The Endoplasmic Reticulum: an emerging player in redox pathophysiology

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No conflicts of interest to declare

Role of disulfide bonds in proteins

- Folding stabilization and functional capacitation of membrane, cell surface or secreted proteins

  Also.....:

- Control of peptide loading in the MHC
- Thiol-mediated ER retention of some proteins (ex: Ero1)
- Thrombogenicity

The endoplasmic reticulum is the major site for protein disulfide introduction and isomerization in eukaryotes

Oxidized Redox State of Glutathione in the Endoplasmic Reticulum

Christopher Hwang,* Anthony J. Sinskey, Harvey F. Lodish

The redox state of the endoplasmic reticulum (ER) was measured with the peptide *N*-Acetyl-Asn-Tyr-Thr-Cys-NH_2. The peptide diffused across cellular membranes; some became glycosylated and thus trapped within the secretory pathway, and its cysteine residue underwent reversible thiol-disulfide exchanges with the surrounding redox buffer. Glycosylated peptides from cells were disulfide-linked to glutathione, indicating that glutathione is the major redox buffer in the secretory pathway. The redox state of the secretory pathway was more oxidative than that of the cytosol; the ratio of reduced glutathione to the disulfide form (GSH/GSSG) within the secretory pathway ranged from 1:1 to 3:1, whereas the overall cellular GSH/GSSG ratio ranged from 30:1 to 100:1. Cytosolic glutathione was also transported into the lumen of microsomes in a cell-free system. Although how the ER maintains an oxidative environment is not known, these results suggest that the demonstrated preferential transport of GSSG compared to GSH into the ER lumen may contribute to this redox compartmentation.

Ancient disulfide relay systems have terminal electron sinks for reduced molecular oxygen...

- Bacterial periplasm:

  Main components: DsbA/DsbB

  Electron sink: ubiquinone / terminal membrane oxidase

- Mitochondrial intermembrane space:

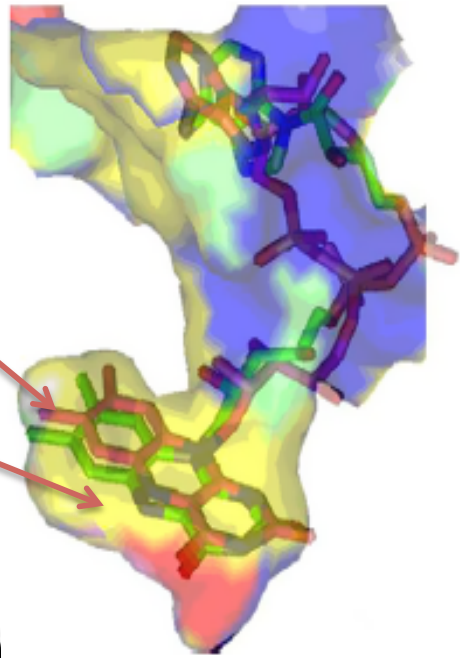
  Main components: Mia40 / Erv1 (FAD)

  Electron sink: cytochrome c / cytochrome oxidase

... and the final product is H_2O

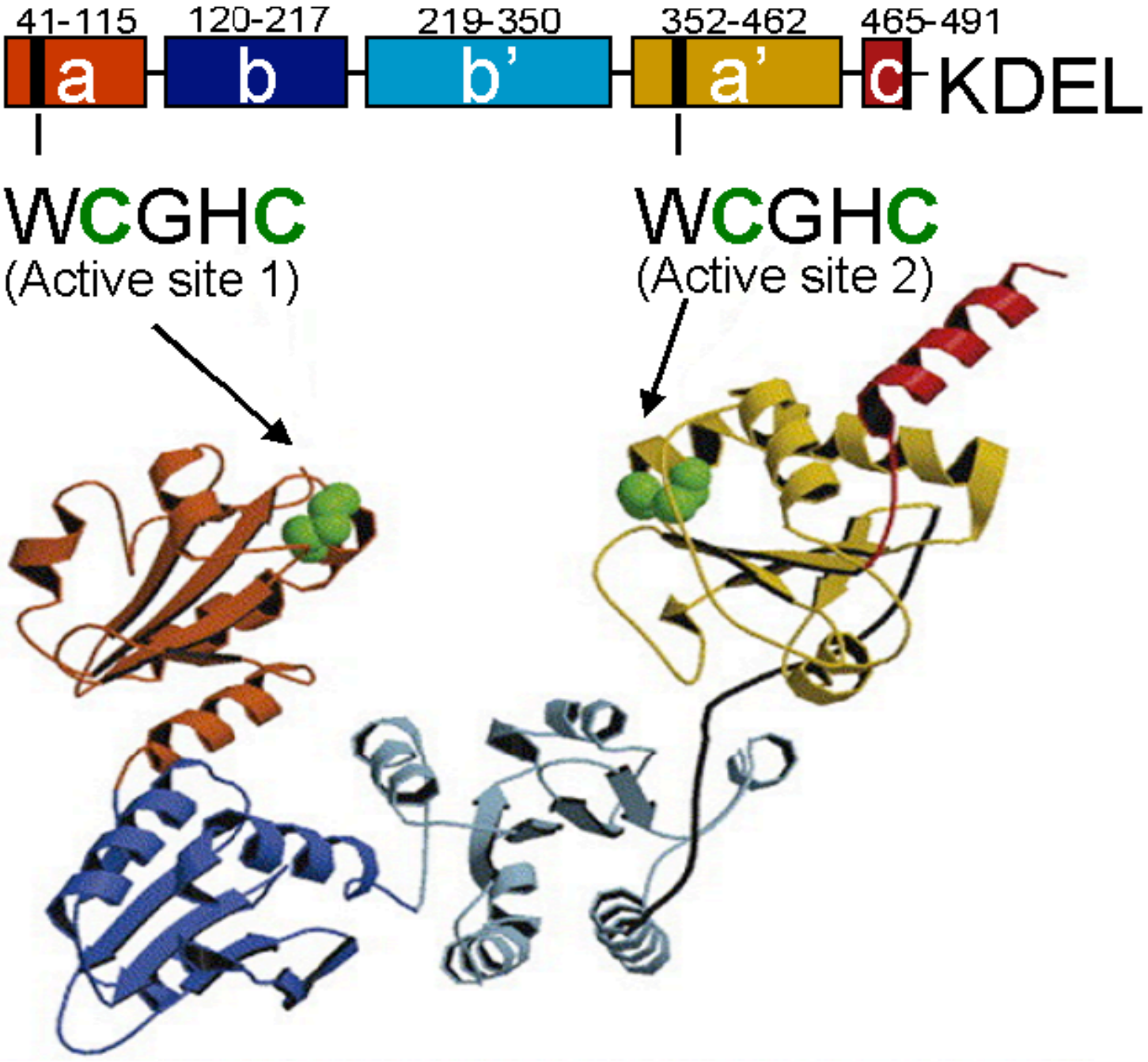
Ero1p oxidizes PDI and transfer electrons to oxygen, generating hydrogen peroxide: catalytic and shuttle disulfides

