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Hypothesis

Oxidative folding in the endoplasmic reticulum: Towards a multiple oxidant hypothesis?

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ABSTRACT

Oxidative protein folding in the luminal compartment of the endoplasmic reticulum is thought to be mediated by a proteinaceous electron relay system composed by PDI and ER oxidoreductin 1 (Ero1), transferring electrons from the cysteinyl residues of substrate proteins to oxygen. However, recent observations revealed that Ero1 isoforms are dispensable. Endoplasmic reticulum 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 is proposed where the oxidative power is provided by the stochastic contribution of prooxidants.

1. Introduction

Investigation of the redox regulation of cellular processes lives its renaissance nowadays. The luminal compartment of the endoplasmic reticulum (ER) attracted great interest as a place of the integration of external and internal stimuli, which can be characterized by a peculiar oxidizing milieu [1,2]. Oxidative protein folding, a key pathway of the ER, acts as a sensor helping the cell in the adaptation to adverse environmental, metabolic or redox conditions. Thus, exploration of the mechanism of disulfide bond formation has a paramount importance in the understanding of the integrator function of the ER. The well-established classic protein-based model of the oxidative folding, however, has been challenged by recent observations.

2. Thesis

In eukaryotic cells, proteins destined to the extracellular space or into the membranes undergo specific posttranslational modifications, such as disulfide bond formation, in the luminal compartment of the ER. In order to achieve their stable, native structure, the nascent polypeptide itself contains all the sufficient information. However, disulfide linked folding is slow due to its dependence on a suitable redox environment and needs to be assisted.

Studies using the classic substrate ribonuclease A led to the identification of protein disulfide isomerase (PDI). PDI has been shown to catalyze disulfide bond formation, isomerization and reduction on a wide range of substrates in vitro [3]. Between PDI and folding intermediates covalent complexes were detected in living mammalian cells [4], and in PDI-deficient microsomes compromised disulfide bond formation was observed [5]. Genetic screens searching for the oxidative source ensures reoxidation of PDI discovered the conserved ER membrane-associated ER oxidoreductin 1p (Ero1p) protein in *Saccharomyces cerevisiae*, which was proven to be necessary for the oxidation of thiol groups [6]. Decreased Ero1p function results in thermosensitivity, increased susceptibility to the reductant dithiothreitol treatment and defective maturation and transport of the disulfide-containing substrate protein carboxypeptidase Y in yeast, whereas overexpression

Abbreviations: ER, endoplasmic reticulum; Ero1, ER oxidoreductin 1; PDI, protein disulfide isomerase; QSOX, quiescin sulfhydryl oxidase; ROS, reactive oxygen species; UPR, unfolded protein response; VKOR, vitamin K epoxide reductase

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confers reverse effects. Direct interactions between PDI and Ero1p were detected both in vivo and in vitro, indicating the presence of mixed disulfides and suggesting an electron flow from PDI to Ero1p [7,8]. Ero1p homologues present in most species define a family of genes with conserved functions. The identified human analogues – Ero1L-α and Ero1L-β – were described to be ER-resident glycoproteins, which when overexpressed in *ero1-1* yeast, compensate for thermo- and dithiothreitol-sensitivity and for defective substrate transport from ER to Golgi [9,10]. The isoforms differ from each other in tissue distribution and inducibility, being Ero1L-β a target of unfolded protein response (UPR). Both isoforms were shown to facilitate oxidative folding and to form mixed disulfides with PDI [11,12]. Eros have been identified as flavoproteins and an efficient pathway for oxidative folding of ribonuclease A as substrate protein was reconstituted in vitro in a system composed by Ero1p or Ero1-Lα plus PDI and FAD [8,13]. Molecular oxygen can be efficiently used in vitro as final electron acceptor both with Ero1p [14] and Ero1-Lα [13], suggesting that molecular oxygen is most likely to be at least one of the electron acceptors for the Ero1 family in vivo.

According to these observations, in the present model of oxidative protein folding (for recent reviews see [15–17]) electrons are carried from substrate proteins towards molecular oxygen by a proteinaceous relay system composed by PDI and Ero1 (Fig. 1, pink planes). PDI is responsible for the formation of disulfide bonds in substrate proteins by a thiol – disulfide exchange mechanism, while Ero1 isoforms transfer the electrons from PDI to oxygen, maintaining the oxidized state of PDI required for further cycles of disulfide formation.

3. Antithesis

Although several other enzymes and small molecular weight prooxidants can contribute to the oxidation of protein thiols, from the discovery of Ero1p the protein relay hypothesis has become dominant. The widely accepted deterministic model of oxidative protein folding has been challenged by the results of a recent work [18]. It was observed that mutant mouse lacking both isoforms of Ero1 is viable; a residual oxidative folding of insulin is still present, and most importantly, the oxidative folding of IgM shows only a modest delay in these mutants. These findings provide strong evidence for Ero1-independent mechanism(s) of oxidative folding.

