain. CHOP mediates the most significant ER stress-induced apoptotic pathway (187, 193, 239, 268). The precise mechanism of the CHOP-mediated apoptosis is unknown. *CHOP*$^{-/-}$cells are protected from ER stress-induced apoptosis indicating the significance of this pathway in ER stress-related apoptosis. From a redox point of view it is important that CHOP activates the transcription of *ERO1*, potentially leading to a hyperoxidizing condition within the ER lumen (193). However, the regulatory mechanisms within Ero1 (see earlier) can at least partially counterbalance the hyperoxidizing effect of the induction.

The IRE1 branch of the UPR can activate the MAP kinase cascade. The complex of IRE1 and TRAF2 interacts with the mitogen-activated protein kinase kinase kinase, ASK1, which by a kinase cascade subsequently phosphorylates and activates JNK (232, 315). Therefore, ER stress-induced JNK activation and apoptosis are reduced in *Ire1*$^{-/-}$ and *Ask1*$^{-/-}$ cells (232, 315). IRE1–TRAF2 complex also activates the transcriptional repressor ATF3 leading to apoptosis in ER stress induced by homocysteine (339). The involvement of PERK (and other eIF2α kinases) has been also reported in the activation of ATF3 with the consecutive induction of CHOP (136). The activation of procaspase-12 can also occur through a TRAF2-dependent mechanism in ER stress (336).

The caspase cascade has been reported to be involved in ER stress-induced cell death (162, 217). Procaspase-12 is an initiator caspase associated with the ER membrane (336) and activated by ER stress-dependent limited proteolysis possibly by calpain (301). Caspase-12 activates the effector caspases (caspase-9, caspase-3) (219) leading to cell death. *Caspase-12*$^{-/-}$ mice are partially resistant to ER stress-induced apoptosis but sensitive to other death stimuli, suggesting that caspase-12 is a regulator specific to ER stress-induced apoptosis (225). However, active caspase-12 is absent in human cells due to several inactivating mutations in its gene (78). Caspase-4 is a potential candidate to fulfill the role of caspase-12 in ER stress-induced apoptosis of human cells (112, 148).

Proapoptotic members of the Bcl-2 family have been shown to be involved in ER stress-induced apoptosis, which provides a mechanistic link between the UPR and the apoptotic pathway (for a review, see Ref. 103). Activation of the IRE1 arm of the UPR is augmented by Bax and Bak as it has been demonstrated in Bax and Bak double knockout mice (108). Consistently, Bax inhibitor-1-deficient cells displayed hyperactivation of IRE1α, leading to upregulation of UPR target genes (179). These findings show that Bcl-2 family proteins are physiological modulators of the ER stress. However, during prolonged ER stress, the UPR initiates apoptotic signaling pathways *via* differential modulation of Bcl-2 family proteins. Bax and Bak regulate activation of the IRE1/TRAF2/ASK1 arm of the UPR, leading to JNK activation. JNK shifts the balance towards proapoptotic members of the Bcl-2 family (BH3-only proteins Bim and Bad) and negatively regulates Bcl-2. Bak and Bax, ER membrane resident proteins, undergo conformational alteration to permit Ca^{2+} efflux. The elevated cytoplasmic Ca^{2+} activates calpain, which cleaves

procaspase-12. This caspase-12 activation is independent from the mitochondrial apoptotic pathway (342, 271). However, Ca^{2+} efflux and Bak/Bax release also favors permeabilization of the mitochondrial outer membrane, and activation of caspases involved in mitochondrium-dependent apoptosis. CHOP, one of the UPR downstream effectors, inhibits the expression of Bcl-2 to promote apoptosis (205).

ER-stress dependent proapoptotic signaling can be interrupted and come to a halt. For instance, ER stress markers were detected but the effector caspases were not activated in a model of acetaminophen hepatotoxicity *in vivo* (224). The phenomenon is probably due to serious redox alterations in the cytosol. The extent of oxidative stress and the consequent depletion of reduced glutathione seem to determine whether apoptotic or necrotic cell death is favored in drug-induced intracellular stress (142).

Besides apoptosis, other forms of programmed cell death should be taken into consideration in redox-dependent ER stress. Inhibition of the ER G6PT results in cell death in glioma (26, 27) or in neutrophil granulocytes (171); it can be supposed that the altered redox state of luminal pyridine nucleotides underlies the phenomenon. Emerging data now indicate that ER stress is also a potent inducer of macroautophagy, a process for recycling macromolecules and organelles in eukaryotic cells (126). Since starvation is the classic inducer of macroautophagy and a nutrient sensor function has been proposed for the luminal pyridine nucleotides (18), it seems plausible that an oxidative shift in the pyridine nucleotide redox system would promote autophagy. In fact, the vesiculation of the sarcoplasmic reticulum has also been observed in the skeletal muscle of H6PD-deficient mice (166), where the depletion of luminal NADPH can be reasonably supposed. It has been recently observed that 7-ketocholesterol causes intense cytoplasmic vacuolization, the processing of the autophagy marker, microtubule-associated protein 1 light chain 3, LC3-I to LC3-II, but little caspase activation in smooth muscle cells, which further supports the proposed model of the ER redox-dependent autophagic mechanism (201). 7-Ketocholesterol is rapidly converted to 7β-hydroxycholesterol by the ER luminal enzyme 11βHSD1, consuming the luminal NADPH pool (279). ER calcium ion stores and their mobilization were also identified as essential components of the autophagic response to nutrient deprivation (38, 126). Pyridine nucleotides were also identified as regulators of ER calcium ion channels, for example, the ryanodine receptor calcium ion channel (13, 76, 206, 338).

V. Pathophysiology of the Endoplasmic Reticulum Redox Homeostasis

ER stress has been implicated in a wide range of diseases, including neurodegeneration, stroke, bipolar disorder, heart diseases, ischemia-reperfusion, atherosclerosis, cancer, diabetes, metabolic syndrome, muscle degeneration, autoimmune diseases, and others (21, 90, 124, 134, 147, 192, 274, 306, 340). ER stress can develop on the ground of a massive oxidative stress and a chronic ER stress can cause or aggravate oxidative stress—a vicious cycle that presumably contributes to cell damage. Although redox alterations are almost obligatory components of ER stress, in this section we discuss only those human diseases where the redox imbalance has a major contribution to the pathomechanism.

A. Hypoxia, ischemia–reperfusion

Molecular oxygen, as a final electron acceptor, is required for the functioning of electron transfer chains in the ER including the one that generates disulfide bridges in the nascent luminal proteins. Insufficient supply of oxygen diminishes the capacity of oxidative folding, and thus disturbs the balance between protein synthesis and protein processing. In addition, ATP depletion in hypoxia may contribute to ER stress, because many chaperones, including BiP (99) and GRP94 (174), are ATPases, and ATP hydrolysis is required for protein folding. Since the imbalance can lead to the accumulation of unfolded proteins in the lumen, with the consequent activation of the UPR (158), the ER itself can be regarded as a direct sensor of hypoxia. Besides the direct effects, the functions of the ER are also greatly affected indirectly by the complex adaptive and corrective cellular responses, which are mediated by the hypoxia-inducible factor 1 (HIF-1) (57). HIF-1 regulates the transcription of genes involved in angiogenesis, vascular reactivity and remodeling, erythropoiesis, iron homeostasis, glucose and energy metabolism, cell proliferation, and survival (284). Inactivation and destabilization of HIF-1α subunit is oxygen-dependent, so the active dimer is formed in hypoxia and induces the expression of several secretory and plasma membrane proteins (285) further increasing the burden on the ER. Important ER proteins such as BiP, 94 kDa glucose-regulated protein (GRP94) (280), 150 kDa oxygen-regulated protein (ORP150) (160), protein disulfide isomerase (302), and Ero1Lα (87) are products of hypoxia-sensitive genes. In summary, ER stress and the UPR can develop in hypoxia due both to impaired oxidative folding and to stimulated protein synthesis.

Stroke and myocardial ischemia are the most important human diseases where hypoxia or ischemia–reperfusion-dependent ER stress plays a major role in the pathomechanism. Upon arterial occlusion or hypotension, reduced blood flow results in tissue hypoxia and hypoglycemia, which cause protein misfolding and ER stress. During reperfusion, oxidative stress is triggered in the affected tissues, with the production of reactive oxygen and nitrogen species such as nitric oxide (NO), a protein nitrosylating agent, and superoxide that alters cellular redox-dependent reactions, interferes with protein disulfide bonding and results in protein misfolding. The integrated stress response in *acute brain ischemia* involves the ER stress and the UPR as indicated by the induction of BiP (237, 265), the activation of the PERK-eIF2α pathway (3, 159, 233), the induction of CHOP (247), and depletion of ER calcium ion stores (106). Proapoptotic signaling is activated; besides CHOP induction and activation, procaspase-12 (221) and ATF4 (100) activation were also observed during cerebral ischemia in rats. The role of ER stress induced pro-apoptotic signaling in ischemia-induced neuronal cell death and in the damage caused by ischemia/stroke was also demonstrated in *CHOP*$^{-/-}$ mice. These animals were more resistant and showed reduced tissue loss after hypoxia-reoxygenation, implying a causal role for this mediator of ER stress in neuronal cell death *in vivo* (298). NO induces CHOP expression in cultured neurons. Accordingly, NOS inhibitors show protective effects in brain ischemia (155), and NOS-2-deficient mice display decreased sensitivity towards brain ischemia (129). Permanent upregulation of CHOP was shown to be crucial for the induction of astrocyte death in a cell

culture model of stroke (29), further suggesting a causal role for CHOP in brain damage. Autophagy was also observed (1) and was proposed to be stimulated by oxidative and ER stresses in cerebral hypoxia (2). The hypoxia-induced ER chaperones, ORP150, GRP94 and BiP proved to be protective against ischemia-induced cell death in brain (14, 237, 300).

Reactive oxygen and nitrogen species are also involved in the PERK branch of ER stress signaling in the brain. The PERK-dependent ER stress response is markedly less pronounced in superoxide dismutase type 1 (SOD1) overexpressing animals, indicating the (likely indirect) role of superoxide anion in PERK activation (100, 101). PERK activation and eIF2α phosphorylation were blocked in endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) knockout mice (65).

Different kinds and experimental models of *ischemic heart disease* have been demonstrated to be associated with ER stress (89, 90). Ischemia-reperfusion injury in myocardial infarction activates the UPR, including the increased expression of X-box binding protein 1 (XBP1), GRP78, and protein disulfide isomerase (304). The induction of the hypoxia-sensitive chaperones such as GRP78 underlies the phenomenon of preconditioning in the heart (*i.e.*, an exposure to a transient episode of brief ischemia provides subsequent protection in a prolonged ischemic period) (292). Recent studies have also demonstrated evidence for the involvement of autophagy in the response to ischemia and reperfusion in the heart (96).

B. Neurodegenerative diseases

Neurodegenerative diseases, despite the heterogeneity of the symptoms, share some common features that can suggest the participation of ER stress, UPR, or ER-dependent cell death in the pathomechanism. These commonalities are the deposition of aggregated proteins, deficiency of the ubiquitin-proteasome system, impairment of mitochondrial functions, and increased oxidative damage (30, 97, 176). The accumulation of misfolded proteins should stimulate the ubiquitin–proteasome system but its activity is depressed by oxidative effects or by protein aggregates. This leads to further accumulation of misfolded proteins and the aggravation of the disease. The development of ER stress in these conditions (177) is not surprising and has been suggested to be involved in the pathomechanism of a variety of human neurodegenerative diseases including Parkinson's (117), Alzheimer's (119), prion (77), polyglutamine (290) diseases, and multiple sclerosis (208). The UPR can be regarded as a principally protective mechanism under physiological conditions, but in these diseases it can contribute to the aggravation of symptoms. The redox imbalance is not an obligatory constituent of the pathomechanism; it can vary in the different neurodegenerative diseases. ER stress in neurodegenerative diseases has been the topic of numerous excellent recent reviews (21, 177, 204, 248, 262, 312, 337). Thus, we only focus on those diseases where redox alterations are crucial or at least important elements of the pathogenesis.

Parkinson's disease is a chronic progressive neurodegenerative disorder characterized by a profound and selective loss of nigrostriatal dopaminergic neurons. Clinical manifestations of this complex disease include motor impairments involving resting tremor, bradykinesia, postural instability, gait difficulty, and rigidity. The disease is associated with oxidative stress in the neurons as indicated by glutathione depletion (249) as well as massive oxidative and nitrosative protein modifications (64). ER luminal proteins protein disulfide isomerase and parkin are also intensively S-nitrosylated, showing that reactive oxygen and/or nitrogen species are likely present in the ER lumen (226, 313). The redox pathomechanism of the disease is further supported by the observation that missense mutations in α-synuclein gene, coding a putative modulator of oxidative damage (153) occur in rare cases of familial Parkinson's disease (255). Moreover, the accumulation of aggregated wild-type α-synuclein *in vivo* blocks vesicular transport between the ER and the Golgi, causing ER stress (50).

Induction of oxidative stress by certain redox-active neurotoxins can mimic the disease in cell cultures and *in vivo*. These model compounds, such as 6-hydroxydopamine and N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or its active derivative, N-methyl-4-phenylpyridinium, have been shown to induce ER stress in cultured neuronal cells, including dopaminergic neurons: upregulation of ER chaperones and CHOP, a negative regulator of transcription as well as autophosphorylation of IRE1 and PERK were detected (115, 272). In line with these observations, 6-hydroxydopamine-generated ROS induces the UPR, which seems to initiate cell death *via* the secondary involvement of the mitochondrial apoptotic pathway (116). However, it is arguable whether chemical parkinsonism induced by various toxins can be regarded as an appropriate model for the idiopathic Parkinson's disease.

PERK and eIF2α phosphorylation has been detected in neuromelanin containing dopaminergic neurons in the substantia nigra of Parkinson's disease patients (117). Therefore, *in vivo* findings support the experimental observations that ER stress and the UPR are present in human Parkinson's disease, while the role of redox imbalance in the development of ER dysfunction and the contribution of the UPR to the neurodegeneration are strongly supported.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive degeneration specifically affecting both upper and lower motor neurons. Since the exact etiology of ALS is unknown, several theories have been proposed regarding the pathomechanism: mitochondrial dysfunction, aggregation of misfolded protein, glutamate toxicity, increased oxidative stress, disturbed intracellular trafficking. A hypothesis nominates the disturbed glutamate metabolism as a primary event of the mechanisms involved in motor neuron degeneration (39). The prolonged stimulation of excitatory amino acid receptors results in increased intracellular calcium ion concentration with the secondary damages of the mitochondria and the ER (322). Moreover, the consequent elevation of ROS production can also contribute to the cell injury by oxidizing crucial proteins and other cell components.

Although SOD1 was identified as an ALS-linked gene as early as in 1993 (66), the presence of ER stress and the UPR was first demonstrated in the transgenic mouse carrying the mutant human SOD1 (mSOD1) as a model of familial ALS. The mutant SOD1 can aggregate and associate with BiP in the ER of spinal cord motor neurons (146), although the presence of wild-type SOD has not been reported in this organelle. ALS-linked mSOD1 sometimes shows no enzymatic activity (37, 66), while in other cases the activity is almost normal (36).