FAD-binding pocket of Ero 1 (Chu Y et al, BBRC 2009)



(Tavender TJ and Bulleid NJ. Antiox Redox Signal 2010; 13: 1177-87)

Protein disulfide isomerase

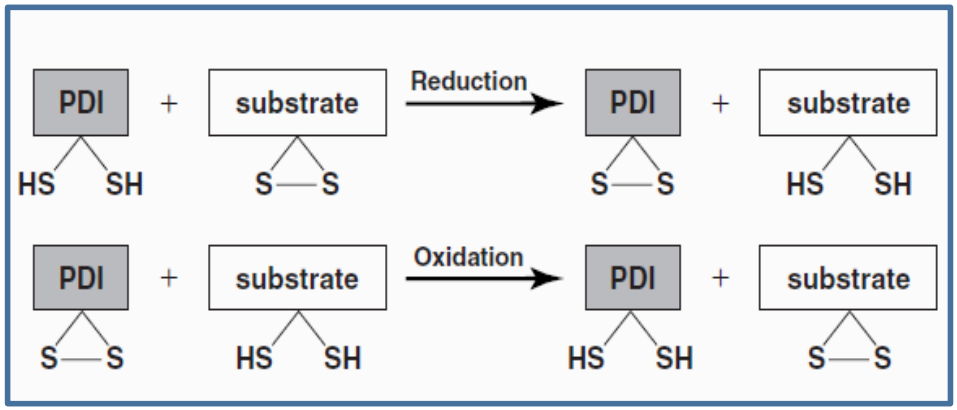


Characteristics of PDI (-SH pKa 6.2; E'_o -180 mV)

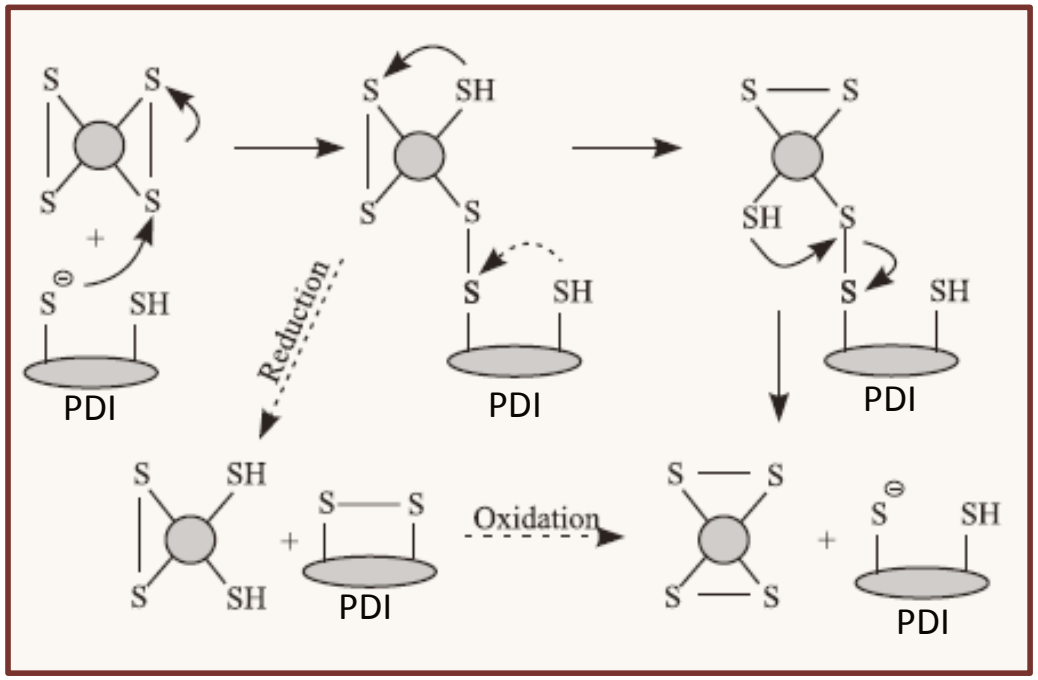
• PDI is a thioredoxin superfamily dithiol-disulfide oxidoreductase chaperone from the endoplasmic reticulum. The PDI family has >17 known proteins.