Beside Ero1, two other groups of enzymes have been proposed as alternative pathways of oxidative folding for some time past (Fig. 1, green symbols). However, their general role is at least dubious. The enzymes of the QSOX (quiescin sulfhydryl oxidase) superfamily are flavin-containing sulfhydryl oxidases, which can catalyze disulfide bond formation [19]. However, they are localized rather to the Golgi apparatus than the ER [20]. Moreover, QSOXs are not ubiquitous; e.g. they are absent in the liver [21]. VKOR (vitamin K epoxide reductase) generates the reduced (hydroquinone) form of vitamin K, which is used by the vitamin K-dependent γ-carboxylase as a cofactor during γ-carboxylation. VKOR is working in a complex with PDI, probably connecting to the vitamin K cycle to disulfide bond formation [22]. Although the gene is expressed ubiquitously [23], γ-carboxylation is obviously a lower capacity pathway than disulfide bond formation, therefore its contribution cannot be fully accountable for the oxidative folding.

Participation of enzymes other than PDI and Ero1 should not necessarily be considered in the oxidative folding. The lumen of the ER is an oxidative environment where low molecular weight oxidants are generated and accumulated. Local oxidoreductases, membrane transporters and the relative absence of antioxidant enzymes all contribute to the generation of an oxidizing milieu [24]. It can be supposed that these seemingly disadvantageous conditions serve a common aim: the facilitation of the oxidative folding. The accumulated prooxidants can act as electron acceptors receiving electrons from substrate proteins, PDI or Ero1. From this aspect, the role of reactive oxygen species (ROS), redox-active vitamins (C, E and K) and metalloproteins has been raised (Fig. 1, orange ovals).

ROS are produced within and around the ER. The organelle is particularly rich in oxygenases and oxidases (e.g. cytochrome P450s, flavin-containing monooxygenases, prolyl and lysyl hydroxylases, NOX4), which often produce ROS as a byproduct [2]. Besides enzymatic processes, hydroxyl radical generation by an iron-dependent Fenton reaction has also been reported in the ER [24]. FAD-containing oxidases including Ero1 family members generate one molecule of hydrogen peroxide per disulfide bond formed [14]. Although H_2O_2 is usually viewed as a harmful byproduct leading to

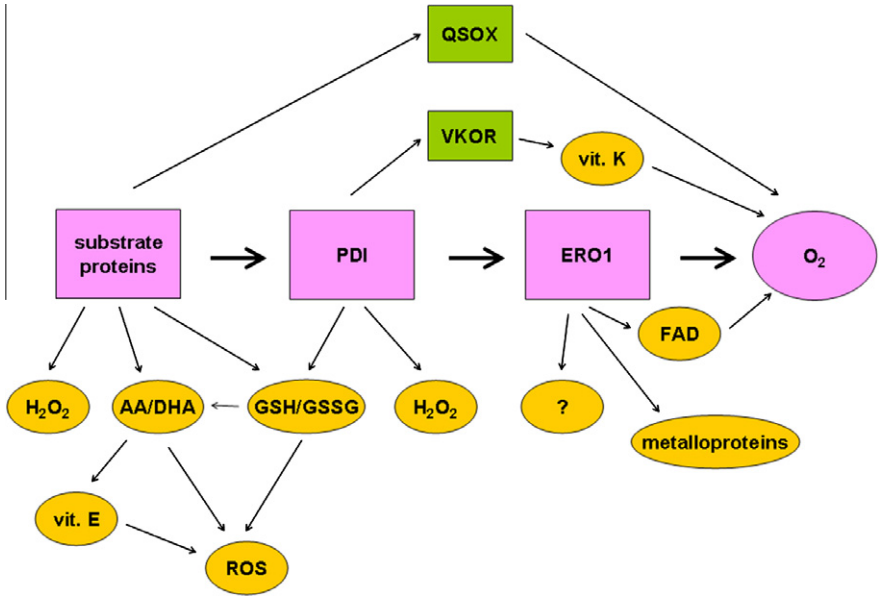

Fig. 1. Multiple oxidant hypothesis of oxidative protein folding. Several prooxidants promote to the oxidative folding in the ER. The classic proteinaceous electron relay pathway (pink planes) is allegedly supported by other ER oxidoreductases (green). Alternative electron acceptors (both low molecular weight compounds and proteins) are indicated as orange ovals. The direction of the electron transfer is marked with arrows. *Abbreviations*: AA, ascorbate; DHA, dehydroascorbic acid; Ero1, ER oxidoreductin 1; PDI, protein disulfide isomerase; QSOX, quiescin sulfhydryl oxidase; ROS, reactive oxygen species; vit, vitamin; VKOR, vitamin K epoxide reductase.