These observations suggest that the luminal accumulation of the mutant protein rather than its enzymatic activity is responsible for the ER stress. The cleavage of procaspase-12 was also observed, which could be attributed to the activation of the calcium dependent protease calpain in the spinal cord of these animals (334). The motor neurons of the spinal cord also showed a pronounced elevation of BiP before the onset of motor symptoms, suggesting the role of ER stress in the pathogenesis (305). Similarly, the increased expression of protein disulfide isomerase and its association with mutant SOD1 aggregates were demonstrated in the motor neurons of transgenic mice and rats (9). The ER resident protein Derlin-1 has been shown to interact with mutant SOD1, which leads to activation of ASK1 (231). Since only 2% of total human ALS can be accounted for by SOD1 mutations, it is remarkable that the induction and/or activation of a wide array of UPR proteins have recently been detected also in patients with the sporadic form of the disease (10). The mutation of a newly identified ALS-causative gene, vesicle-associated membrane protein-associated protein B (VAPB), results in motoneuronal vulnerability to ER stress (295). VABP is thought to play a pivotal role in the UPR, a physiological reaction to ER stress (295) but the effect of VAPB on redox signaling has not been explored yet. The elevated reactive species, the increased NOS activity and the enhanced nitration and oxidation of proteins in the motor neurons of the transgenic mouse model of ALS (181) suggest the possible role of the altered antioxidant protection of the ER and the consequent redox changes in the mechanism of ER stress. In summary, an interplay between oxidative and ER stress has been evidenced in the pathomechanism of ALS (130, 140).

Alzheimer's disease (AD), one of the most common causes of adult dementia, is a protein misfolding disease caused by the accumulation of abnormally folded amyloid β (Aβ) protein in the brain. Aβ is a proteolytic product of the transmembrane protein amyloid precursor protein (APP). Aβ monomers are soluble; however, at sufficiently high concentration, they undergo a dramatic conformational change to form aggregated amyloid fibrils. Deposition of aggregated fibrillar Aβ is toxic to the neurons and greatly enhances the production of ROS (137). Increased levels of BiP and activation of PERK were observed in the neurons of AD patients, which suggests the development of UPR early in neurodegeneration (119, 314). Whether the ER stress response delays (121) or accelerates (118) neuronal cell death in Alzheimer's disease, as well as the contribution of redox mechanisms remains to be clarified.

Subcellular Ca^{2+} dynamics are also thought to be involved in the pathomechanism of AD (32). Presenilins, a family of multi-pass transmembrane proteins that function as a part of the gamma-secretase protease complex, are mutated in the rare hereditary form of AD. Mutations of APP and presenilins alter Ca^{2+} fluxes in the ER. Aβ treatment of cortical neurons results in the release of Ca^{2+} from the ER by ryanodine receptor and InsP3R, induces ER stress and finally leads to increased cytosolic Ca^{2+} and ROS levels (77). Cells expressing mutant presenilin-1 have perturbed cellular calcium regulation and oxidative stress, which sensitize them to apoptosis induced by trophic factor withdrawal, by Aβ (95) or by ER/Golgi stressors (303).

Although several elements of the pathomechanism have been characterized, the connection between the redox alterations and ER stress in AD remain to be elucidated. Recent observations suggest a possible link: prooxidant effects in AD patients result in the NO-dependent S-nitrosylation of PDI and hence inhibit PDI activity. Therefore, the protective effect of PDI against neuronal cell death triggered by ER stress, misfolded proteins or proteasome inhibition is reduced in AD (312).

C. Metabolic diseases

Metabolic conditions profoundly affect the ER. Since both carbohydrate and lipid metabolisms have ER-resident reactions, nutrient (*i.e.*, reducing equivalent) supply influences the redox environment of the lumen. These circumstances make the ER an excellent nutrient sensor; recent experimental findings explicitly support this assumption. Altered functions of the ER were mainly shown in pancreatic β-cells, adipocytes and hepatocytes in pathological metabolic conditions. The normal functioning of the ER is based on a balanced nutrient supply. When the provided amount of reducing equivalents is too high or too low, the ER initiates intracellular signaling cascades or autocrine/paracrine effects that normally regulate cellular metabolism; however, these cascades have also been implicated in the development or in the aggravation of metabolic diseases, such as the metabolic syndrome. Glucotoxicity, lipotoxicity, and glucolipotoxicity have been known as pathogenic factors that play a role in type 2 diabetes and related metabolic diseases. ER stress is a main component of the pathomechanism in both pancreatic β-cells and peripheral tissues such as the liver, adipose tissue, and skeletal muscle. Persistently high glucose (125) and fatty acid (52, 145, 252) levels reportedly induce ER stress in β-cells, thereby playing a role in the development of type 2 diabetes. Sustained or excessive ER stress reduces insulin expression (141) and increases apoptosis (168), ultimately decreasing β-cell mass. ER stress, through JNK activation, suppresses insulin biosynthesis at the transcriptional level (141). UPR markers have been detected in β-cells of type 2 diabetic patients (168). According with its involvement in ER stress, CHOP-mediated apoptosis seems to be a fundamental contributing factor to β-cell failure in the disease (274). Although only modest signs of ER stress can be detected in β-cells derived from type 2 diabetic patients, these cells are more sensitive to ER stress inducers in vitro (191).

Since destructive ER stress can be triggered by repetitive or sustained hyperglycemia and/or hyper-fatty-acidemia, the reduction of β-cell mass in type 2 diabetes may be due to gluco- and lipotoxicity (256). Hyperglycemia results in accelerated oxidative phosphorylation that is accompanied by enhanced production of reactive oxygen species (266). Pancreatic β-cells are particularly sensitive to oxidative injuries due to low expression of antioxidant enzymes (*e.g.*, catalase, glutathione peroxidase) (266, 274). Hyperglycemia-induced proinsulin synthesis also stimulates ROS production due to excessive activity of the oxidative protein folding (274).

Besides β-cells, the ER stress response was also detected upon overfeeding in liver and adipose tissue in a mouse model of obesity (241), and ER stress markers have recently been found in adipose tissue in obese patients (289). Excessive caloric intake stimulates the synthesis and storage of triglycerides in adipose tissue. Increase of the adipose tissue mass is due to both hypertrophy and hyperplasia associated with

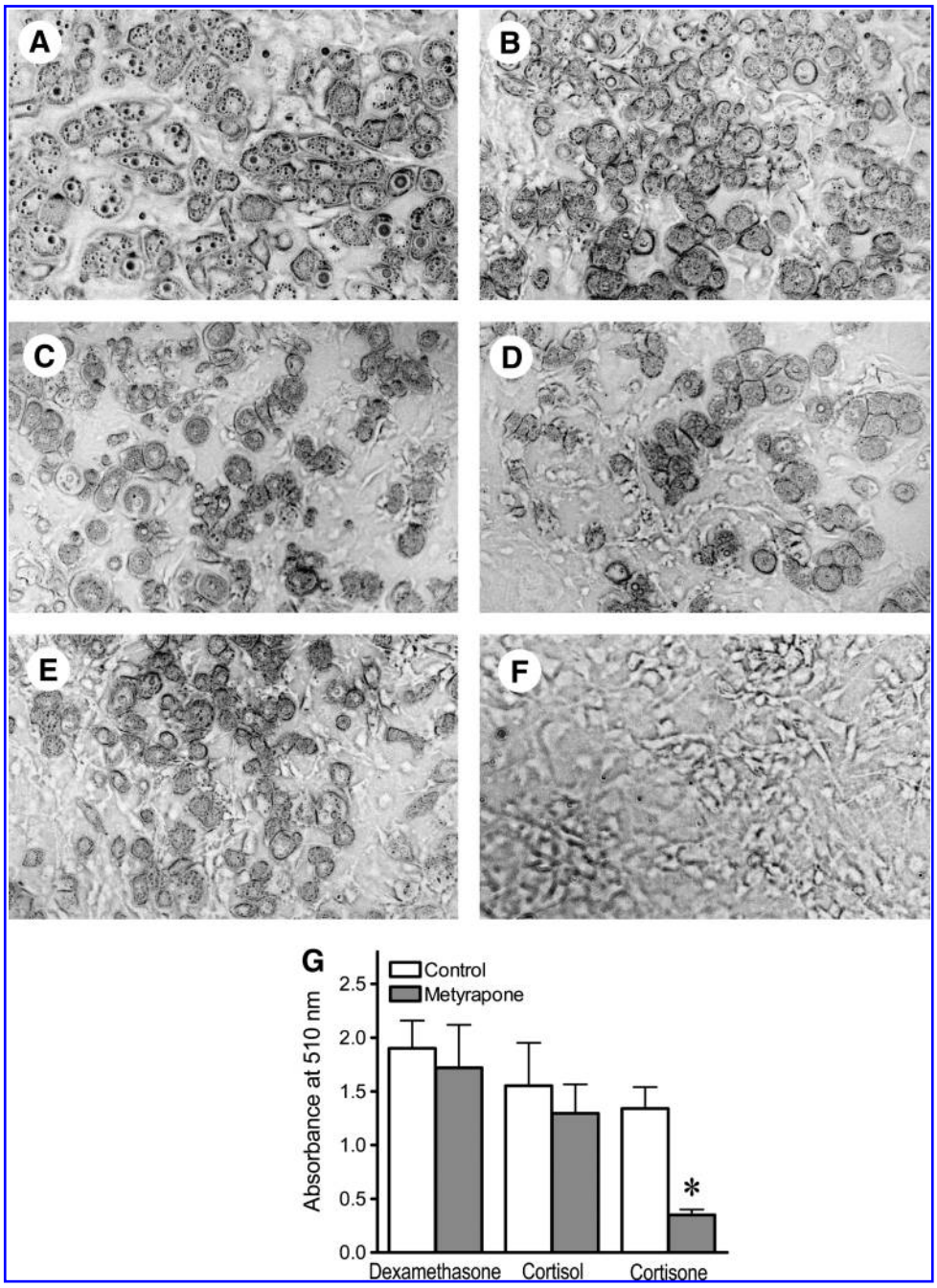

FIG. 14. Reduced state of the luminal pyridine nucleotides is a prerequisite for preadipocyte differentiation. The effect of metyrapone—a luminal NADPH depleting agent—was investigated on cortisone-induced adipogenic differentiation of 3T3-L1 cells. Adipogenesis was induced in 3T3-L1 cells with a medium containing 0.5 μM dexamethasone (**A** and **B**), cortisol (**C** and **D**), or cortisone (**E** and **F**). Metyrapone (50 μM) was added together with the steroids to cells shown in **B**, **D**, and **F**. Cellular lipids were stained with Oil Red O and examined by phase contrast microscopy after 7 days. The diagram (**G**) shows the absorbance at 510 nm of the Oil Red O dye extracted with isopropanol from the cells treated as indicated. Data are mean ± S.D. from three experiments. *$p < 0.01$ *vs.* control. (Adapted from Ref. 195.)

the growth of fat droplets in adipocytes. The enhanced triglyceride synthesis causes ER stress and provokes certain elements of the UPR. Among others, CHOP induction was shown and implicated in the reduction of adiponectin production in mouse models of obesity (122).

ER redox conditions play a fundamental role in preadipocyte differentiation, which is a prerequisite for the development of obesity. The enhancement of local glucocorticoid production is an important event in preadipocyte differentiation. The capacity of the ER to convert cortisone to active cortisol is enhanced during preadipocyte differentiation by a remarkable induction of 11βHSD1 (286). It has been demonstrated that the redox state of the ER luminal pyridine nucleotides is a key factor of prereceptorial hormone activation (195); reduced state favors, while oxidized state counteracts the local activation of glucocorticoids (Fig. 14). Since

luminal NADPH is generated at the expense of glucose-6-phosphate, it is plausible that hyperglycemia (*i.e.*, an increased supply of glucose-6-phosphate), favors preadipocyte differentiation by means of increased local cortisol production (Fig. 14). It has been recently demonstrated that the adipose tissue of obese mice is hypoxic (122), which may also contribute to metabolic dysregulation and even to development of ER stress (158).

ER stress and the UPR in the insulin-sensitive tissues interfere with insulin signaling to induce insulin resistance. The activation of JNK in the ER stress response leads to inhibitory serine phosphorylation of insulin receptor substrate-1 (IRS-1), thereby reducing the insulin responsiveness of the cells (111, 241). This observation provides a possible link between obesity and type 2 diabetes because the enlargement of fat stores in adipose tissue is associated with elevated glucose and FFA levels in the circulation, which threatens with the development of a vicious cycle, since hyperglycemia and hyper-fatty-acidemia induce ER stress, and ER stress can further increase insulin resistance (92). TNFα-dependent ROS generation converts the catalytic cysteine to sulfenic acid in JNK phosphatases; this mechanism also leads to sustained JNK activity contributing to insulin resistance (138). Hepatocytes are also affected in these conditions. Obesity-induced ER stress causes insulin resistance through JNK-dependent IRS-1 phosphorylation (124), and leads to increased hepatic glucose production through the induction of glucose-6-phosphatase (135). The enhancement of gluconeogenesis can be expected to favor local cortisol production by the G6PT-H6PD-11βHSD1 triad due to the increased glucose-6-phosphate supply, and the metabolic effects of cortisol can worsen insulin resistance.

Atherosclerosis also involves redox alterations in the ER. Oxysterols, the products of enzymatic or nonenzymatic cholesterol oxidation, may be involved in the initiation and progression of atherosclerosis (317). Oxysterols can be rapidly metabolized in the ER lumen in NADPH-consuming reactions (279). Hypercholesterolemia and elevated triglycerides may also induce ER stress in vascular cells (90). Oxidized lipids stimulate the UPR in endothelial cells, and UPR components ATF4 and XBP1 have been implicated in ER-stress-induced cytokine production by these vascular cells (84). Cholesterol induces ER stress in macrophages and promotes cytokine expression by a CHOP-dependent manner (173), which further implicates the UPR in atherosclerosis mechanisms. Atherosclerosis is also a possible consequence of hyperhomocysteinemia (170). Hyperhomocysteinemia, an integral component of several disorders including cardiovascular disease, neurodegeneration, diabetes, alcoholic liver disease, and obesity (134), induces ER stress in vascular endothelial cells, and triggers apoptosis through an IRE1-dependent mechanism (339). Hyperhomocysteinemia interferes with oxidative protein folding, influences the cellular redox status of thiols and the intracellular glutathione concentrations (154). In addition, incorporation of nitrosylated homocysteine into proteins may lead to protein misfolding (132). These effects should be considered in the ER stress provoking machinery.

D. Cancer

Tumor cells usually need to adopt to a hypoxic and hypoglycemic environment due to their rapid growth and the insufficient vascularization. This suggests that a certain level of ER stress should be present in tumors, followed by a protective UPR. The mechanism helps the tumor cells to survive in a nutrient- and oxygen-deprived environment. In fact, UPR marker proteins such as GRP78 and XBP1 are overexpressed in tumors or in cultured cancer cells exposed to hypoxia (82, 293). Supporting the protective role of the UPR in cancer, GRP78, XBP1, or PERK-deficient transformed cell lines showed remarkably reduced tumorigenesis in mice (33, 133, 267). GRP78 seems to play a pivotal role in tumor survival, proliferation, metastatic potential, and resistance to therapies. Thus, GRP78 expression is an important marker to estimate tumor malignancy, and it is a target for cancer treatment (24, 169). These findings indicate that GRP78 plays an important role in the antiapoptotic defense of tumor cells, beyond its participation in the ER stress response. In summary, inhibition of the UPR might be a possible new direction in cancer therapy. However, in certain cases the aggravation of the existing ER stress and the UPR may also turn the tumors towards apoptosis (46, 188).