• PDI may complex with other chaperones: Grp78, Grp94, P5, ERdj3, cyclophilin B, Erp72, Grp170, UGGT, SDF2 (Meunier et al 2002)

• PDI may act as thiol reductase or oxidase, but the hallmark of PDI family is the isomerase activity:



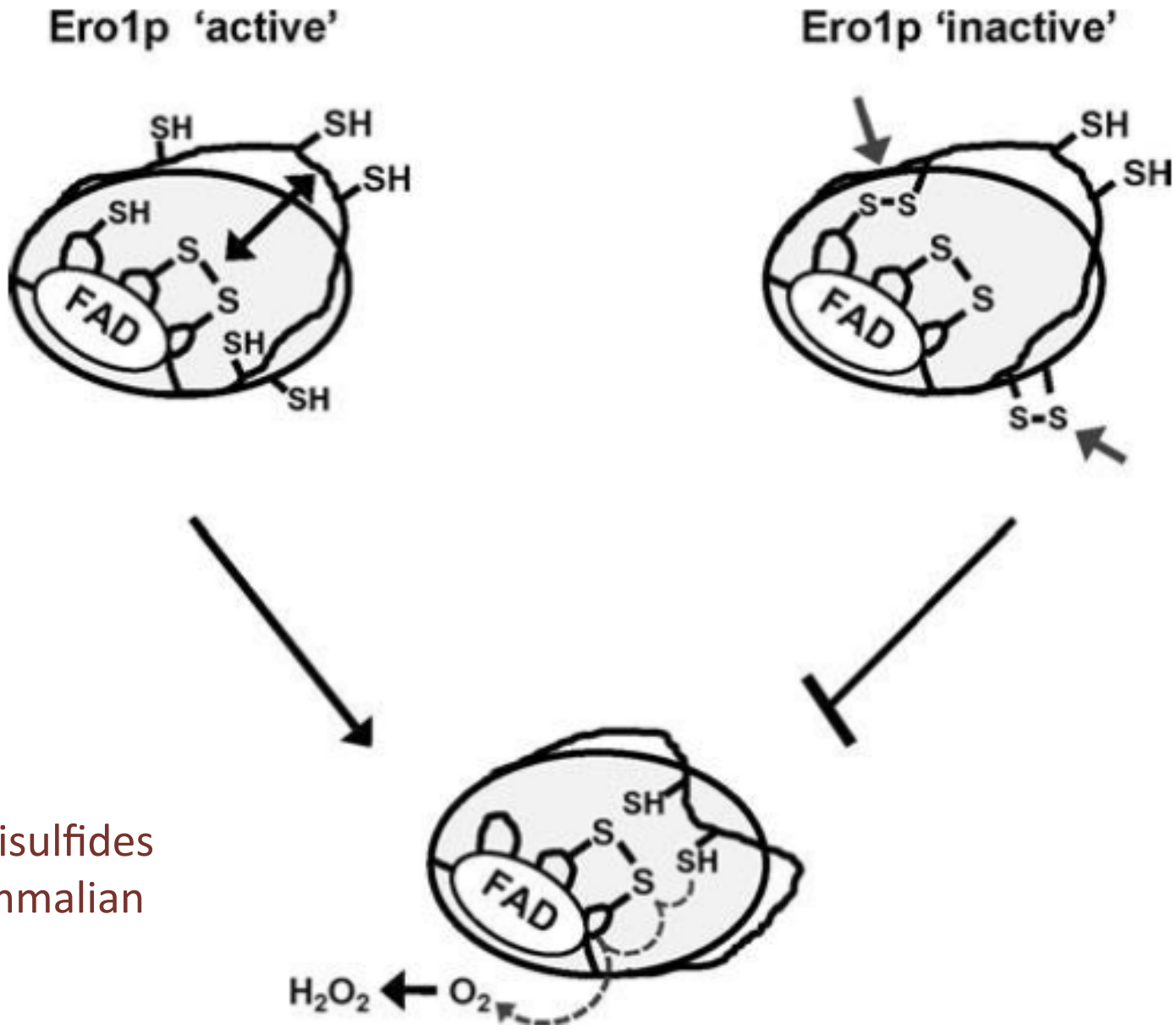
Laurindo et al 2008



Modif. from Hatahet and Ruddock 2009

• PDI also has chaperone activity, which is in itself independent on thiol groups, but is synergic with disulfide transfer.

