The endoplasmic reticulum (ER) is central for many essential cellular activities, such as folding, assembly and quality control of secretory and membrane proteins, disulfide bond formation, glycosylation, lipid biosynthesis, Ca^{2+} storage and signaling. In addition, this multifunctional organelle integrates many adaptive and/or maladaptive signaling cues reporting on metabolism, proteostasis, Ca^{2+} and redox homeostasis. We are beginning to understand how these functions and pathways are integrated with one another to regulate homeostasis at cell, tissue and organism levels. The mechanisms underlying the introduction of the proper set of disulfide bonds into secretory proteins (oxidative folding) are strictly related to redox homeostasis, ER stress sensing and signaling and provide a good example of the integration systems operative in the early secretory compartment.

Functioning of the oxidative protein folding (and presumably some ER oxidases) generates hydrogen peroxide (H_2O_2) within the lumen. H_2O_2 being a reactive oxygen species is thought to be a dangerous byproduct; however, as a prooxidant, can be effectively reutilize in oxidative reactions such as disulfide formation. The aim of our studies was to clarify the possible effects of luminal H_2O_2 production. As specific aims, the effect of oxidative stimuli on ER Ca^{2+} fluxes and on the efficacy of oxidative folding were investigated.

To perform its multiple tasks, the ER is composed of distinct, specialized subregions, amongst which mitochondrial-associated ER membranes (MAM) emerge as key signaling hubs. How these multiple functions are integrated with one another in living cells remains unclear. We showed that Ero1α, a component of the electron transfer chain of oxidative folding and a key controller of ER redox homeostasis, is enriched in MAM and regulates Ca^{2+} fluxes. Downregulation of Ero1α by RNA interference inhibited mitochondrial Ca^{2+} fluxes and modifies the activity of mitochondrial Ca^{2+} uniporters. The overexpression of redox active Ero1α increased passive Ca^{2+} efflux from the ER, lowering $[Ca^{2+}]$(ER) and mitochondrial Ca^{2+} fluxes in response to IP3 agonists. The unexpected observation that Ca^{2+} fluxes were affected by either increasing or decreasing the levels of Ero1α reveals a pivotal role for this oxidase in the early secretory compartment and implies a strict control of its amounts. Taken together, our results indicate that the levels, subcellular localization, and activity of Ero1α coordinately regulate Ca^{2+} and redox homeostasis and signaling in the early secretory compartment (4).

We examined the role of H_2O_2 produced in the lumen, which on one hand can lead to redox imbalance and hence can contribute to ER stress caused by overproduction of secretory proteins; on the other hand, as an excellent electron acceptor, H_2O_2 might serve as an additional prooxidant in physiological oxidative folding. We used gulonolactone-treated mice

for the elevation of hepatic luminal H_2O_2 levels. Gulonolactone administration resulted in a decrease in microsomal GSH and protein-thiol contents and in a redox shift of certain luminal oxidoreductases. The oxidative wave was accompanied only by moderate signs of ER stress and reversible dilation of ER *cisternae*, all effects prevented by concomitant reducing treatment. The imbalance also affected the redox state of pyridine nucleotides in the ER. Antibody producing cells artificially engineered with powerful luminal H_2O_2 eliminating system (overexpressing an ER-targeted catalase) showed diminished secretion of mature antibody polymers, while incomplete antibody monomers/dimers were accumulated and/or secreted. The results indicate that local H_2O_2 production promotes, while quenching of H_2O_2 impairs disulfide formation. The contribution of H_2O_2 to disulfide bond formation previously observed *in vitro* can be also shown in cellular and *in vivo* systems (7).

We published three reviews on the redox conditions in the ER (1,2,5). In a Hypothesis paper (1) we proposed a new paradigm for the oxidative folding: the multiple oxidant hypothesis. Oxidative protein folding in the luminal compartment of the ER is thought to be mediated by a proteinaceous electron relay system composed by PDI and Ero1, transferring electrons from the cysteinyl residues of substrate proteins to oxygen. However, recent observations revealed that Ero1 isoforms are dispensable. ER is known as a generator and accumulator of low molecular weight oxidants; some of them have already been shown to promote oxidative folding. On the basis of these observations a new theory of oxidative folding was proposed where the oxidative power is provided by the stochastic contribution of prooxidants (1).

In a comprehensive review we summarized the present knowledge on the major redox systems in the ER (2). The lumen of the ER constitutes a separate compartment with a special proteome and metabolome. The characteristic redox environment required for the optimal functioning of local pathways is defined by the redox couples of the main electron carriers. In a third review we focused on the three most important electron carriers of the ER (5). These molecules, glutathione, pyridine nucleotides and ascorbic acid, are present within the ER, but their composition, concentration and redox state are characteristically different from those observed in other subcellular compartments. Spatial and kinetic barriers contribute to the generation and maintenance of this special redox environment. The ER redox has usually been considered from the perspective of oxidative protein folding, one of the major functions of the ER. Thus, the lumen has been described as a relatively oxidizing subcellular compartment. The ER redoxome has been scantily mapped. However, recent observations suggest that the redox systems in reduced and oxidized states are present simultaneously. The concerted actions of transmembrane uptake processes and local oxidoreductases as well as

the absence of specific transport and enzyme activities maintain the oxidized state of the thiol-disulfide systems and the reduced state of the pyridine nucleotide redox systems. These states are prerequisites for the normal redox reactions localized in the ER. An outline of the interactions between the major electron carriers of the ER will contribute to a better understanding of human diseases related to ER redox homeostasis (2,5).

We summarized the facts showing the importance of compartmentation in two other reviews (3,6). Proteins destined to secretion and exposure on the cell surface are synthesized and processed in the extracellular-like environment of the ER (or more generally in the early secretory compartment) of higher eukaryotic cells (3). Compartmentation plays a crucial role in the posttranslational modifications, such as oxidative folding and N-glycosylation in the ER lumen. Transport of the required intermediates across the ER membrane and maintenance of the luminal redox conditions and Ca^{2+} ion concentration are indispensable for appropriate protein maturation. Cooperation of enzymes and transporters to maintain a thiol-oxidizing milieu in the ER lumen has been recently elucidated. Ca^{2+} dependence of certain ER chaperones is a subject of intensive research. Mounting evidence supports the existence of a real barrier between the ER lumen and the cytosol. The unique set of enzymes, selection of metabolites and characteristic ion and redox milieu of the luminal compartment strongly argue against the general permeability of the ER membrane. Alterations in the luminal environment can trigger the unfolded protein response, a common event in a variety of pathological conditions. Therefore, redox and calcium homeostasis and protein glycosylation in the ER provide novel drug-targets for medical treatment in a wide array of diseases (3,6).

In summary, our studies revealed that intraluminal hydrogen peroxide plays an important role in the regulation of ER Ca^{2+} movements and in the catalysis of disulfide bond formation. These functions provide a connection between redox and Ca^{2+} regulation in the ER.

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