VI. Conclusions

The redox reactions in the ER lumen are arranged around a bicentral network. Protein processing is tightly linked to the thiol–disulfide (including GSH–GSSG) couple; while the reactions of carbohydrate and lipid metabolism, as well as those of biotransformation, employ pyridine nucleotides. In spite of the weak links between the two modules, their redox states seem to be independent and fundamentally different. The oxidized/oxidizing thiol–disulfide system is essential for protein maturation while reduced/reducing pyridine nucleotides allow local steroid activation and fatty acid desaturation.

The redox conditions of the ER lumen are sensitive to alterations in the balance between the supply of electron donors and electron acceptors. The thiol–disulfide module is affected by the nascent protein load and the oxygen tension while the pyridine nucleotide module is particularly dependent on the

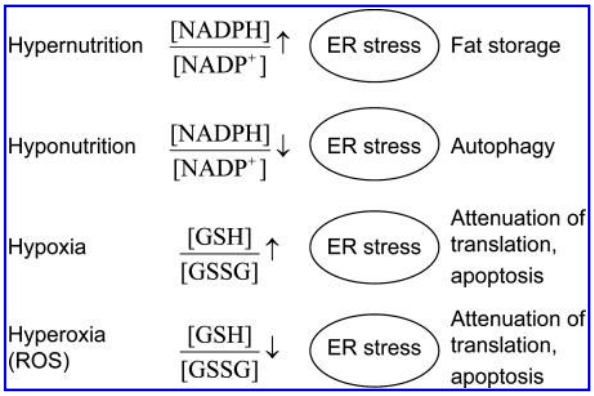

FIG. 15. ER luminal redox environment, as an integrative sensor of external and internal stimuli. The imbalance of the main redox systems of the ER lumen provoked by exogenous or endogenous factors leads to the disturbed functioning of the organelle (collectively termed as ER stress) and to the initiation of restitution mechanisms. The possible outcome of the *in vivo* stress seems to be dependent on the redox systems affected.

abundance of carbohydrates and lipids. Both systems are able to initiate or modulate signaling mechanisms (*i.e.*, the UPR and glucocorticoid action) with the capacity to trigger major cell responses, such as the induction of metabolic enzymes or chaperones, attenuation of protein synthesis or even the initiation of apoptosis. The evoked response is not uniform; the organelle seems to induce the most adequate changes depending on the original stimulus (Fig. 15). Therefore, the ER is equipped with suitable sensors and appropriate effectors required for a central redox-based nutrient and metabolic regulator of the cell.

The primary aim of the ER-derived control mechanisms is to adjust the metabolic capacities to the demands and vice versa. It is now evident that the redox-based metabolic control in the ER is an important component of the physiological adaptation. However, when the ER stress induced signaling fails to recover the balance, positive feedback loops can develop and destructive responses become dominant. Growing evidence supports the role of the ER stress and the UPR in the development and/or aggravation of a number of pathological conditions. Some of the most important and best known examples are discussed in this review. Investigtions have shed light recently on the role of ER dysfunctions in these pathological conditions. The intensive research in this novel field provides new perspectives for the understanding of major medical problems, such as diabetes mellitus and neurodegenerative diseases, and hopefully facilitates the search for potential therapeutic targets as well.

Acknowledgments

Thanks are due to the János Bolyai Research Scholarship of the Hungarian Academy of Sciences for supporting M. Cs. Support was also given by the Hungarian Scientific Research Fund (NN 78300 and 75275). We thank Balazs Enyedi (Dept. of Physiology, Semmelweis University, Budapest) for critical reading of the article.

References

1. Adhami F, Liao G, Morozov YM, Schloemer A, Schmithorst VJ, Lorenz JN, Dunn RS, Vorhees CV, Wills–Karp M, Degen JL, Davis RJ, Mizushima N, Rakic P, Dardzinski BJ, Holland SK, Sharp FR, and Kuan CY. Cerebral ischemia-hypoxia induces intravascular coagulation and autophagy. *Am J Pathol* 169: 566–583, 2006.
2. Adhami F, Schloemer A, and Kuan CY. The roles of autophagy in cerebral ischemia. *Autophagy* 3: 42–44, 2007.
3. Althausen S, Mengesdorf T, Mies G, Olah L, Nairn AC, Proud CG, and Paschen W. Changes in the phosphorylation of initiation factor eIF-2α, elongation factor eEF-2 and p70 S6 kinase after transient focal cerebral ischaemia in mice. *J Neurochem* 78: 779–787, 2001.
4. Anelli T, Alessio M, Bachi A, Bergamelli L, Bertoli G, Camerini S, Mezghrani A, Ruffato E, Simmen T, and Sitia R. Thiol-mediated protein retention in the endoplasmic reticulum: The role of ERp44. *EMBO J* 22: 5015–5022, 2003.
5. Anelli T, Alessio M, Mezghrani A, Simmen T, Talamo F, Bachi A, and Sitia R. ERp44, a novel endoplasmic reticulum folding assistant of the thioredoxin family. *EMBO J* 21: 835–844, 2002.
6. Anelli T and Sitia R. Protein quality control in the early secretory pathway. *EMBO J* 27: 315–327, 2008.
7. Appenzeller–Herzog C and Ellgaard L. The human PDI family: Versatility packed into a single fold. *Biochim Biophys Acta* 1783: 535–548, 2008.
8. Appenzeller–Herzog C, Riemer J, Christensen B, Sorensen ES, and Ellgaard L. A novel disulfide switch mechanism in Ero1α balances ER oxidation in human cells. *EMBO J* 27: 2977–2987, 2008.
9. Atkin JD, Farg MA, Turner BJ, Tomas D, Lysaght JA, Nunan J, Rembach A, Nagley P, Beart PM, Cheema SS, and Horne MK. Induction of the unfolded protein response in familial amyotrophic lateral sclerosis and association of protein-disulfide isomerase with superoxide dismutase 1. *J Biol Chem* 281: 30152–30165, 2006.
10. Atkin JD, Farg MA, Walker AK, McLean C, Tomas D, and Horne MK. Endoplasmic reticulum stress and induction of the unfolded protein response in human sporadic amyotrophic lateral sclerosis. *Neurobiol Dis* 30: 400–407, 2008.
11. Baines AC and Zhang B. Receptor-mediated protein transport in the early secretory pathway. *Trends Biochem Sci* 32: 381–388, 2007.
12. Baker KM, Chakravarthi S, Langton KP, Sheppard AM, Lu H, and Bulleid NJ. Low reduction potential of Ero1α regulatory disulfides ensures tight control of substrate oxidation. *EMBO J* 27: 2988–2997, 2008.
13. Baker ML, Serysheva, II, Sencer S, Wu Y, Ludtke SJ, Jiang W, Hamilton SL, and Chiu W. The skeletal muscle Ca^{2+} release channel has an oxidoreductase-like domain. *Proc Natl Acad Sci U S A* 99: 12155–12160, 2002.
14. Bando Y, Katayama T, Kasai K, Taniguchi M, Tamatani M, and Tohyama M. GRP94 (94 kDa glucose-regulated protein) suppresses ischemic neuronal cell death against ischemia/reperfusion injury. *Eur J Neurosci* 18: 829–840, 2003.
15. Banhegyi G, Benedetti A, Csala M, and Mandl J. Stress on redox. *FEBS Lett* 581: 3634–3640, 2007.
16. Banhegyi G, Benedetti A, Fulceri R, and Senesi S. 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, 2004.
17. Banhegyi G, Braun L, Csala M, Puskas F, and Mandl J. Ascorbate metabolism and its regulation in animals. *Free Radic Biol Med* 23: 793–803, 1997.
18. Banhegyi G, Csala M, and Benedetti A. Hexose-6-phosphate dehydrogenase: linking endocrinology and metabolism in the endopsmic reticulum. *J Mol Endocrinol* 42: 283–289, 2009.
19. Banhegyi G, Csala M, Braun L, Garzo T, and Mandl J. Ascorbate synthesis-dependent glutathione consumption in mouse liver. *FEBS Lett* 381: 39–41, 1996.
20. Banhegyi G, Lusini L, Puskas F, Rossi R, Fulceri R, Braun L, Mile V, di Simplicio P, Mandl J, and Benedetti A. Preferential transport of glutathione versus glutathione disulfide in rat liver microsomal vesicles. *J Biol Chem* 274: 12213–12216, 1999.
21. Banhegyi G, Mandl J, and Csala M. Redox-based endoplasmic reticulum dysfunction in neurological diseases. *J Neurochem* 107: 20–34, 2008.
22. Banhegyi G, Marcolongo P, Puskas F, Fulceri R, Mandl J, and Benedetti A. Dehydroascorbate and ascorbate transport in rat liver microsomal vesicles. *J Biol Chem* 273: 2758–2762, 1998.
23. Bass R, Ruddock LW, Klappa P, and Freedman RB. A major fraction of endoplasmic reticulum-located glutathione is present as mixed disulfides with protein. *J Biol Chem* 279: 5257–5262, 2004.

24. Baumeister P, Dong D, Fu Y, and Lee AS. Transcriptional induction of GRP78/BiP by histone deacetylase inhibitors and resistance to histone deacetylase inhibitor-induced apoptosis. *Mol Cancer Ther* 8: 1086–1094, 2009.
25. Bedard K, Szabo E, Michalak M, and Opas M. Cellular functions of endoplasmic reticulum chaperones calreticulin, calnexin, and ERp57. *Int Rev Cytol* 245: 91–121, 2005.
26. Belkaid A, Copland IB, Massillon D, and Annabi B. Silencing of the human microsomal glucose-6-phosphate translocase induces glioma cell death: Potential new anticancer target for curcumin. *FEBS Lett* 580: 3746–3752, 2006.
27. Belkaid A, Currie JC, Desgagnes J, and Annabi B. 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, 2006.
28. Belousov VV, Fradkov AF, Lukyanov KA, Staroverov DB, Shakhbazov KS, Terskikh AV, and Lukyanov S. Genetically encoded fluorescent indicator for intracellular hydrogen peroxide. *Nat Methods* 3: 281–286, 2006.
29. Benavides A, Pastor D, Santos P, Tranque P, and Calvo S. CHOP plays a pivotal role in the astrocyte death induced by oxygen and glucose deprivation. *Glia* 52: 261–275, 2005.
30. Bence NF, Sampat RM, and Kopito RR. Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* 292: 1552–1555, 2001.
31. Berndt C, Lillig CH, and Holmgren A. Thioredoxins and glutaredoxins as facilitators of protein folding. *Biochim Biophys Acta* 1783: 641–650, 2008.
32. Bezprozvanny I, and Mattson MP. Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. *Trends Neurosci* 31: 454–463, 2008.
33. Bi M, Naczki C, Koritzinsky M, Fels D, Blais J, Hu N, Harding H, Novoa I, Varia M, Raleigh J, Scheuner D, Kaufman RJ, Bell J, Ron D, Wouters BG, and Koumenis C. ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumor growth. *EMBO J* 24: 3470–3481, 2005.
34. Bjorneboe A, Bjorneboe GE, and Drevon CA. Absorption, transport and distribution of vitamin E. *J Nutr* 120: 233–242, 1990.
35. Bock KW and Kohle C. Topological aspects of oligomeric UDP-glucuronosyltransferases in endoplasmic reticulum membranes: Advances and open questions. *Biochem Pharmacol* 77: 1458–146, 2009.
36. Borchelt DR, Lee MK, Slunt HS, Guarnieri M, Xu ZS, Wong PC, Brown RH, Jr., Price DL, Sisodia SS, and Cleveland DW. Superoxide dismutase 1 with mutations linked to familial amyotrophic lateral sclerosis possesses significant activity. *Proc Natl Acad Sci USA* 91: 8292–8296, 1994.
37. Bowling AC, Barkowski EE, McKenna–Yasek D, Sapp P, Horvitz HR, Beal MF, and Brown RH, Jr. Superoxide dismutase concentration and activity in familial amyotrophic lateral sclerosis. *J Neurochem* 64: 2366–2369, 1995.
38. Brady NR, Hamacher–Brady A, Yuan H, and Gottlieb RA. The autophagic response to nutrient deprivation in the hl-1 cardiac myocyte is modulated by Bcl-2 and sarco/endoplasmic reticulum calcium stores. *FEBS J* 274: 3184–3197, 2007.
39. Bruijn LI, Miller TM, and Cleveland DW. Unraveling the mechanisms involved in motor neuron degeneration in ALS. *Annu Rev Neurosci* 27: 723–749, 2004.
40. Bublitz C and Lawler CA. The levels of nicotinamide nucleotides in liver microsomes and their possible significance to the function of hexose phosphate dehydrogenase. *Biochem J* 245: 263–267, 1987.
41. Buettner GR. The pecking order of free radicals and antioxidants: Lipid peroxidation, α-tocopherol, and ascorbate. *Arch Biochem Biophys* 300: 535–543, 1993.
42. Cabibbo A, Pagani M, Fabbri M, Rocchi M, Farmery MR, Bulleid NJ, and Sitia R. ERO1-L, a human protein that favors disulfide bond formation in the endoplasmic reticulum. *J Biol Chem* 275: 4827–4833, 2000.
43. Camporeale G and Zempleni J. 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, 2003.
44. Cannon MB and Remington SJ. Redox-sensitive green fluorescent protein: Probes for dynamic intracellular redox responses. A review. *Methods Mol Biol* 476: 51–65, 2008.
45. Carrasco DR, Sukhdeo K, Protopopova M, Sinha R, Enos M, Carrasco DE, Zheng M, Mani M, Henderson J, Pinkus GS, Munshi N, Horner J, Ivanova EV, Protopopov A, Anderson KC, Tonon G, and DePinho RA. The differentiation and stress response factor XBP-1 drives multiple myeloma pathogenesis. *Cancer Cell* 11: 349–360, 2007.
46. Cenci S and Sitia R. Managing and exploiting stress in the antibody factory. *FEBS Lett* 581: 3652–3657, 2007.
47. Chou JY, Matern D, Mansfield BC, and Chen YT. Type I glycogen storage diseases: Disorders of the glucose-6-phosphatase complex. *Curr Mol Med* 2: 121–143, 2002.
48. Clarke JL and Mason PJ. Murine hexose-6-phosphate dehydrogenase: A bifunctional enzyme with broad substrate specificity and 6-phosphogluconolactonase activity. *Arch Biochem Biophys* 415: 229–234, 2003.
49. Clerc S, Hirsch C, Oggier DM, Deprez P, Jakob C, Sommer T, and Aebi M. Htm1 protein generates the N-glycan signal for glycoprotein degradation in the endoplasmic reticulum. *J Cell Biol* 184: 159–172, 2009.
50. Cooper AA, Gitler AD, Cashikar A, Haynes CM, Hill KJ, Bhullar B, Liu K, Xu K, Strathearn KE, Liu F, Cao S, Caldwell KA, Caldwell GA, Marsischky G, Kolodner RD, Labaer J, Rochet JC, Bonini NM, and Lindquist S. α-Synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science* 313: 324–328, 2006.
51. Cribb AE, Peyrou M, Muruganandan S, and Schneider L. The endoplasmic reticulum in xenobiotic toxicity. *Drug Metab Rev* 37: 405–442, 2005.
52. Cunha DA, Hekerman P, Ladriere L, Bazarra–Castro A, Ortis F, Wakeham MC, Moore F, Rasschaert J, Cardozo AK, Bellomo E, Overbergh L, Mathieu C, Lupi R, Hai T, Herchuelz A, Marchetti P, Rutter GA, Eizirik DL, and Cnop M. Initiation and execution of lipotoxic ER stress in pancreatic β-cells. *J Cell Sci* 121: 2308–2318, 2008.
53. Cuozzo JW and Kaiser CA. Competition between glutathione and protein thiols for disulfide-bond formation. *Nat Cell Biol* 1: 130–135, 1999.
54. Cullinan SB, Zhang D, Hannink M, Arvisais E, Kaufman RJ, and Diehl JA. Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. *Mol Cell Biol* 23: 7198–7209, 2003.
55. Cullinan SB and Diehl JA. PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. *J Biol Chem* 279: 20108–20117, 2004.
56. Czegle I, Piccirella S, Senesi S, Csala M, Mandl J, Banhegyi G, Fulceri R, and Benedetti A. Cooperativity between