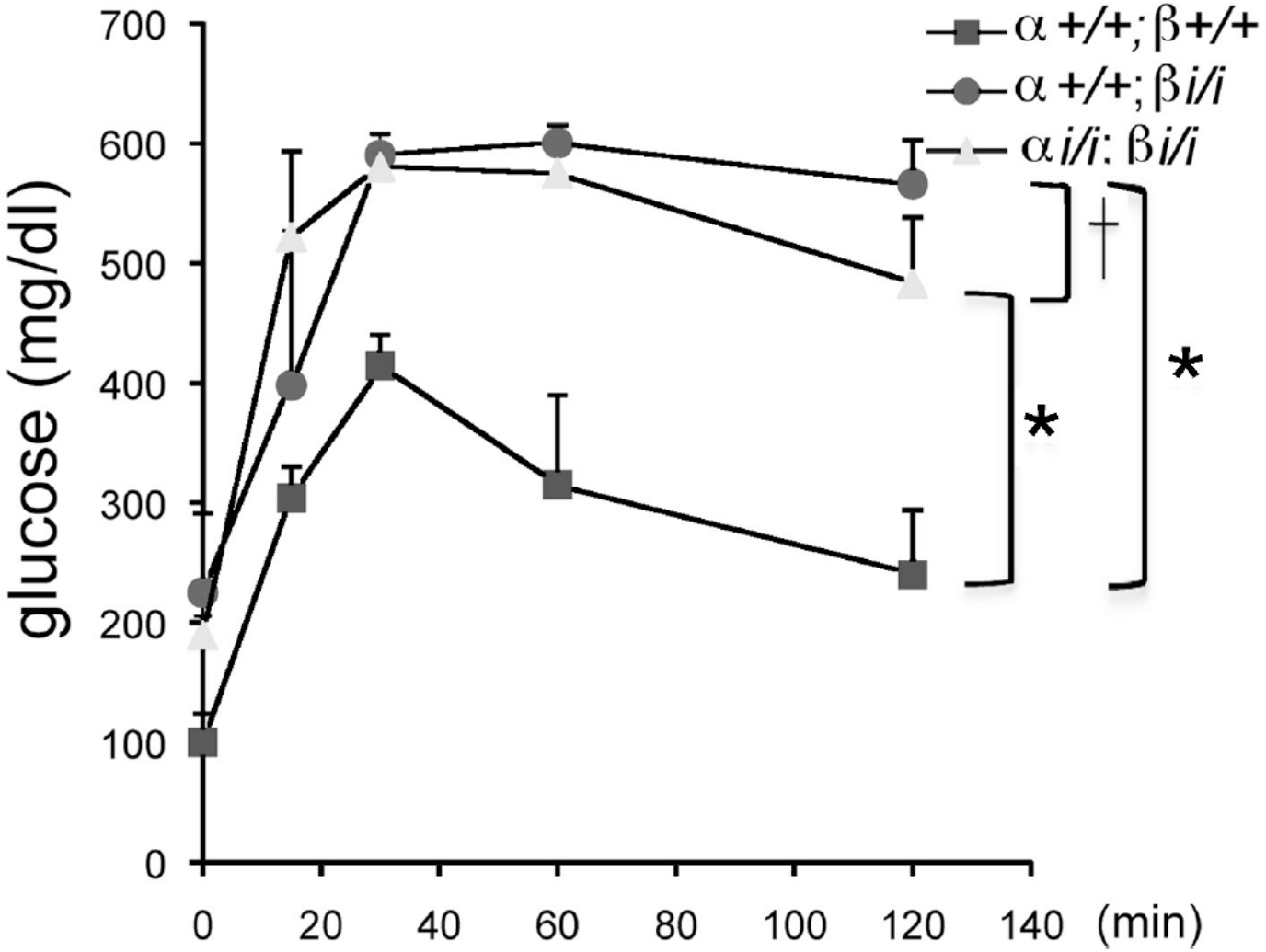
Regulatory disulfides in Ero1p *



*analogous disulfides exist for mammalian Ero1α

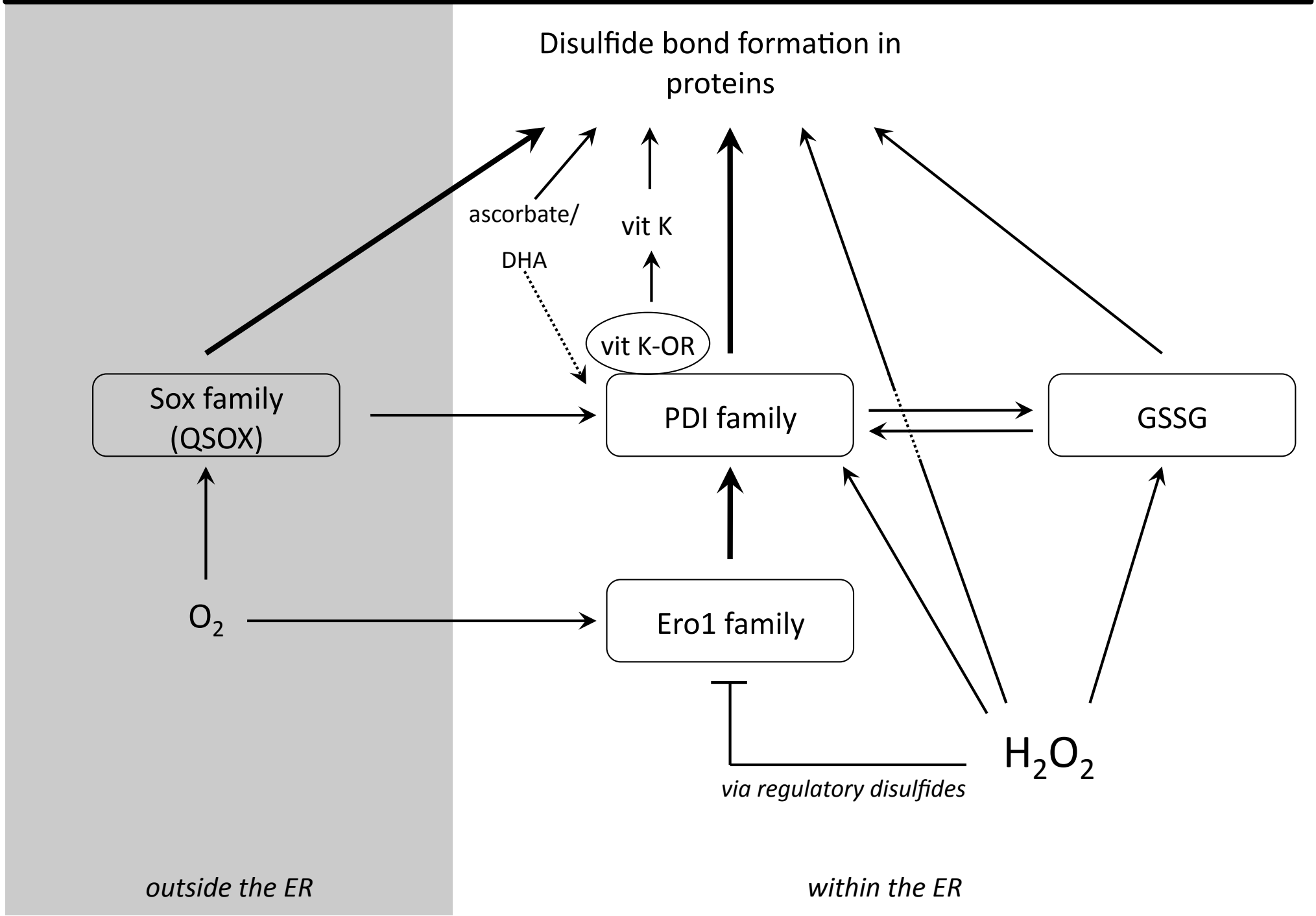
Tavender TJ and Bulleid NJ. Antiox Redox Signal 2010; 13: 1177-87

Genetic deletion of Ero1β promotes glucose intolerance, but mice with double Ero1 β/Ero1α deletion are viable and have no additional phenotype :
another challenge to the concept of Ero1-PDI as the only disulfide-generating system



Zito et al. J Cell Biol 2009; 188: 821-32)

Summary of pathways for protein disulfide formation

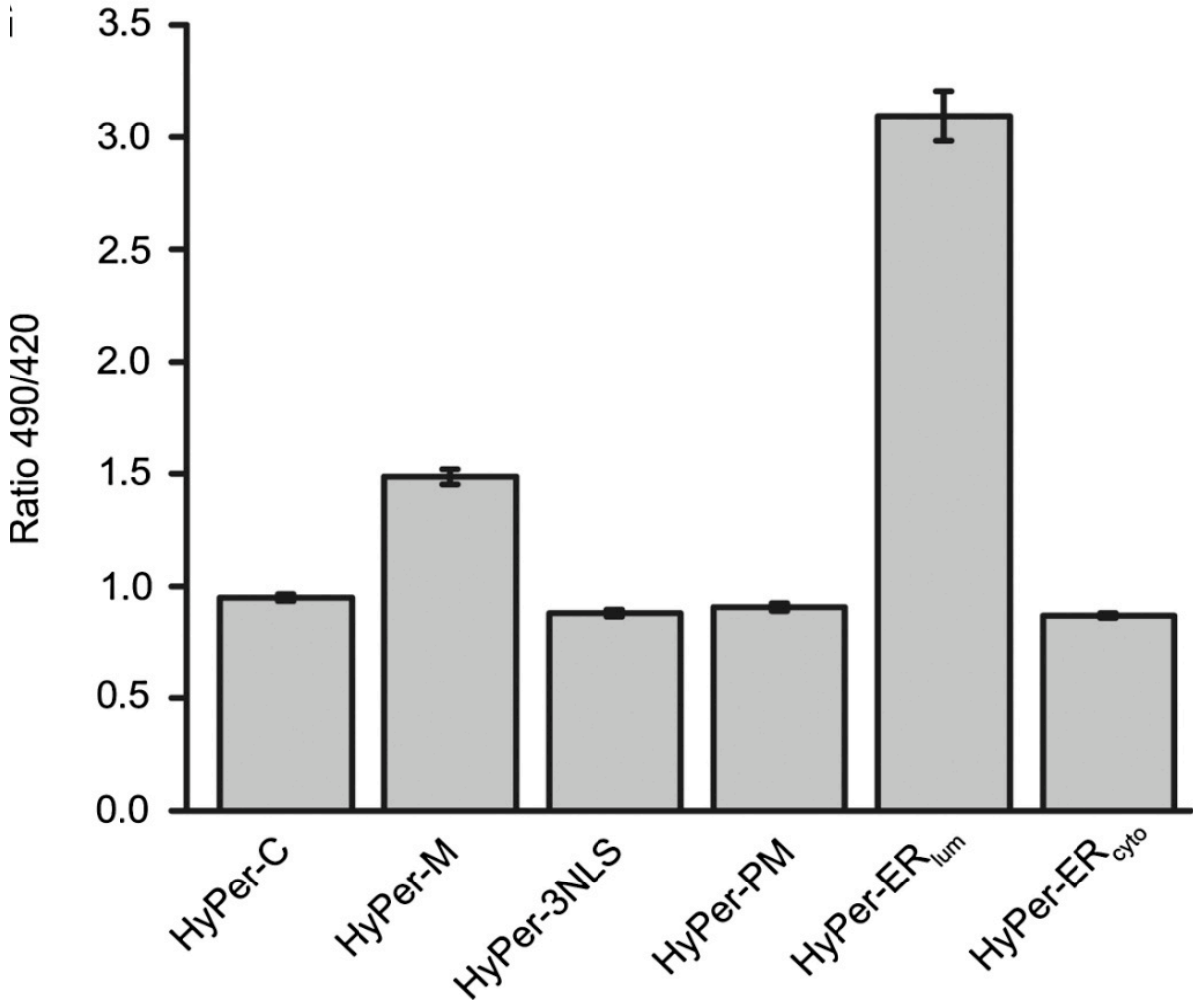


Endoplasmic reticulum: an underappreciated source of ROS

- Quantitation
  - Superficial calculation suggests up to 25% of cell ROS *(Tu and Weissman . J Cell Biol 2004)*
- Low antioxidant concentration
  - Very low or nonexistent SODs, catalase or glutathione reductase; little reference to GPx(s), Trx/TrxR; glutathione transferase only in hepatocytes *(for references, see Santos CX et al, ARS 2009)*
  - Secreted ER-specific PrxIV forms decamers with no obvious antioxidant activities *(Tavender et al 2008)*, although some reports do suggest antioxidant effect *(Okado-Matsumoto et al, 2000)*
- ER: the major source of GSSG in the cell (in yeast, via Ero1p – *Cuozzo and Kaiser, Nature Cell Biol 1999*)