11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase is based on a common pyridine nucleotide pool in the lumen of the endoplasmic reticulum. *Mol Cell Endocrinol* 248: 24–25, 2006.
57. Czyzyk–Krzeska MF. Molecular aspects of oxygen sensing in physiological adaptation to hypoxia. *Respir Physiol* 110: 99–111, 1997.
58. Csala M, Banhegyi G, and Benedetti A. Endoplasmic reticulum: A metabolic compartment. *FEBS Lett* 580: 2160–2165, 2006.
59. Csala M, Banhegyi G, Braun L, Szirmai R, Burchell A, Burchell B, Benedetti A, and Mandl J. β-Glucuronidase latency in isolated murine hepatocytes. *Biochem Pharmacol* 59: 801–805, 2000.
60. Csala M, Braun L, Mile V, Kardon T, Szarka A, Kupcsulik P, Mandl J, and Banhegyi G. Ascorbate-mediated electron transfer in protein thiol oxidation in the endoplasmic reticulum. *FEBS Lett* 460: 539–543, 1999.
61. Csala M, Marcolongo P, Lizak B, Senesi S, Margittai E, Fulceri R, Magyar JE, Benedetti A, and Banhegyi G. Transport and transporters in the endoplasmic reticulum. *Biochim Biophys Acta* 1768: 1325–1341, 2007.
62. Csala M, Mile V, Benedetti A, Mandl J, and Banhegyi G. Ascorbate oxidation is a prerequisite for its transport into rat liver microsomal vesicles. *Biochem J* 349: 413–415, 2000.
63. Csala M, Szarka A, Margittai E, Mile V, Kardon T, Braun L, Mandl J, and Banhegyi G. Role of vitamin E in ascorbate-dependent protein thiol oxidation in rat liver endoplasmic reticulum. *Arch Biochem Biophys* 388: 55–59, 2001.
64. Danielson SR and Andersen JK. Oxidative and nitrative protein modifications in Parkinson's disease. *Free Radic Biol Med* 44: 1787–1794, 2008.
65. DeGracia DJ and Montie HL. Cerebral ischemia and the unfolded protein response. *J Neurochem* 91: 1–8, 2004.
66. Deng HX, Hentati A, Tainer JA, Iqbal Z, Cayabyab A, Hung WY, Getzoff ED, Hu P, Herzfeldt B, Roos RP, and *et al*. Amyotrophic lateral sclerosis and structural defects in Cu,Zn superoxide dismutase. *Science* 261: 1047–1051, 1993.
67. Deng J, Lu PD, Zhang Y, Scheuner D, Kaufman RJ, Sonenberg N, Harding HP, and Ron D. Translational repression mediates activation of nuclear factor κ B by phosphorylated translation initiation factor 2. *Mol Cell Biol* 24: 10161–10168, 2004.
68. Denton RM, Yorke RE, and Randle PJ. Measurement of concentrations of metabolites in adipose tissue and effects of insulin, alloxan-diabetes and adrenaline. *Biochem J* 100: 407–419, 1966.
69. Dixon BM, Heath SH, Kim R, Suh JH, and Hagen TM. Assessment of endoplasmic reticulum glutathione redox status is confounded by extensive ex vivo oxidation. *Antioxid Redox Signal* 10: 963–972, 2008.
70. Dollins DE, Warren JJ, Immormino RM, and Gewirth DT. Structures of GRP94-nucleotide complexes reveal mechanistic differences between the hsp90 chaperones. *Mol Cell* 28: 41–56, 2007.
71. Draper N and Stewart PM. 11β-hydroxysteroid dehydrogenase and the pre-receptor regulation of corticosteroid hormone action. *J Endocrinol* 186: 251–271, 2005.
72. Duennwald ML, and Lindquist S. Impaired ERAD and ER stress are early and specific events in polyglutamine toxicity. *Genes Dev* 22: 3308–3319, 2008.
73. Dzyakanchuk AA, Balazs Z, Nashev LG, Amrein KE, and Odermatt A. 11β-Hydroxysteroid dehydrogenase 1 reductase activity is dependent on a high ratio of NADPH/$NADP^+$ and is stimulated by extracellular glucose. *Mol Cell Endocrinol* 301: 137–141, 2009.
74. Ellgaard L and Ruddock LW. The human protein disulfide isomerase family: Substrate interactions and functional properties. *EMBO Rep* 6: 28–32, 2005.
75. Fagioli C, Mezghrani A, and Sitia R. Reduction of interchain disulfide bonds precedes the dislocation of Ig-μ chains from the endoplasmic reticulum to the cytosol for proteasomal degradation. *J Biol Chem* 276: 40962–40967, 2001.
76. Feng W, Liu G, Allen PD, and Pessah IN. Transmembrane redox sensor of ryanodine receptor complex. *J Biol Chem* 275: 35902–35907, 2000.
77. Ferreiro E, Resende R, Costa R, Oliveira CR, and Pereira CM. An endoplasmic-reticulum-specific apoptotic pathway is involved in prion and amyloid-β peptides neurotoxicity. *Neurobiol Dis* 23: 669–678, 2006.
78. Fischer H, Koenig U, Eckhart L, and Tschachler E. Human caspase 12 has acquired deleterious mutations. *Biochem Biophys Res Commun* 293: 722–726, 2002.
79. Fraldi A, Zito E, Annunziata F, Lombardi A, Cozzolino M, Monti M, Spampanato C, Ballabio A, Pucci P, Sitia R, and Cosma MP. Multistep, sequential control of the trafficking and function of the multiple sulfatase deficiency gene product, SUMF1 by PDI, ERGIC-53 and ERp44. *Hum Mol Genet* 17: 2610–2621, 2008.
80. Frand AR and Kaiser CA. The ERO1 gene of yeast is required for oxidation of protein dithiols in the endoplasmic reticulum. *Mol Cell* 1: 161–170, 1998.
81. Fujimoto T, Ohsaki Y, Cheng J, Suzuki M, and Shinohara Y. Lipid droplets: A classic organelle with new outfits. *Histochem Cell Biol* 130: 263–279, 2008.
82. Fujimoto T, Onda M, Nagai H, Nagahata T, Ogawa K, and Emi M. Upregulation and overexpression of human X-box binding protein 1 (hXBP-1) gene in primary breast cancers. *Breast Cancer* 10: 301–306, 2003.
83. Garcia AA and Reitsma PH. VKORC1 and the vitamin K cycle. *Vitam Horm* 78: 23–33, 2008.
84. Gargalovic PS, Gharavi NM, Clark MJ, Pagnon J, Yang WP, He A, Truong A, Baruch–Oren T, Berliner JA, Kirchgessner TG, and Lusis AJ. The unfolded protein response is an important regulator of inflammatory genes in endothelial cells. *Arterioscler Thromb Vasc Biol* 26: 2490–2496, 2006.
85. Gerin I and Van Schaftingen E. Evidence for glucose-6-phosphate transport in rat liver microsomes. *FEBS Lett* 517: 257–260, 2002.
86. Gerin I, Veiga-da-Cunha M, Achouri Y, Collet JF, and Van Schaftingen E. Sequence of a putative glucose 6-phosphate translocase, mutated in glycogen storage disease type Ib. *FEBS Lett* 419: 235–238, 1997.
87. Gess B, Hofbauer KH, Wenger RH, Lohaus C, Meyer HE, and Kurtz A. The cellular oxygen tension regulates expression of the endoplasmic oxidoreductase ERO1-Lα. *Eur J Biochem* 270: 2228–2235, 2003.
88. Ghosh D. Human sulfatases: A structural perspective to catalysis. *Cell Mol Life Sci* 64: 2013–2022, 2007.
89. Glembotski CC. Endoplasmic reticulum stress in the heart. *Circ Res* 101: 975–984, 2007.
90. Glembotski CC. The role of the unfolded protein response in the heart. *J Mol Cell Cardiol* 44: 453–459, 2008.
91. Gooding JM, Shayeghi M, and Saggerson ED. Membrane transport of fatty acylcarnitine and free L-carnitine by rat liver microsomes. *Eur J Biochem* 271: 954–961, 2004.

92. Gregor MF and Hotamisligil GS. Thematic review series: Adipocyte Biology. Adipocyte stress: The endoplasmic reticulum and metabolic disease. *J Lipid Res* 48: 1905–1914, 2007.
93. Gross E, Sevier CS, Heldman N, Vitu E, Bentzur M, Kaiser CA, Thorpe C, and Fass D. Generating disulfides enzymatically: Reaction products and electron acceptors of the endoplasmic reticulum thiol oxidase Ero1p. *Proc Natl Acad Sci USA* 103: 299–304, 2006.
94. Guerre–Millo M. Adiponectin: Aan update. *Diabetes Metab* 34: 12–18, 2008.
95. Guo Q, Sopher BL, Furukawa K, Pham DG, Robinson N, Martin GM, and Mattson MP. Alzheimer's presenilin mutation sensitizes neural cells to apoptosis induced by trophic factor withdrawal and amyloid β-peptide: involvement of calcium and oxyradicals. *J Neurosci* 17: 4212–4222, 1997.
96. Gustafsson AB and Gottlieb RA. Autophagy in ischemic heart disease. *Circ Res* 104: 150–158, 2009.
97. Halliwell B. Oxidative stress and neurodegeneration: Where are we now? *J Neurochem* 97: 1634–1658, 2006.
98. Han D, Lerner AG, Vande Walle L, Upton JP, Xu W, Hagen A, Backes BJ, Oakes SA, and Papa FR. IRE1α kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. *Cell* 138: 562–575, 2009
99. Hartl FU. Molecular chaperones in cellular protein folding. *Nature* 381: 571–579, 1996.
100. Hayashi T, Saito A, Okuno S, Ferrand–Drake M, Dodd RL, and Chan PH. Damage to the endoplasmic reticulum and activation of apoptotic machinery by oxidative stress in ischemic neurons. *J Cereb Blood Flow Metab* 25: 41–53, 2005.
101. Hayashi T, Saito A, Okuno S, Ferrand–Drake M, Dodd RL, Nishi T, Maier CM, Kinouchi H, and Chan PH. Oxidative damage to the endoplasmic reticulum is implicated in ischemic neuronal cell death. *J Cereb Blood Flow Metab* 23: 1117–1128, 2003.
102. He CH, Gong P, Hu B, Stewart D, Choi ME, Choi AM, and Alam J. Identification of activating transcription factor 4 (ATF4) as an Nrf2-interacting protein. Implication for heme oxygenase-1 gene regulation. *J Biol Chem* 276: 20858–20865, 2001.
103. Heath–Engel HM, Chang NC, and Shore GC. The endoplasmic reticulum in apoptosis and autophagy: Role of the BCL-2 protein family. Oncogene 27: 6419–6433, 2008.
104. Hebert DN and Molinari M. In and out of the ER: Protein folding, quality control, degradation, and related human diseases. *Physiol Rev* 87: 1377–1408, 2007.
105. Heckler EJ, Rancy PC, Kodali VK, and Thorpe C. Generating disulfides with the Quiescin-sulfhydryl oxidases. *Biochim Biophys Acta* 1783: 567–577, 2008.
106. Henrich M and Buckler KJ. Effects of anoxia and aglycaemia on cytosolic calcium regulation in rat sensory neurons. *J Neurophysiol* 100: 456–473, 2008.
107. Heritage D and Wonderlin WF. Translocon pores in the endoplasmic reticulum are permeable to a neutral, polar molecule. *J Biol Chem* 276: 22655–22662, 2001.
108. Hetz C, Bernasconi P, Fisher J, Lee AH, Bassik MC, Antonsson B, Brandt GS, Iwakoshi NN, Schinzel A, Glimcher LH, and Korsmeyer SJ. Proapoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1α. *Science* 312: 572–576, 2006.
109. Hewitt KN, Walker EA, and Stewart PM. Minireview: Hexose-6-phosphate dehydrogenase and redox control of 11β-hydroxysteroid dehydrogenase type 1 activity. *Endocrinology* 146: 2539–2543, 2005.
110. Higo T, Hattori M, Nakamura T, Natsume T, Michikawa T, and Mikoshiba K. Subtype-specific and ER lumenal environment-dependent regulation of inositol 1,4,5-trisphosphate receptor type 1 by ERp44. *Cell* 120: 85–98, 2005.
111. Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, and Hotamisligil GS. A central role for JNK in obesity and insulin resistance. *Nature* 420: 333–336, 2002.
112. Hitomi J, Katayama T, Eguchi Y, Kudo T, Taniguchi M, Koyama Y, Manabe T, Yamagishi S, Bando Y, Imaizumi K, Tsujimoto Y, and Tohyama M. Involvement of caspase-4 in endoplasmic reticulum stress-induced apoptosis and Aβ-induced cell death. *J Cell Biol* 165: 347–356, 2004.
113. Hoffmann F and Maser E. Carbonyl reductases and pluripotent hydroxysteroid dehydrogenases of the short-chain dehydrogenase/reductase superfamily. *Drug Metab Rev* 39: 87–144, 2007.
114. Holmes MC, Yau JL, Kotelevtsev Y, Mullins JJ, and Seckl JR. 11 β-Hydroxysteroid dehydrogenases in the brain: Two enzymes two roles. *Ann NY Acad Sci* 1007: 357–366, 2003.
115. Holtz WA and O'Malley KL. Parkinsonian mimetics induce aspects of unfolded protein response in death of dopaminergic neurons. *J Biol Chem* 278: 19367–19377, 2003.
116. Holtz WA, Turetzky JM, Jong YJ, and O'Malley KL. Oxidative stress-triggered unfolded protein response is upstream of intrinsic cell death evoked by parkinsonian mimetics. *J Neurochem* 99: 54–69, 2006.
117. Hoozemans JJ, van Haastert ES, Eikelenboom P, de Vos RA, Rozemuller JM, and Scheper W. Activation of the unfolded protein response in Parkinson's disease. *Biochem Biophys Res Commun* 354: 707–711, 2007.
118. Hoozemans JJ, Stieler J, van Haastert ES, Veerhuis R, Rozemuller AJ, Baas F, Eikelenboom P, Arendt T, and Scheper W. The unfolded protein response affects neuronal cell cycle protein expression: Implications for Alzheimer's disease pathogenesis. *Exp Gerontol* 41: 380–386, 2006.
119. Hoozemans JJ, Veerhuis R, Van Haastert ES, Rozemuller JM, Baas F, Eikelenboom P, and Scheper W. The unfolded protein response is activated in Alzheimer's disease. *Acta Neuropathol* 110: 165–172, 2005.
120. Horemans N, Foyer CH, and Asard H. Transport and action of ascorbate at the plant plasma membrane. *Trends Plant Sci* 5: 263–267, 2000.
121. Hoshino T, Nakaya T, Araki W, Suzuki K, Suzuki T, and Mizushima T. Endoplasmic reticulum chaperones inhibit the production of amyloid-β peptides. *Biochem J* 402: 581–589, 2007.
122. Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, Furukawa S, Tochino Y, Komuro R, Matsuda M, and Shimomura I. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes* 56: 901–911, 2007.
123. Hosokawa N and Nagata K. Procollagen binds to both prolyl 4-hydroxylase/protein disulfide isomerase and HSP47 within the endoplasmic reticulum in the absence of ascorbate. *FEBS Lett* 466: 19–25, 2000.
124. Hotamisligil GS. Role of endoplasmic reticulum stress and c-Jun NH2-terminal kinase pathways in inflammation and origin of obesity and diabetes. *Diabetes* 54 Suppl 2: S73–78, 2005.