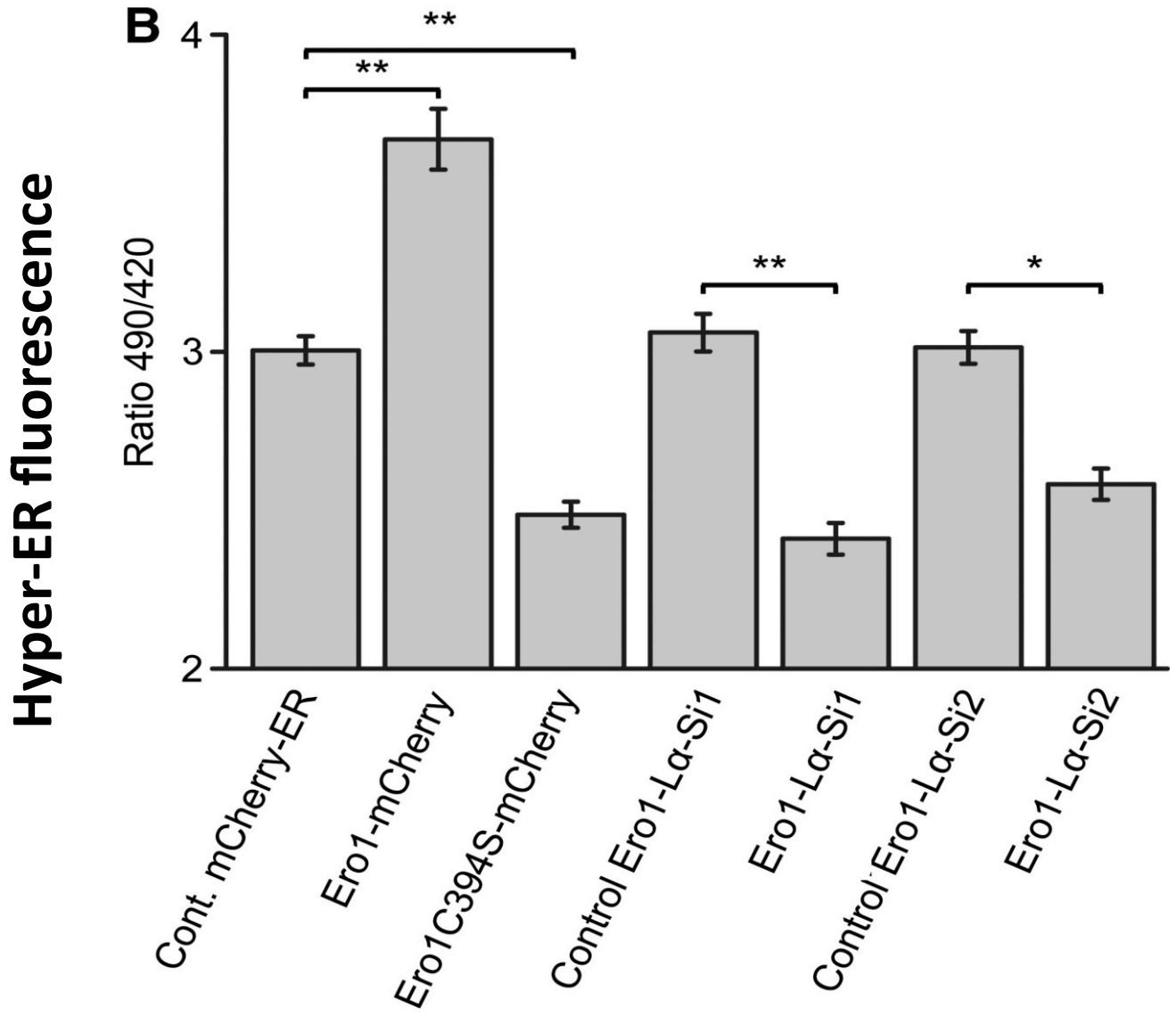
The ER lumen is a relevant source of H_2O_2:

studies with HyPer sensor targeted to cytosol, mitochondria, nucleus, plasma membrane, ER lumen or ER cytosolic surface



Enyedi B et al.
Antiox Redox Signal 2010; 13: 721

Ero1α regulates ER levels of H_2O_2



Enyedi B et al.
Antiox Redox Signal 2010; 13: 721

Un/misfolded protein accumulation at the ER lumen

↓

Endoplasmic reticulum (ER) stress

↓

Unfolded protein response ***(UPR)***

1- Arrest in mRNA translation and synthesis of new protein
2- ER expansion
3- Overexpression of ER chaperones
4- Apoptosis

The 4 "A"s of the UPR *(Rutkowski & Kaufman, TBS 2007):*

A*ctivation*

A*cute response*

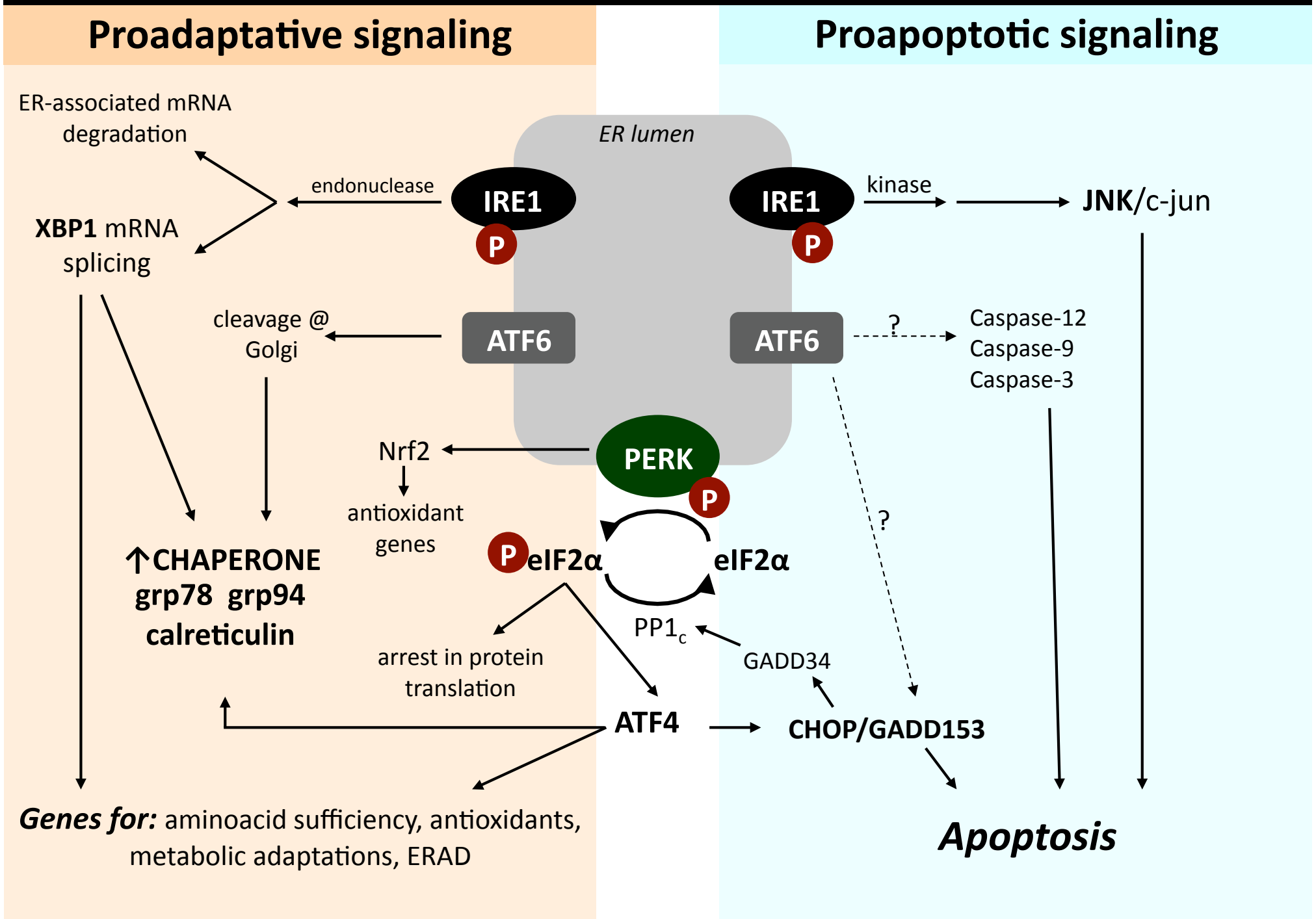
A*daptation*

A*poptosis*

Many diseases/pathogenetic mechanisms are associated with ER stress signaling

- **Tumors** *(Herr et al, Blood 2001)*
- **Parkinson´s disease and Neurodegenerative Diseases** *(Paschen & Doutheil, JCBFM 1999; Zhao & Ackerman, Curr Opin Cell Biol 2006)*
- **α-1 antitrypsin deficiency** *(Lawless et al, J Immunol 2004)*
- **Viral infection** *(He, Cell Death Differ 2006)*
- **Inflammation** *(for a review, see Zhang K and Kaufman R, Nature 2008)*
- **Atrial fibrillation** *(Vitadello et al, Circulation 2001)*
- **Cardiac hypertrophy and failure** *(Okada et al, Circulation 2004)*
- **Hyperhomocysteinemia** *(Werstuck et al, J Clin Invest 2001)*
- **Obesity, diabetes and insulin resistance** *(Ozcan et al, Science 2004 & 2006; for a review, see Scheuner and Kaufman , Endocr Rev 2008)*
- **Atherosclerosis** *(Zhou et al, Circulation 2005)*
- **Cholesterol toxicity / lipid metabolism** *(reviewed by Marciniak and Ron, Physiol Rev 2006)*

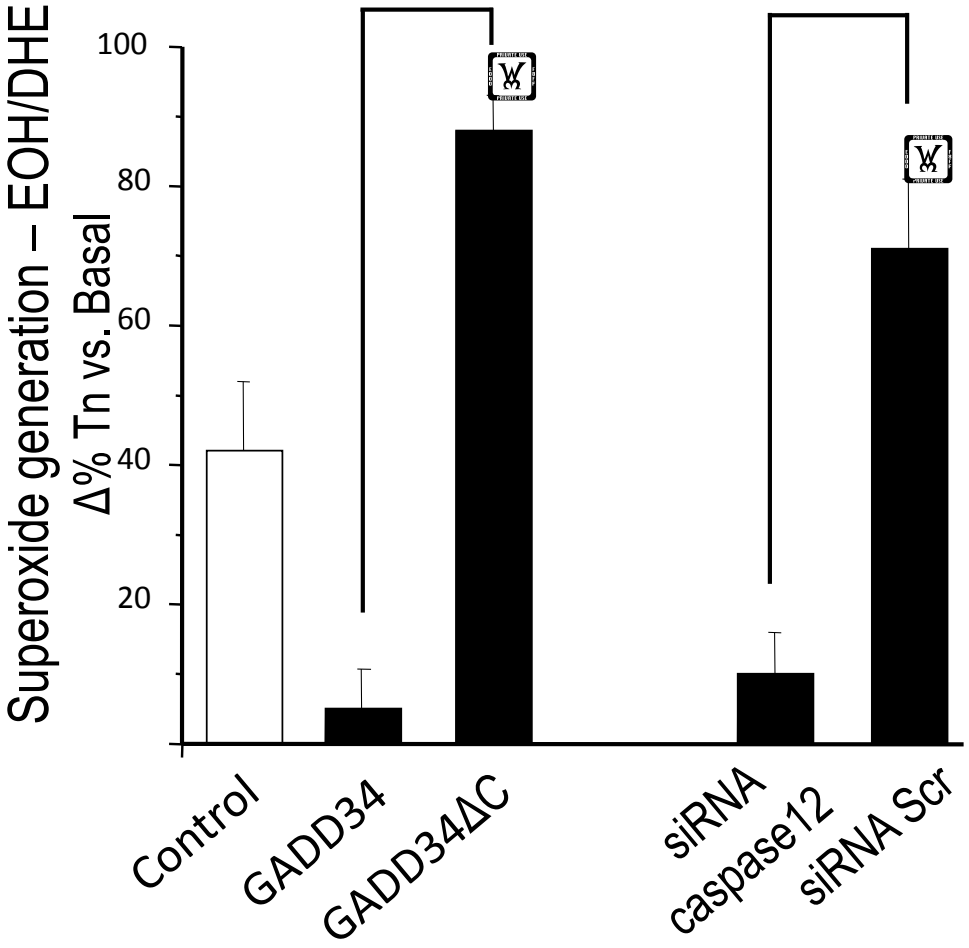
UPR signaling induced by ER stress *(modif. from Santos et al, ARS 2009)*