125. Hou ZQ, Li HL, Gao L, Pan L, Zhao JJ, and Li GW. Involvement of chronic stresses in rat islet and INS-1 cell glucotoxicity induced by intermittent high glucose. *Mol Cell Endocrinol* 291: 71–78, 2008.
126. Hoyer–Hansen M and Jaattela M. Connecting endoplasmic reticulum stress to autophagy by unfolded protein response and calcium. *Cell Death Differ* 14: 1576–1582, 2007.
127. Hu P, Han Z, Couvillon AD, Kaufman RJ, and Exton JH. Autocrine tumor necrosis factor α links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1α-mediated NF-κB activation and down-regulation of TRAF2 expression. *Mol Cell Biol* 26: 3071–3084, 2006.
128. Hwang C, Sinskey AJ, and Lodish HF. Oxidized redox state of glutathione in the endoplasmic reticulum. *Science* 257: 1496–1502, 1992.
129. Iadecola C, Zhang F, Casey R, Nagayama M, and Ross ME. Delayed reduction of ischemic brain injury and neurological deficits in mice lacking the inducible nitric oxide synthase gene. *J Neurosci* 17: 9157–9164, 1997.
130. Ilieva EV, Ayala V, Jove M, Dalfo E, Cacabelos D, Povedano M, Bellmunt MJ, Ferrer I, Pamplona R, and Portero–Otin M. Oxidative and endoplasmic reticulum stress interplay in sporadic amyotrophic lateral sclerosis. *Brain* 130: 3111–3123, 2007.
131. Iyanagi T. Molecular mechanism of phase I and phase II drug-metabolizing enzymes: Implications for detoxification. *Int Rev Cytol* 260: 35–112, 2007.
132. Jakubowski H. Molecular basis of homocysteine toxicity in humans. *Cell Mol Life Sci* 61: 470–487, 2004.
133. Jamora C, Dennert G, and Lee AS. Inhibition of tumor progression by suppression of stress protein GRP78/BiP induction in fibrosarcoma B/C10ME. *Proc Natl Acad Sci USA* 93: 7690–7694, 1996.
134. Ji C. Dissection of endoplasmic reticulum stress signaling in alcoholic and non-alcoholic liver injury. *J Gastroenterol Hepatol* 23 Suppl 1: S16–24, 2008.
135. Ji C and Kaplowitz N. ER stress: Can the liver cope? *J Hepatol* 45: 321–333, 2006.
136. Jiang HY, Wek SA, McGrath BC, Lu D, Hai T, Harding HP, Wang X, Ron D, Cavener DR, and Wek RC. Activating transcription factor 3 is integral to the eukaryotic initiation factor 2 kinase stress response. *Mol Cell Biol* 24: 1365–1377, 2004.
137. Kadowaki H, Nishitoh H, Urano F, Sadamitsu C, Matsuzawa A, Takeda K, Masutani H, Yodoi J, Urano Y, Nagano T, and Ichijo H. Amyloid β induces neuronal cell death through ROS-mediated ASK1 activation. *Cell Death Differ* 12: 19–24, 2005.
138. Kamata H, Honda S, Maeda S, Chang L, Hirata H, and Karin M. Reactive oxygen species promote TNFα-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* 120: 649–661, 2005.
139. Kanehara K, Kawaguchi S, and Ng DT. The EDEM and Yos9p families of lectin-like ERAD factors. *Semin Cell Dev Biol* 18: 743–750, 2007.
140. Kanekura K, Suzuki H, Aiso S, and Matsuoka M. ER stress and unfolded protein response in amyotrophic lateral sclerosis. *Mol Neurobiol* 39: 81–89, 2009.
141. Kaneto H, Nakatani Y, Kawamori D, Miyatsuka T, Matsuoka TA, Matsuhisa M, and Yamasaki Y. Role of oxidative stress, endoplasmic reticulum stress, and c-Jun N-terminal kinase in pancreatic β-cell dysfunction and insulin resistance. *Int J Biochem Cell Biol* 38: 782–793, 2006.
142. Kaplowitz N. Biochemical and cellular mechanisms of toxic liver injury. *Semin Liver Dis* 22: 137–144, 2002.
143. Karala AR, Lappi AK, Saaranen M, and Ruddock LW. Efficient peroxide mediated oxidative refolding of a protein at physiological pH and implications for oxidative folding in the endoplasmic reticulum. *Antioxid Redox Signal* 11: 963–970, 2009.
144. Kardon T, Senesi S, Marcolongo P, Legeza B, Banhegyi G, Mandl J, Fulceri R, and Benedetti A. Maintenance of luminal NADPH in the endoplasmic reticulum promotes the survival of human neutrophil granulocytes. *FEBS Lett* 582: 1809–1815, 2008.
145. Kharroubi I, Ladriere L, Cardozo AK, Dogusan Z, Cnop M, and Eizirik DL. Free fatty acids and cytokines induce pancreatic β-cell apoptosis by different mechanisms: Role of nuclear factor-κB and endoplasmic reticulum stress. *Endocrinology* 145: 5087–5096, 2004.
146. Kikuchi H, Almer G, Yamashita S, Guegan C, Nagai M, Xu Z, Sosunov AA, McKhann GM, 2nd, and Przedborski S. Spinal cord endoplasmic reticulum stress associated with a microsomal accumulation of mutant superoxide dismutase-1 in an ALS model. *Proc Natl Acad Sci USA* 103: 6025–6030, 2006.
147. Kim I, Xu W, and Reed JC. Cell death and endoplasmic reticulum stress: Disease relevance and therapeutic opportunities. *Nat Rev Drug Discov* 7: 1013–1030, 2008.
148. Kim SJ, Zhang Z, Hitomi E, Lee YC, and Mukherjee AB. Endoplasmic reticulum stress-induced caspase-4 activation mediates apoptosis and neurodegeneration in INCL. *Hum Mol Genet* 15: 1826–1834, 2006.
149. Kim YK, Kim KS, and Lee AS. Regulation of the glucose-regulated protein genes by β-mercaptoethanol requires de novo protein synthesis and correlates with inhibition of protein glycosylation. *J Cell Physiol* 133: 553–559, 1987.
150. Kimata Y, Oikawa D, Shimizu Y, Ishiwata–Kimata Y, and Kohno K. A role for BiP as an adjustor for the endoplasmic reticulum stress-sensing protein Ire1. *J Cell Biol* 167: 445–456, 2004.
151. Kiuchi K, Nishikimi M, and Yagi K. Purification and characterization of L-gulonolactone oxidase from chicken kidney microsomes. *Biochemistry* 21: 5076–5082, 1982.
152. Kivirikko KI, Myllyla R, and Pihlajaniemi T. Protein hydroxylation: Prolyl 4-hydroxylase, an enzyme with four cosubstrates and a multifunctional subunit. *FASEB J* 3: 1609–1617, 1989.
153. Klivenyi P, Siwek D, Gardian G, Yang L, Starkov A, Cleren C, Ferrante RJ, Kowall NW, Abeliovich A, and Beal MF. Mice lacking α-synuclein are resistant to mitochondrial toxins. *Neurobiol Dis* 21: 541–548, 2006.
154. Koch HG, Goebeler M, Marquardt T, Roth J, and Harms E. The redox status of aminothiols as a clue to homocysteine-induced vascular damage? *Eur J Pediatr* 157: S102–106, 1998.
155. Kohno K, Higuchi T, Ohta S, Kohno K, Kumon Y, and Sakaki S. Neuroprotective nitric oxide synthase inhibitor reduces intracellular calcium accumulation following transient global ischemia in the gerbil. *Neurosci Lett* 224: 17–20, 1997.
156. Kondo S, Murakami T, Tatsumi K, Ogata M, Kanemoto S, Otori K, Iseki K, Wanaka A, and Imaizumi K. OASIS, a CREB/ATF-family member, modulates UPR signalling in astrocytes. *Nat Cell Biol* 7: 186–194, 2005.
157. Kondo S, Saito A, Hino S, Murakami T, Ogata M, Kanemoto S, Nara S, Yamashita A, Yoshinaga K, Hara H, and

Imaizumi K. BBF2H7, a novel transmembrane bZIP transcription factor, is a new type of endoplasmic reticulum stress transducer. *Mol Cell Biol* 27: 1716–1729, 2007.
158. Koumenis C and Wouters BG. "Translating" tumor hypoxia: Unfolded protein response (UPR)-dependent and UPR-independent pathways. *Mol Cancer Res* 4: 423–436, 2006.
159. Kumar R, Azam S, Sullivan JM, Owen C, Cavener DR, Zhang P, Ron D, Harding HP, Chen JJ, Han A, White BC, Krause GS, and DeGracia DJ. Brain ischemia and reperfusion activates the eukaryotic initiation factor 2α kinase, PERK. *J Neurochem* 77: 1418–1421, 2001.
160. Kuwabara K, Matsumoto M, Ikeda J, Hori O, Ogawa S, Maeda Y, Kitagawa K, Imuta N, Kinoshita T, Stern DM, Yanagi H, and Kamada T. Purification and characterization of a novel stress protein, the 150-kDa oxygen-regulated protein (ORP150), from cultured rat astrocytes and its expression in ischemic mouse brain. *J Biol Chem* 271: 5025–5032, 1996.
161. Lamb HK, Mee C, Xu W, Liu L, Blond S, Cooper A, Charles IG, and Hawkins AR. The affinity of a major Ca^{2+} binding site on GRP78 is differentially enhanced by ADP and ATP. *J Biol Chem* 281: 8796–8805, 2006.
162. Lamkanfi M, Festjens N, Declercq W, Vanden Berghe T, and Vandenabeele P. Caspases in cell survival, proliferation and differentiation. *Cell Death Differ* 14: 44–55, 2007.
163. Larade K, Jiang Z, Zhang Y, Wang W, Bonner-Weir S, Zhu H, and Bunn HF. Loss of Ncb5or results in impaired fatty acid desaturation, lipoatrophy, and diabetes. *J Biol Chem* 283: 29285–29291, 2008.
164. Larade K, Jiang ZG, Dejam A, Zhu H, and Bunn HF. The reductase NCB5OR is responsive to the redox status in β-cells and is not involved in the ER stress response. *Biochem J* 404: 467–476, 2007.
165. Lavery GG, Walker EA, Draper N, Jeyasuria P, Marcos J, Shackleton CH, Parker KL, White PC, and Stewart PM. Hexose-6-phosphate dehydrogenase knock-out mice lack 11β-hydroxysteroid dehydrogenase type 1-mediated glucocorticoid generation. *J Biol Chem* 281: 6546–6551, 2006.
166. Lavery GG, Walker EA, Turan N, Rogoff D, Ryder JW, Shelton JM, Richardson JA, Falciani F, White PC, Stewart PM, Parker KL, and McMillan DR. Deletion of hexose-6-phosphate dehydrogenase activates the unfolded protein response pathway and induces skeletal myopathy. *J Biol Chem* 283: 8453–8461, 2008.
167. Lavoie C and Paiement J. Topology of molecular machines of the endoplasmic reticulum: A compilation of proteomics and cytological data. *Histochem Cell Biol* 129: 117–128, 2008.
168. Laybutt DR, Preston AM, Akerfeldt MC, Kench JG, Busch AK, Biankin AV, and Biden TJ. Endoplasmic reticulum stress contributes to β cell apoptosis in type 2 diabetes. *Diabetologia* 50: 752–763, 2007.
169. Lee AS. GRP78 induction in cancer: Therapeutic and prognostic implications. *Cancer Res* 67: 3496–3499, 2007.
170. Lentz SR. Mechanisms of homocysteine-induced atherothrombosis. *J Thromb Haemost* 3: 1646–1654, 2005.
171. Leuzzi R, Banhegyi G, Kardon T, Marcolongo P, Capecchi PL, Burger HJ, Benedetti A, and Fulceri R. 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, 2003.
172. Li Y and Camacho P. Ca^{2+}-dependent redox modulation of SERCA 2b by ERp57. *J Cell Biol* 164: 35–46, 2004.
173. Li Y, Schwabe RF, DeVries–Seimon T, Yao PM, Gerbod–Giannone MC, Tall AR, Davis RJ, Flavell R, Brenner DA, and Tabas I. Free cholesterol-loaded macrophages are an abundant source of tumor necrosis factor-α and interleukin-6: Model of NF-κB- and map kinase-dependent inflammation in advanced atherosclerosis. *J Biol Chem* 280: 21763–21772, 2005.
174. Li Z and Srivastava PK. Tumor rejection antigen gp96/grp94 is an ATPase: implications for protein folding and antigen presentation. *EMBO J* 12: 3143–3151, 1993.
175. Lin JH, Li H, Zhang Y, Ron D, and Walter P. Divergent effects of PERK and IRE1 signaling on cell viability. *PLoS ONE* 4: e4170. 2009.
176. Lin MT and Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* 443: 787–795, 2006.
177. Lindholm D, Wootz H, and Korhonen L. ER stress and neurodegenerative diseases. *Cell Death Differ* 13: 385–392, 2006.
178. Linster CL and Van Schaftingen E. Vitamin C. Biosynthesis, recycling and degradation in mammals. *FEBS J* 274: 1–22, 2007.
179. Lisbona F, Rojas–Rivera D, Thielen P, Zamorano S, Todd D, Martinon F, Glavic A, Kress C, Lin JH, Walter P, Reed JC, Glimcher LH, and Hetz C. BAX inhibitor-1 is a negative regulator of the ER stress sensor IRE1α. *Mol Cell* 33: 679–691, 2009.
180. Little E, Ramakrishnan M, Roy B, Gazit G, and Lee AS. The glucose-regulated proteins (GRP78 and GRP94): Functions, gene regulation, and applications. *Crit Rev Eukaryot Gene Expr* 4: 1–18, 1994.
181. Liu D, Bao F, Wen J, and Liu J. Mutation of superoxide dismutase elevates reactive species: Comparison of nitration and oxidation of proteins in different brain regions of transgenic mice with amyotrophic lateral sclerosis. *Neuroscience* 146: 255–264, 2007.
182. Liu Q, Berchner–Pfannschmidt U, Moller U, Brecht M, Wotzlaw C, Acker H, Jungermann K, and Kietzmann T. A Fenton reaction at the endoplasmic reticulum is involved in the redox control of hypoxia-inducible gene expression. *Proc Natl Acad Sci USA* 101: 4302–4307, 2004.
183. Lizak B, Czegle I, Csala M, Benedetti A, Mandl J, and Banhegyi G. Translocon pores in the endoplasmic reticulum are permeable to small anions. *Am J Physiol Cell Physiol* 291: C511–517, 2006.
184. Lizak B, Csala M, Benedetti A, and Banhegyi G. The translocon and the non-specific transport of small molecules in the endoplasmic reticulum. *Mol Membr Biol* 25: 95–101, 2008.
185. Lundstrom–Ljung J, Birnbach U, Rupp K, Soling HD, and Holmgren A. Two resident ER-proteins, CaBP1 and CaBP2, with thioredoxin domains, are substrates for thioredoxin reductase: Comparison with protein disulfide isomerase. *FEBS Lett* 357: 305–308, 1995.
186. Luu–The V. Analysis and characteristics of multiple types of human 17β-hydroxysteroid dehydrogenase. *J Steroid Biochem Mol Biol* 76: 143–151, 2001.
187. Ma Y, Brewer JW, Diehl JA, and Hendershot LM. Two distinct stress signaling pathways converge upon the CHOP promoter during the mammalian unfolded protein response. *J Mol Biol* 318: 1351–1365, 2002.
188. Magyar JE, Gamberucci A, Konta L, Margittai E, Mandl J, Banhegyi G, Benedetti A, and Csala M. Endoplasmic reticulum stress underlying the pro-apoptotic effect of epigallocatechin gallate in mouse hepatoma cells. *Int J Biochem Cell Biol* 41: 694–700, 2009.

189. Malhotra JD and Kaufman RJ. Endoplasmic reticulum stress and oxidative stress: A vicious cycle or a double-edged sword? *Antioxid Redox Signal* 9: 2277–2293, 2007.
190. Manthey KC, Chew YC, and Zempleni J. Riboflavin deficiency impairs oxidative folding and secretion of apolipoprotein B-100 in HepG2 cells, triggering stress response systems. *J Nutr* 135: 978–982, 2005.
191. Marchetti P, Bugliani M, Lupi R, Marselli L, Masini M, Boggi U, Filipponi F, Weir GC, Eizirik DL, and Cnop M. The endoplasmic reticulum in pancreatic β cells of type 2 diabetes patients. *Diabetologia* 50: 2486–2494, 2007.
192. Marciniak SJ and Ron D. Endoplasmic reticulum stress signaling in disease. *Physiol Rev* 86: 1133–1149, 2006.
193. Marciniak SJ, Yun CY, Oyadomari S, Novoa I, Zhang Y, Jungreis R, Nagata K, Harding HP, and Ron D. CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev* 18: 3066–3077, 2004.
194. Marcolongo P, Piccirella S, Senesi S, Wunderlich L, Gerin I, Mandl J, Fulceri R, Banhegyi G, and Benedetti A. The glucose-6-phosphate transporter–hexose-6-phosphate dehydrogenase–11β-hydroxysteroid dehydrogenase type 1 system of the adipose tissue. *Endocrinology* 148: 2487–2495, 2007.
195. Marcolongo P, Senesi S, Gava B, Fulceri R, Sorrentino V, Margittai E, Lizak B, Csala M, Banhegyi G, and Benedetti A. Metyrapone prevents cortisone-induced preadipocyte differentiation by depleting luminal NADPH of the endoplasmic reticulum. *Biochem Pharmacol* 76: 382–390, 2008.
196. Margittai E and Banhegyi G. Isocitrate dehydrogenase: A NADPH-generating enzyme in the lumen of the endoplasmic reticulum. *Arch Biochem Biophys* 471: 184–190, 2008.
197. Margittai E, Banhegyi G, Kiss A, Nagy G, Mandl J, Schaff Z, and Csala M. Scurvy leads to endoplasmic reticulum stress and apoptosis in the liver of Guinea pigs. *J Nutr* 135: 2530–2534, 2005.
198. Margittai E, Low P, Szarka A, Csala M, Benedetti A, and Banhegyi G. Intraluminal hydrogen peroxide induces a permeability change of the endoplasmic reticulum membrane. *FEBS Lett* 582: 4131–4136, 2008.
199. Mariappan M, Radhakrishnan K, Dierks T, Schmidt B, and von Figura K. ERp44 mediates a thiol-independent retention of formylglycine-generating enzyme in the endoplasmic reticulum. *J Biol Chem* 283: 6375–6383, 2008.
200. Marinho HS, Antunes F, and Pinto RE. Role of glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase in the reduction of lysophospholipid hydroperoxides. *Free Radic Biol Med* 22: 871–883, 1997.
201. Martinet W, Schrijvers DM, Timmermans JP, and Bult H. Interactions between cell death induced by statins and 7-ketocholesterol in rabbit aorta smooth muscle cells. *Br J Pharmacol* 154: 1236–1246, 2008.
202. Mason PJ, Stevens D, Diez A, Knight SW, Scopes DA, and Vulliamy TJ. Human hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase) encoded at 1p36: Coding sequence and expression. *Blood Cells Mol Dis* 25: 30–37, 1999.
203. Matsumoto A, Okado A, Fujii T, Fujii J, Egashira M, Niikawa N, and Taniguchi N. Cloning of the peroxiredoxin gene family in rats and characterization of the fourth member. *FEBS Lett* 443: 246–250, 1999.
204. Matus S, Lisbona F, Torres M, Leon C, Thielen P, and Hetz C. The stress rheostat: An interplay between the unfolded protein response (UPR) and autophagy in neurodegeneration. *Curr Mol Med* 8: 157–172, 2008.
205. McCullough KD, Martindale JL, Klotz LO, Aw TY, and Holbrook NJ. Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state. *Mol Cell Biol* 21: 1249–1259, 2001.
206. Meissner G. NADH, a new player in the cardiac ryanodine receptor? *Circ Res* 94: 418–419, 2004.
207. Merksamer PI, Trusina A, and Papa FR. Real-time redox measurements during endoplasmic reticulum stress reveal interlinked protein folding functions. *Cell* 135: 933–947, 2008.
208. Mhaille AN, McQuaid S, Windebank A, Cunnea P, McMahon J, Samali A, and FitzGerald U. Increased expression of endoplasmic reticulum stress-related signaling pathway molecules in multiple sclerosis lesions. *J Neuropathol Exp Neurol* 67: 200–211, 2008.
209. Michalak M, Groenendyk J, Szabo E, Gold LI, and Opas M. Calreticulin, a multi-process calcium-buffering chaperone of the endoplasmic reticulum. *Biochem J* 417: 651–666, 2009.
210. Mikoshiba K. The IP3 receptor/Ca^{2+} channel and its cellular function. *Biochem Soc Symp* 9–22, 2007.
211. Mikoshiba K. IP3 receptor/Ca^{2+} channel: From discovery to new signaling concepts. *J Neurochem* 102: 1426–1446, 2007.
212. Miller WL. Minireview: Regulation of steroidogenesis by electron transfer. *Endocrinology* 146: 2544–2550, 2005.
213. Mintz M, Vanderver A, Brown KJ, Lin J, Wang Z, Kaneski C, Schiffmann R, Nagaraju K, Hoffman EP, and Hathout Y. Time series proteome profiling to study endoplasmic reticulum stress response. *J Proteome Res* 7: 2435–2444, 2008.
214. Mizutani A, Sanuki R, Kakimoto K, Kojo S, and Taketani S. Involvement of 101F6, a homologue of cytochrome b561, in the reduction of ferric ions. *J Biochem* 142: 699–705, 2007.
215. Molinari M and Sitia R. The secretory capacity of a cell depends on the efficiency of endoplasmic reticulum-associated degradation. *Curr Top Microbiol Immunol* 300: 1-15, 2005.
216. Molteni SN, Fassio A, Ciriolo MR, Filomeni G, Pasqualetto E, Fagioli C, and Sitia R. Glutathione limits Ero1-dependent oxidation in the endoplasmic reticulum. *J Biol Chem* 279: 32667–32673, 2004.
217. Momoi T. Caspases involved in ER stress-mediated cell death. *J Chem Neuroanat* 28: 101–105, 2004.
218. Monetti M, Levin MC, Watt MJ, Sajan MP, Marmor S, Hubbard BK, Stevens RD, Bain JR, Newgard CB, Farese RV Sr, Hevener AL, and Farese RV Jr. Dissociation of hepatic steatosis and insulin resistance in mice overexpressing DGAT in the liver. *Cell Metab* 6: 69–78, 2007.
219. Morishima N, Nakanishi K, Takenouchi H, Shibata T, and Yasuhiko Y. An endoplasmic reticulum stress-specific caspase cascade in apoptosis. Cytochrome c-independent activation of caspase-9 by caspase-12. *J Biol Chem* 277: 34287–34294, 2002.
220. Morton NM and Seckl JR. 11β-Hydroxysteroid dehydrogenase type 1 and obesity. *Front Horm Res* 36: 146–164, 2008.
221. Mouw G, Zechel JL, Gamboa J, Lust WD, Selman WR, and Ratcheson RA. Activation of caspase-12, an endoplasmic reticulum resident caspase, after permanent focal ischemia in rat. *Neuroreport* 14: 183–186, 2003.
222. Nadanaka S, Okada T, Yoshida H, and Mori K. Role of disulfide bridges formed in the luminal domain of ATF6 in sensing endoplasmic reticulum stress. *Mol Cell Biol* 27: 1027–1043, 2007.
223. Nadanaka S, Yoshida H, and Mori K. Reduction of disulfide bridges in the lumenal domain of ATF6 in response to glucose starvation. *Cell Struct Funct* 31: 127–134, 2006.

224. Nagy G, Kardon T, Wunderlich L, Szarka A, Kiss A, Schaff Z, Banhegyi G, and Mandl J. Acetaminophen induces ER dependent signaling in mouse liver. *Arch Biochem Biophys* 459: 273–279, 2007.
225. Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, and Yuan J. Caspase-12 mediates endoplasmic reticulum-specific apoptosis and cytotoxicity by amyloid-β. *Nature* 403: 98–103, 2000.
226. Nakamura T and Lipton SA. Emerging roles of S-nitrosylation in protein misfolding and neurodegenerative diseases. *Antioxid Redox Signal* 10: 87–101, 2008.
227. Nardai G, Braun L, Csala M, Mile V, Csermely P, Benedetti A, Mandl J, and Banhegyi G. 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, 2001.
228. Ni M and Lee AS. ER chaperones in mammalian development and human diseases. *FEBS Lett* 581: 3641–3651, 2007.
229. Nishikimi M, Noguchi E, and Yagi K. Occurrence in yeast of L-galactonolactone oxidase which is similar to a key enzyme for ascorbic acid biosynthesis in animals, L-gulonolactone oxidase. *Arch Biochem Biophys* 191: 479–486, 1978.
230. Nishikimi M and Yagi K. Molecular basis for the deficiency in humans of gulonolactone oxidase, a key enzyme for ascorbic acid biosynthesis. *Am J Clin Nutr* 54: 1203S–1208S, 1991.
231. Nishitoh H, Kadowaki H, Nagai A, Maruyama T, Yokota T, Fukutomi H, Noguchi T, Matsuzawa A, Takeda K, and Ichijo H. ALS-linked mutant SOD1 induces ER stress- and ASK1-dependent motor neuron death by targeting Derlin-1. *Genes Dev* 22: 1451–1464, 2008.
232. Nishitoh H, Matsuzawa A, Tobiume K, Saegusa K, Takeda K, Inoue K, Hori S, Kakizuka A, and Ichijo H. ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. *Genes Dev* 16: 1345–1355, 2002.
233. Nowak TS, Jr., Fried RL, Lust WD, and Passonneau JV. Changes in brain energy metabolism and protein synthesis following transient bilateral ischemia in the gerbil. *J Neurochem* 44: 487–494, 1985.
234. Odermatt A, Atanasov AG, Balazs Z, Schweizer RA, Nashev LG, Schuster D, and Langer T. 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, 2006.
235. Ohsaki Y, Cheng J, Suzuki M, Fujita A, and Fujimoto T. Lipid droplets are arrested in the ER membrane by tight binding of lipidated apolipoprotein B-100. *J Cell Sci* 121: 2415–2422, 2008.
236. Ohsaki Y, Cheng J, Suzuki M, Shinohara Y, Fujita A, and Fujimoto T. Biogenesis of cytoplasmic lipid droplets: From the lipid ester globule in the membrane to the visible structure. *Biochim Biophys Acta* 1791: 399–407, 2009.
237. Oida Y, Izuta H, Oyagi A, Shimazawa M, Kudo T, Imaizumi K, and Hara H. Induction of BiP, an ER-resident protein, prevents the neuronal death induced by transient forebrain ischemia in gerbil. *Brain Res* 1208: 217–224, 2008.
238. Otsu M, Bertoli G, Fagioli C, Guerini–Rocco E, Nerini–Molteni S, Ruffato E, and Sitia R. Dynamic retention of Ero1α and Ero1β in the endoplasmic reticulum by interactions with PDI and ERp44. *Antioxid Redox Signal* 8: 274–282, 2006.
239. Oyadomari S and Mori M. Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell Death Differ* 11: 381–389, 2004.
240. Oyadomari S, Harding HP, Zhang Y, Oyadomari M, and Ron D. Dephosphorylation of translation initiation factor 2α enhances glucose tolerance and attenuates hepatosteatosis in mice. *Cell Metab* 7: 520–532, 2008.
241. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, and Hotamisligil GS. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 306: 457–461, 2004.
242. Ozols J. Isolation and the complete amino acid sequence of lumenal endoplasmic reticulum glucose-6-phosphate dehydrogenase. *Proc Natl Acad Sci USA* 90: 5302–5306, 1993.
243. Ozols J. Lumenal orientation and post-translational modifications of the liver microsomal 11β-hydroxysteroid dehydrogenase. *J Biol Chem* 270: 10360, 1995.
244. Pahl HL and Baeuerle PA. Activation of NF-κB by ER stress requires both Ca^{2+} and reactive oxygen intermediates as messengers. *FEBS Lett* 392: 129–136, 1996.
245. Papp E, Nardai G, Soti C, and Csermely P. Molecular chaperones, stress proteins and redox homeostasis. *Biofactors* 17: 249–257, 2003.
246. Papp E, Szaraz P, Korcsmaros T, and Csermely P. Changes of endoplasmic reticulum chaperone complexes, redox state, and impaired protein disulfide reductase activity in misfolding α1-antitrypsin transgenic mice. *FASEB J* 20: 1018–1020, 2006.
247. Paschen W, Gissel C, Linden T, Althausen S, and Doutheil J. Activation of gadd153 expression through transient cerebral ischemia: Evidence that ischemia causes endoplasmic reticulum dysfunction. *Brain Res Mol Brain Res* 60: 115–122, 1998.
248. Paschen W and Mengesdorf T. Endoplasmic reticulum stress response and neurodegeneration. *Cell Calcium* 38: 409–415, 2005.
249. Perry TL, Godin DV, and Hansen S. Parkinson's disease: A disorder due to nigral glutathione deficiency? *Neurosci Lett* 33: 305–310, 1982.
250. Petrelli MD, Lim–Tio SS, Condon J, Hewison M, and Stewart PM. Differential expression of nuclear 11β-hydroxysteroid dehydrogenase type 2 in mineralocorticoid receptor positive and negative tissues. *Endocrinology* 138: 3077–3080, 1997.
251. Piccirella S, Czegle I, Lizak B, Margittai E, Senesi S, Papp E, Csala M, Fulceri R, Csermely P, Mandl J, Benedetti A, and Banhegyi G. Uncoupled redox systems in the lumen of the endoplasmic reticulum. Pyridine nucleotides stay reduced in an oxidative environment. *J Biol Chem* 281: 4671–4677, 2006.
252. Pirot P, Ortis F, Cnop M, Ma Y, Hendershot LM, Eizirik DL, and Cardozo AK. Transcriptional regulation of the endoplasmic reticulum stress gene chop in pancreatic insulin-producing cells. *Diabetes* 56: 1069–1077, 2007.
253. Pollak N, Dolle C, and Ziegler M. The power to reduce: Pyridine nucleotides–small molecules with a multitude of functions. *Biochem J* 402: 205–218, 2007.
254. Pollard MG, Travers KJ, and Weissman JS. Ero1p: A novel and ubiquitous protein with an essential role in oxidative protein folding in the endoplasmic reticulum. *Mol Cell* 1: 171–182, 1998.
255. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papape-

tropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, and Nussbaum RL. Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science* 276: 2045–2047, 1997.
256. Prentki M and Nolan CJ. Islet β cell failure in type 2 diabetes. *J Clin Invest* 116: 1802–1812, 2006.
257. Puskas F, Braun L, Csala M, Kardon T, Marcolongo P, Benedetti A, Mandl J, and Banhegyi G. Gulonolactone oxidase activity-dependent intravesicular glutathione oxidation in rat liver microsomes. *FEBS Lett* 430: 293–296, 1998.
258. Quan EM, Kamiya Y, Kamiya D, Denic V, Weibezahn J, Kato K, and Weissman JS. Defining the glycan destruction signal for endoplasmic reticulum-associated degradation. *Mol Cell* 32: 870–877, 2008.
259. Radominska–Pandya A, Czernik PJ, Little JM, Battaglia E, and Mackenzie PI. Structural and functional studies of UDP-glucuronosyltransferases. *Drug Metab Rev* 31: 817–899, 1999.
260. Randle PJ. Regulatory interactions between lipids and carbohydrates: The glucose fatty acid cycle after 35 years. *Diabetes Metab Rev* 14: 263–283, 1998.
261. Randle PJ, Garland PB, Hales CN, and Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1: 785–789, 1963.
262. Rao RV and Bredesen DE. Misfolded proteins, endoplasmic reticulum stress and neurodegeneration. *Curr Opin Cell Biol* 16: 653–662, 2004.
263. Revesz K, Tutto A, Margittai E, Banhegyi G, Magyar JE, Mandl J, and Csala M. Glucuronide transport across the endoplasmic reticulum membrane is inhibited by epigallocatechin gallate and other green tea polyphenols. *Int J Biochem Cell Biol* 39: 922–930, 2007.
264. Rhee SG, Chae HZ, and Kim K. Peroxiredoxins: A historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radic Biol Med* 38: 1543–1552, 2005.
265. Roberts GG, Di Loreto MJ, Marshall M, Wang J, and DeGracia DJ. Hippocampal cellular stress responses after global brain ischemia and reperfusion. *Antioxid Redox Signal* 9: 2265–2275, 2007.
266. Robertson RP, Harmon J, Tran PO, and Poitout V. β-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes* 53 Suppl 1: S119–124, 2004.
267. Romero–Ramirez L, Cao H, Nelson D, Hammond E, Lee AH, Yoshida H, Mori K, Glimcher LH, Denko NC, Giaccia AJ, Le QT, and Koong AC. XBP1 is essential for survival under hypoxic conditions and is required for tumor growth. *Cancer Res* 64: 5943–5947, 2004.
268. Ron D and Habener JF. CHOP, a novel developmentally regulated nuclear protein that dimerizes with transcription factors C/EBP and LAP and functions as a dominant-negative inhibitor of gene transcription. *Genes Dev* 6: 439–453, 1992.
269. Ron D and Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 8: 519–529, 2007.
270. Ruddock LW and Molinari M. N-glycan processing in ER quality control. *J Cell Sci* 119: 4373–4380, 2006.
271. Ruiz–Vela A, Opferman JT, Cheng EH, and Korsmeyer SJ. Proapoptotic BAX and BAK control multiple initiator caspases. *EMBO Rep* 6: 379–385, 2005.
272. Ryu EJ, Harding HP, Angelastro JM, Vitolo OV, Ron D, and Greene LA. Endoplasmic reticulum stress and the unfolded protein response in cellular models of Parkinson's disease. *J Neurosci* 22: 10690–10698, 2002.
273. Saaranen MJ, Karala AR, Lappi AK, and Ruddock LW. The role of dehydroascorbate in disulfide bond formation. *Antioxid Redox Signal* 12: 15–25, 2010.
274. Scheuner D and Kaufman RJ. The unfolded protein response: A pathway that links insulin demand with β-cell failure and diabetes. *Endocr Rev* 29: 317–333, 2008.
275. Schrag JD, Procopio DO, Cygler M, Thomas DY, and Bergeron JJ. Lectin control of protein folding and sorting in the secretory pathway. *Trends Biochem Sci* 28: 49–57, 2003.
276. Schroder M. Endoplasmic reticulum stress responses. *Cell Mol Life Sci* 65: 862–894, 2008.
277. Schroder M and Kaufman RJ. ER stress and the unfolded protein response. *Mutat Res* 569: 29–63, 2005.
278. Schwarzlander M, Fricker MD, Muller C, Marty L, Brach T, Novak J, Sweetlove LJ, Hell R, and Meyer AJ. Confocal imaging of glutathione redox potential in living plant cells. *J Microsc* 231: 299–316, 2008.
279. Schweizer RA, Zurcher M, Balazs Z, Dick B, and Odermatt A. Rapid hepatic metabolism of 7-ketocholesterol by 11β-hydroxysteroid dehydrogenase type 1: Species-specific differences between the rat, human, and hamster enzyme. *J Biol Chem* 279: 18415–18424, 2004.
280. Sciandra JJ, Subjeck JR, and Hughes CS. Induction of glucose-regulated proteins during anaerobic exposure and of heat-shock proteins after reoxygenation. *Proc Natl Acad Sci USA* 81: 4843–4847, 1984.
281. Seckl JR. 11β-hydroxysteroid dehydrogenases: Changing glucocorticoid action. *Curr Opin Pharmacol* 4: 597–602, 2004.
282. Seckl JR and Walker BR. 11β-hydroxysteroid dehydrogenase type 1 as a modulator of glucocorticoid action: From metabolism to memory. *Trends Endocrinol Metab* 15: 418–424, 2004.
283. Sekar N, Qian S, and Shechter Y. Vanadate elevates lipogenicity of starved rat adipose tissue: Mechanism of action. *Endocrinology* 139: 2514–2518, 1998.
284. Semenza GL. HIF-1: Mediator of physiological and pathophysiological responses to hypoxia. *J Appl Physiol* 88: 1474–1480, 2000.
285. Semenza GL. Targeting HIF-1 for cancer therapy. *Nat Rev Cancer* 3: 721–732, 2003.
286. Senesi S, Marcolongo P, Manini I, Fulceri R, Sorrentino V, Csala M, Banhegyi G, and Benedetti A. Constant expression of hexose-6-phosphate dehydrogenase during differentiation of human adipose-derived mesenchymal stem cells. *J Mol Endocrinol* 41: 125–133, 2008.
287. Sevier CS, Qu H, Heldman N, Gross E, Fass D, and Kaiser CA. Modulation of cellular disulfide-bond formation and the ER redox environment by feedback regulation of Ero1. *Cell* 129: 333–344, 2007.
288. Sharma MK and Buettner GR. Interaction of vitamin C and vitamin E during free radical stress in plasma: An ESR study. *Free Radic Biol Med* 14: 649–653, 1993.
289. Sharma NK, Das SK, Mondal AK, Hackney OG, Chu WS, Kern PA, Rasouli N, Spencer HJ, Yao–Borengasser A, and Elbein SC. Endoplasmic reticulum stress markers are associated with obesity in nondiabetic subjects. *J Clin Endocrinol Metab* 93: 4532–4541, 2008.
290. Shastry BS. Neurodegenerative disorders of protein aggregation. *Neurochem Int* 43: 1–7, 2003.
291. Shen J, Snapp EL, Lippincott-Schwartz J, and Prywes R. Stable binding of ATF6 to BiP in the endoplasmic reticulum stress response. *Mol Cell Biol* 25: 921–932, 2005.

292. Shintani–Ishida K, Nakajima M, Uemura K, and Yoshida K. Ischemic preconditioning protects cardiomyocytes against ischemic injury by inducing GRP78. *Biochem Biophys Res Commun* 345: 1600–1605, 2006.
293. Shuda M, Kondoh N, Imazeki N, Tanaka K, Okada T, Mori K, Hada A, Arai M, Wakatsuki T, Matsubara O, Yamamoto N, and Yamamoto M. Activation of the ATF6, XBP1 and grp78 genes in human hepatocellular carcinoma: A possible involvement of the ER stress pathway in hepatocarcinogenesis. *J Hepatol* 38: 605–614, 2003.
294. Sitia R and Molteni SN. Stress, protein (mis)folding, and signaling: The redox connection. *Sci STKE* 2004: pe27, 2004.
295. Suzuki H, Kanekura K, Levine TP, Kohno K, Olkkonen VM, Aiso S, and Matsuoka M. ALS-linked P56S-VAPB, an aggregated loss-of-function mutant of VAPB, predisposes motor neurons to ER stress-related death by inducing aggregation of co-expressed wild-type VAPB. *J Neurochem* 108: 973–985, 2009.
296. Szarka A, Stadler K, Jenei V, Margittai E, Csala M, Jakus J, Mandl J, and Banhegyi G. Ascorbyl free radical and dehydroascorbate formation in rat liver endoplasmic reticulum. *J Bioenerg Biomembr* 34: 317–323, 2002.
297. Szegezdi E, Logue SE, Gorman AM, and Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Rep* 7: 880–885, 2006.
298. Tajiri S, Oyadomari S, Yano S, Morioka M, Gotoh T, Hamada JI, Ushio Y, and Mori M. Ischemia-induced neuronal cell death is mediated by the endoplasmic reticulum stress pathway involving CHOP. *Cell Death Differ* 11: 403–415, 2004.
299. Takahashi T and Hori SH. Intramembraneous localization of rat liver microsomal hexose-6-phosphate dehydrogenase and membrane permeability to its substrates. *Biochim Biophys Acta* 524: 262–276, 1978.
300. Tamatani M, Matsuyama T, Yamaguchi A, Mitsuda N, Tsukamoto Y, Taniguchi M, Che YH, Ozawa K, Hori O, Nishimura H, Yamashita A, Okabe M, Yanagi H, Stern DM, Ogawa S, and Tohyama M. ORP150 protects against hypoxia/ischemia-induced neuronal death. *Nat Med* 7: 317–323, 2001.
301. Tan Y, Dourdin N, Wu C, De Veyra T, Elce JS, and Greer PA. Ubiquitous calpains promote caspase-12 and JNK activation during endoplasmic reticulum stress-induced apoptosis. *J Biol Chem* 281: 16016–16024, 2006.
302. Tanaka S, Uehara T, and Nomura Y. Up-regulation of protein-disulfide isomerase in response to hypoxia/brain ischemia and its protective effect against apoptotic cell death. *J Biol Chem* 275: 10388–10393, 2000.
303. Terro F, Czech C, Esclaire F, Elyaman W, Yardin C, Baclet MC, Touchet N, Tremp G, Pradier L, and Hugon J. Neurons overexpressing mutant presenilin-1 are more sensitive to apoptosis induced by endoplasmic reticulum–Golgi stress. *J Neurosci Res* 69: 530–539, 2002.
304. Thuerauf DJ, Marcinko M, Gude N, Rubio M, Sussman MA, and Glembotski CC. Activation of the unfolded protein response in infarcted mouse heart and hypoxic cultured cardiac myocytes. *Circ Res* 99: 275–282, 2006.
305. Tobisawa S, Hozumi Y, Arawaka S, Koyama S, Wada M, Nagai M, Aoki M, Itoyama Y, Goto K, and Kato T. Mutant SOD1 linked to familial amyotrophic lateral sclerosis, but not wild-type SOD1, induces ER stress in COS7 cells and transgenic mice. *Biochem Biophys Res Commun* 303: 496–503, 2003.
306. Todd DJ, Lee AH, and Glimcher LH. The endoplasmic reticulum stress response in immunity and autoimmunity. *Nat Rev Immunol* 8: 663–674, 2008.
307. Tomlinson JW. 11β-Hydroxysteroid dehydrogenase type 1 in human disease: A novel therapeutic target. *Minerva Endocrinol* 30: 37–46, 2005.
308. Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Lavery GG, Cooper MS, Hewison M, and Stewart PM. 11β-Hydroxysteroid dehydrogenase type 1: A tissue-specific regulator of glucocorticoid response. *Endocr Rev* 25: 831–866, 2004.
309. Tu BP, Ho–Schleyer SC, Travers KJ, and Weissman JS. Biochemical basis of oxidative protein folding in the endoplasmic reticulum. *Science* 290: 1571–1574, 2000.
310. Tu BP and Weissman JS. The FAD- and O_2-dependent reaction cycle of Ero1-mediated oxidative protein folding in the endoplasmic reticulum. *Mol Cell* 10: 983–994, 2002.
311. Tu BP and Weissman JS. Oxidative protein folding in eukaryotes: Mechanisms and consequences. *J Cell Biol* 164: 341–346, 2004.
312. Uehara T. Accumulation of misfolded protein through nitrosative stress linked to neurodegenerative disorders. *Antioxid Redox Signal* 9: 597–601, 2007.
313. Uehara T, Nakamura T, Yao D, Shi ZQ, Gu Z, Ma Y, Masliah E, Nomura Y, and Lipton SA. S-nitrosylated protein–disulfide isomerase links protein misfolding to neurodegeneration. *Nature* 441: 513–517, 2006.
314. Unterberger U, Höftberger R, Gelpi E, Flicker H, Budka H, and Voigtländer T. Endoplasmic reticulum stress features are prominent in Alzheimer disease but not in prion diseases *in vivo*. *J Neuropathol Exp Neurol* 65: 348–357, 2006.
315. Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, and Ron D. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* 287: 664–666, 2000.
316. Ushioda R, Hoseki J, Araki K, Jansen G, Thomas DY, and Nagata K. ERdj5 is required as a disulfide reductase for degradation of misfolded proteins in the ER. *Science* 321: 569–572, 2008.
317. van Reyk DM, Brown AJ, Hult'en LM, Dean RT, and Jessup W. Oxysterols in biological systems: Sources, metabolism and pathophysiological relevance. *Redox Rep* 11: 255–262, 2006.
318. van Schaftingen E and Gerin I. The glucose-6-phosphatase system. *Biochem J* 362: 513–532, 2002.
319. Varsanyi M, Szarka A, Papp E, Makai D, Nardai G, Fulceri R, Csermely P, Mandl J, Benedetti A, and Banhegyi G. FAD transport and FAD-dependent protein thiol oxidation in rat liver microsomes. *J Biol Chem* 279: 3370–3374, 2004.
320. Veiga–da–Cunha M, Gerin I, Chen YT, de Barsy T, de Lonlay P, Dionisi–Vici C, Fenske CD, Lee PJ, Leonard JV, Maire I, McConkie–Rosell A, Schweitzer S, Vikkula M, and Van Schaftingen E. A gene on chromosome 11q23 coding for a putative glucose- 6-phosphate translocase is mutated in glycogen-storage disease types Ib and Ic. *Am J Hum Genet* 63: 976–983, 1998.
321. Vembar SS and Brodsky JL. One step at a time: Endoplasmic reticulum-associated degradation. *Nat Rev Mol Cell Biol* 9: 944–957, 2008.
322. Verkhratsky A. Physiology and pathophysiology of the calcium store in the endoplasmic reticulum of neurons. *Physiol Rev* 85: 201–279, 2005.
323. Wahlman J, DeMartino GN, Skach WR, Bulleid NJ, Brodsky JL, and Johnson AE. Real-time fluorescence detec-

tion of ERAD substrate retrotranslocation in a mammalian *in vitro* system. *Cell* 129: 943–955, 2007.
324. Wajih N, Hutson SM, and Wallin R. 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, 2007.
325. Wallin R and Martin LF. Warfarin poisoning and vitamin K antagonism in rat and human liver. Design of a system *in vitro* that mimics the situation *in vivo*. *Biochem J* 241: 389–396, 1987.
326. Wang Y, Lam KS, Yau MH, and Xu A. Post-translational modifications of adiponectin: Mechanisms and functional implications. *Biochem J* 409: 623–633, 2008.
327. Wang ZV, Schraw TD, Kim JY, Khan T, Rajala MW, Follenzi A, and Scherer PE. Secretion of the adipocyte-specific secretory protein adiponectin critically depends on thiol-mediated protein retention. *Mol Cell Biol* 27: 3716–3731, 2007.
328. Wells WW, Xu DP, Yang YF, and Rocque PA. Mammalian thioltransferase (glutaredoxin) and protein disulfide isomerase have dehydroascorbate reductase activity. *J Biol Chem* 265: 15361–15364, 1990.
329. Werner R, Manthey KC, Griffin JB, and Zempleni J. HepG2 cells develop signs of riboflavin deficiency within 4 days of culture in riboflavin-deficient medium. *J Nutr Biochem* 16: 617–624, 2005.
330. White PC, Rogoff D, McMillan DR, and Lavery GG. Hexose 6-phosphate dehydrogenase (H6PD) and corticosteroid metabolism. *Mol Cell Endocrinol* 265–266: 89–92, 2007.
331. Williams DB. Beyond lectins: The calnexin/calreticulin chaperone system of the endoplasmic reticulum. *J Cell Sci* 119: 615–623, 2006.
332. Wolf G. New insights into thiol-mediated regulation of adiponectin secretion. *Nutr Rev* 66: 642–645, 2008.
333. Wonderlin WF. Constitutive, translation-independent opening of the protein-conducting channel in the endoplasmic reticulum. *Pflugers Arch* 457: 917–930, 2009.
334. Wootz H, Hansson I, Korhonen L, Napankangas U, and Lindholm D. Caspase-12 cleavage and increased oxidative stress during motoneuron degeneration in transgenic mouse model of ALS. *Biochem Biophys Res Commun* 322: 281–286, 2004.
335. Ye J, Li JZ, Liu Y, Li X, Yang T, Ma X, Li Q, Yao Z, and Li P. Cideb, an ER- and lipid droplet-associated protein, mediates VLDL lipidation and maturation by interacting with apolipoprotein B. *Cell Metab* 9: 177–190, 2009.
336. Yoneda T, Imaizumi K, Oono K, Yui D, Gomi F, Katayama T, and Tohyama M. Activation of caspase-12, an endoplastic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. *J Biol Chem* 276: 13935–13940, 2001.
337. Yoshida H. ER stress and diseases. *FEBS J* 274: 630–658, 2007.
338. Zable AC, Favero TG, and Abramson JJ. 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, 1997.
339. Zhang C, Kawauchi J, Adachi MT, Hashimoto Y, Oshiro S, Aso T, and Kitajima S. Activation of JNK and transcriptional repressor ATF3/LRF1 through the IRE1/TRAF2 pathway is implicated in human vascular endothelial cell death by homocysteine. *Biochem Biophys Res Commun* 289: 718–724, 2001.
340. Zhang K and Kaufman RJ. From endoplasmic reticulum stress to the inflammatory response. *Nature* 454: 455–462, 2008.
341. Zhu H, Larade K, Jackson TA, Xie J, Ladoux A, Acker H, Berchner-Pfannschmidt U, Fandrey J, Cross AR, Lukat–Rodgers GS, Rodgers KR, and Bunn HF. NCB5OR is a novel soluble NAD(P)H reductase localized in the endoplasmic reticulum. *J Biol Chem* 279: 30316–30325, 2004.
342. Zong WX, Li C, Hatzivassiliou G, Lindsten T, Yu QC, Yuan J, and Thompson CB. Bax and Bak can localize to the endoplasmic reticulum to initiate apoptosis. *J Cell Biol* 162: 59–69, 2003.

Address correspondence to:
Gábor Bánhegyi
Department of Medical Chemistry, Molecular Biology and Pathobiochemistry
Semmelweis University
1444 Budapest
POB 260
Hungary

E-mail: banhegyi@unisi.it

Date of first submission to ARS Central, February 23, 2009; date of final revised submission, October 5, 2009; date of acceptance, October 30, 2009.

Abbreviations Used

11βHSD1 = 11β-hydroxysteroid dehydrogenase type 1
11βHSD2 = 11β-hydroxysteroid dehydrogenase type 2
AA = ascorbate
$^{\cdot}$AA = ascorbyl radical
ALS = amyotrophic lateral sclerosis
Ascox = ascorbate oxidase
ASK1 = apoptosis signal-regulating kinase-1
ATF4 = activating transcription factor 4
ATF6 = activating transcription factor 6
BBF2H7 = BBF2 human homolog on chromosome 7
BiP = immunoglobulin heavy chain-binding protein
bZIP = basic leucine zipper
CHOP = C/EBP homologous protein, also known as growth arrest- and DNA damage-inducible gene 153 (GADD153)
CYP450 = cytochrome P450
DAA = dehydroascorbate
EDEM = ER degradation enhancing α-mannosidase-like protein
eIF2α = eukaryotic initiation factor 2α
eNOS = endothelial nitric oxide synthase
ER = endoplasmic reticulum
ERAD = ER-associated degradation
Ero = ER oxidoreductin
F1,6bP = fructose-1,6-bisphosphate
F6P = fructose-6-phosphate
FFA = free fatty acid
G6P = glucose-6-phosphate
G6Pase = glucose-6-phosphatase
G6PT = glucose-6-phosphate transporter
GA = glucuronate

Abbreviations Used (cont.)

GDase = β-glucuronidase
GGCX = glutamate γ-carboxylase
Gla = γ-carboxyglutamate
Glc = glucose
GLO = gulonolactone oxidase
GR = glucocorticoid receptor
GSD = glycogen storage disease
GSH = glutathione
GSSG = glutathione disulfide
H6PD = hexose-6-phosphate dehydrogenase
HIF-1 = hypoxia-inducible factor 1
HK = hexokinase
InsP3 = inositol 1,4,5-trisphosphate
InsP3R1 = inositol 1,4,5-trisphosphate receptor type 1
IR = insulin receptor
IRE1 = inositol-requiring enzyme 1
IRS-1 = insulin receptor substrate-1
JNK = c-jun N-terminal kinase
LC3 = microtubule-associated protein 1 light chain 3
mSOD1 = mutant human superoxide dismutase 1
MT = mitochondrion
Ncb5or = NADPH cytochrome b_5 oxidoreductase
nNOS = neuronal nitric oxide synthase
OAA = oxaloacetate
OASIS = old astrocyte specifically induced substance
ORP150 = 150kDa oxygen-regulated protein
PDH = pyruvate dehydrogenase
PDI = protein disulfide isomerase
PERK = PKR-like ER protein kinase
PFK = phosphofructokinase
Pyr = pyruvate
QSOX = quiescin sulfhydryl oxidase
SERCA2b = sarco/endoplasmic reticulum calcium ion ATPase type 2b
SOD = superoxide dismutase
TRAF2 = tumor necrosis factor receptor-associated factor 2
UGA = UDP-glucuronate
UGT = UDP-glucuronosyl transferase
UPR = unfolded protein response
VK = vitamin K
VKE = vitamin K epoxide
VKHQ = vitamin K hydoroquinone
VKORC1 = vitamin K epoxide reductase
XBP1 = X-box binding protein 1

This article has been cited by:

1. Yuanning Cao, Zhiyong Zhao, Richard L. Eckert, E. Albert Reece. 2012. The essential role of protein kinase C# in diabetes-induced neural tube defects. *Journal of Maternal-Fetal and Neonatal Medicine* **25**:10, 2020-2024. [CrossRef]
2. L. Tomanek. 2012. Environmental Proteomics of the Mussel Mytilus: Implications for Tolerance to Stress and Change in Limits of Biogeographic Ranges in Response to Climate Change. *Integrative and Comparative Biology* . [CrossRef]
3. Arisa Higa, Eric Chevet. 2012. Redox signaling loops in the unfolded protein response. *Cellular Signalling* **24**:8, 1548-1555. [CrossRef]
4. Miklós Csala , Éva Kereszturi , József Mandl , Gábor Bánhegyi . 2012. The Endoplasmic Reticulum As the Extracellular Space Inside the Cell: Role in Protein Folding and Glycosylation. *Antioxidants & Redox Signaling* **16**:10, 1100-1108. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
5. Masatoshi Hagiwara , Kazuhiro Nagata . 2012. Redox-Dependent Protein Quality Control in the Endoplasmic Reticulum: Folding to Degradation. *Antioxidants & Redox Signaling* **16**:10, 1119-1128. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
6. Ronald E. SavageConjugation, Transport, and Elimination of Drugs in Humans . [CrossRef]
7. Marizela Delic, Corinna Rebnegger, Franziska Wanka, Verena Puxbaum, Christina Haberhauer-Troyer, Stephan Hann, Gunda Köllensperger, Diethard Mattanovich, Brigitte Gasser. 2012. Oxidative protein folding and unfolded protein response elicit differing redox regulation in endoplasmic reticulum and cytosol of yeast. *Free Radical Biology and Medicine* **52**:9, 2000-2012. [CrossRef]
8. Tyler Carlage, Rashmi Kshirsagar, Li Zang, Vijay Janakiraman, Marina Hincapie, Yelena Lyubarskaya, Andy Weiskopf, William S. Hancock. 2012. Analysis of dynamic changes in the proteome of a Bcl-XL overexpressing Chinese hamster ovary cell culture during exponential and stationary phases. *Biotechnology Progress* **28**:3, 814-823. [CrossRef]
9. Gábor Bánhegyi , Éva Margittai , András Szarka , József Mandl , Miklós Csala . 2012. Crosstalk and Barriers Between the Electron Carriers of the Endoplasmic Reticulum. *Antioxidants & Redox Signaling* **16**:8, 772-780. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
10. Taichi Kakihana , Kazuhiro Nagata , Roberto Sitia . 2012. Peroxides and Peroxidases in the Endoplasmic Reticulum: Integrating Redox Homeostasis and Oxidative Folding. *Antioxidants & Redox Signaling* **16**:8, 763-771. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
11. James J. Galligan, Rebecca L. Smathers, Colin T. Shearn, Kristofer S. Fritz, Donald S. Backos, Hua Jiang, Christopher C. Franklin, David J. Orlicky, Kenneth N. MacLean, Dennis R. Petersen. 2012. Oxidative Stress and the ER Stress Response in a Murine Model for Early-Stage Alcoholic Liver Disease. *Journal of Toxicology* **2012**, 1-12. [CrossRef]
12. Katalin Révész, Anna Tütt#, Péter Szelényi, Laura Konta. 2011. Tea flavan-3-ols as modulating factors in endoplasmic reticulum function. *Nutrition Research* **31**:10, 731-740. [CrossRef]
13. Alexander R. Lippert, Genevieve C. Van de Bittner, Christopher J. Chang. 2011. Boronate Oxidation as a Bioorthogonal Reaction Approach for Studying the Chemistry of Hydrogen Peroxide in Living Systems. *Accounts of Chemical Research* **44**:9, 793-804. [CrossRef]
14. András Szarka, Gábor Bánhegyi. 2011. Oxidative folding: recent developments. *BioMolecular Concepts* ---. [CrossRef]
15. Neil J. Bulleid, Lars Ellgaard. 2011. Multiple ways to make disulfides. *Trends in Biochemical Sciences* . [CrossRef]
16. Peter F. Davies , Mete Civelek . Endoplasmic Reticulum Stress, Redox, and a Proinflammatory Environment in Athero-Susceptible Endothelium In Vivo at Sites of Complex Hemodynamic Shear Stress. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
17. Kara L. Gordon, Kevin A. Glenn, Pedro Gonzalez-Alegre. 2011. Exploring the Influence of TorsinA Expression on Protein Quality Control. *Neurochemical Research* **36**:3, 452-459. [CrossRef]
18. Yingfeng Deng, Philipp E. Scherer. 2010. Adipokines as novel biomarkers and regulators of the metabolic syndrome. *Annals of the New York Academy of Sciences* **1212**:1, E1-E19. [CrossRef]
19. Éva Kereszturi, Fanni S. Kálmán, Tamás Kardon, Miklós Csala, Gábor Bánhegyi. 2010. Decreased prereceptorial glucocorticoid activating capacity in starvation due to an oxidative shift of pyridine nucleotides in the endoplasmic reticulum. *FEBS Letters* **584**:22, 4703-4708. [CrossRef]