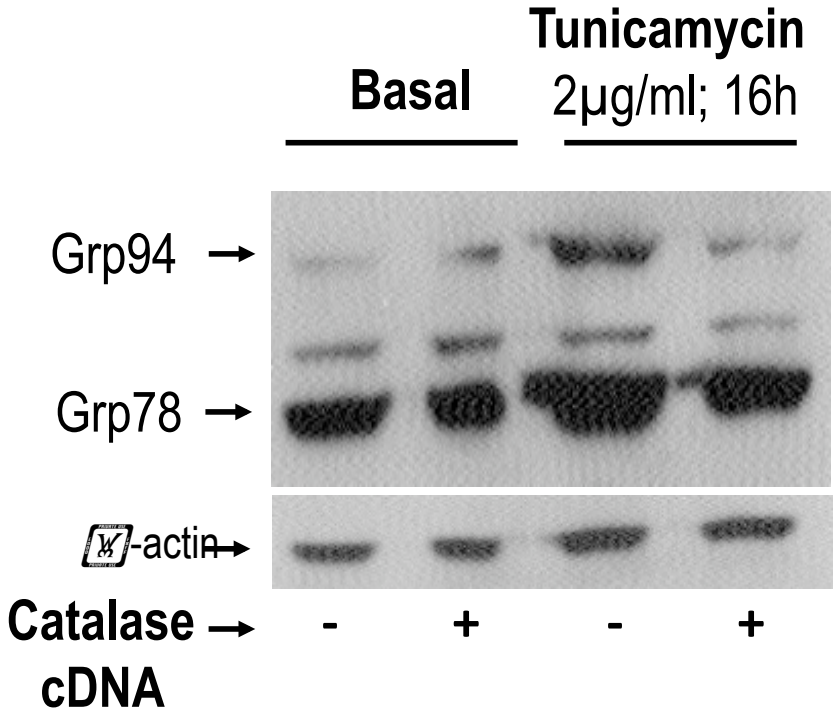


Main operational markers of the UPR

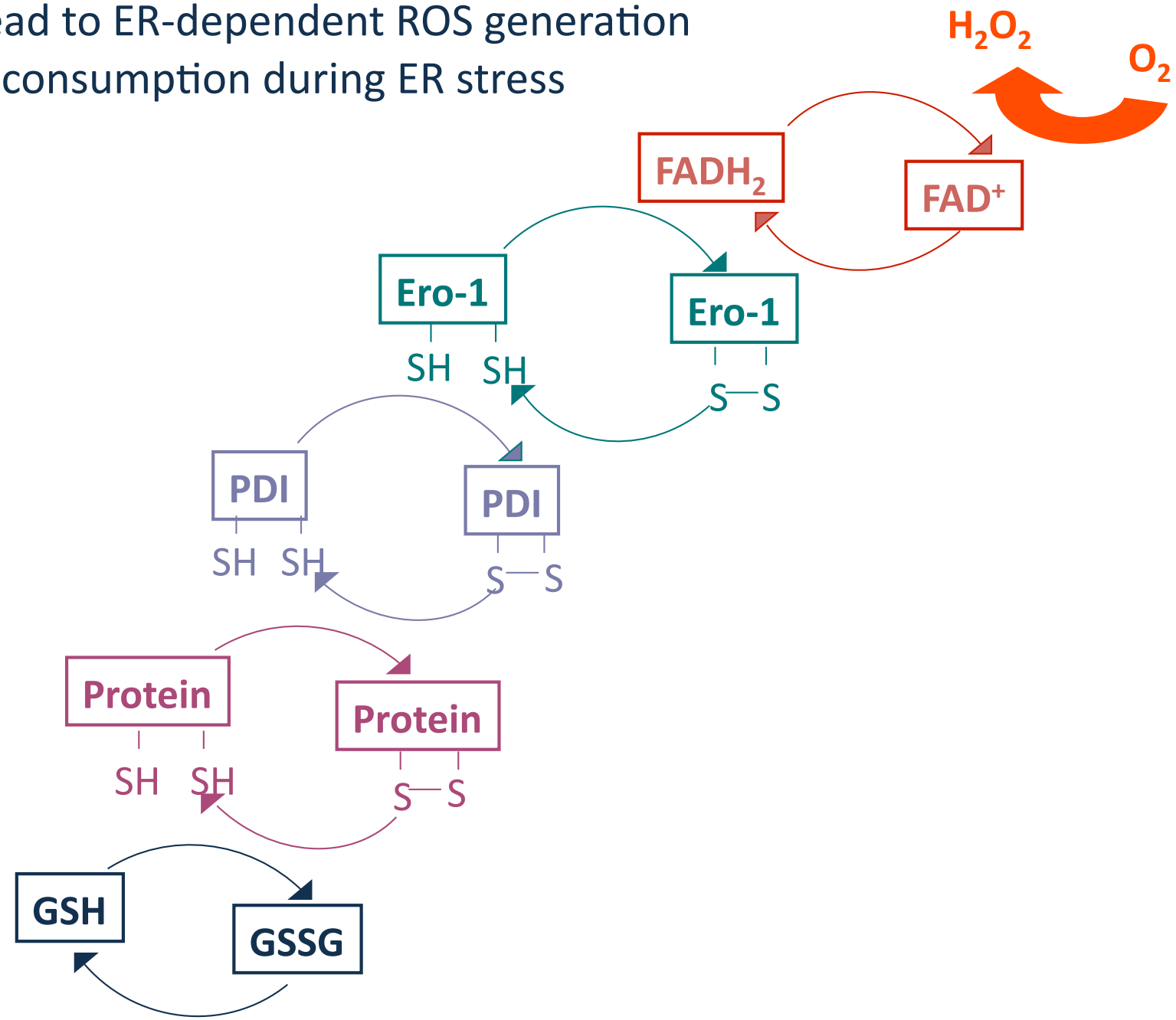
- ER stress sensors
  - IRE1 phosphorylation
  - PERK phosphorylation
  - ATF6 cleavage, nuclear migration
- UPR pathways
  - eIF2α phosphorylation
  - XBP1 mRNA (and protein) splicing
  - ATF4 nuclear expression
  - CHOP/GADD153 nuclear expression
  - Expression of KDEL-bearing chaperones: Grp78 (BIP), Grp94, calreticulin, Orp150

The UPR generates ROS at several levels and ROS feedback on the UPR *(for a review of many other examples, see Santos CX et al, ARS 2009)*





Futile Ero1/PDI/glutathione cycles of attempted folding lead to ER-dependent ROS generation and GSH consumption during ER stress



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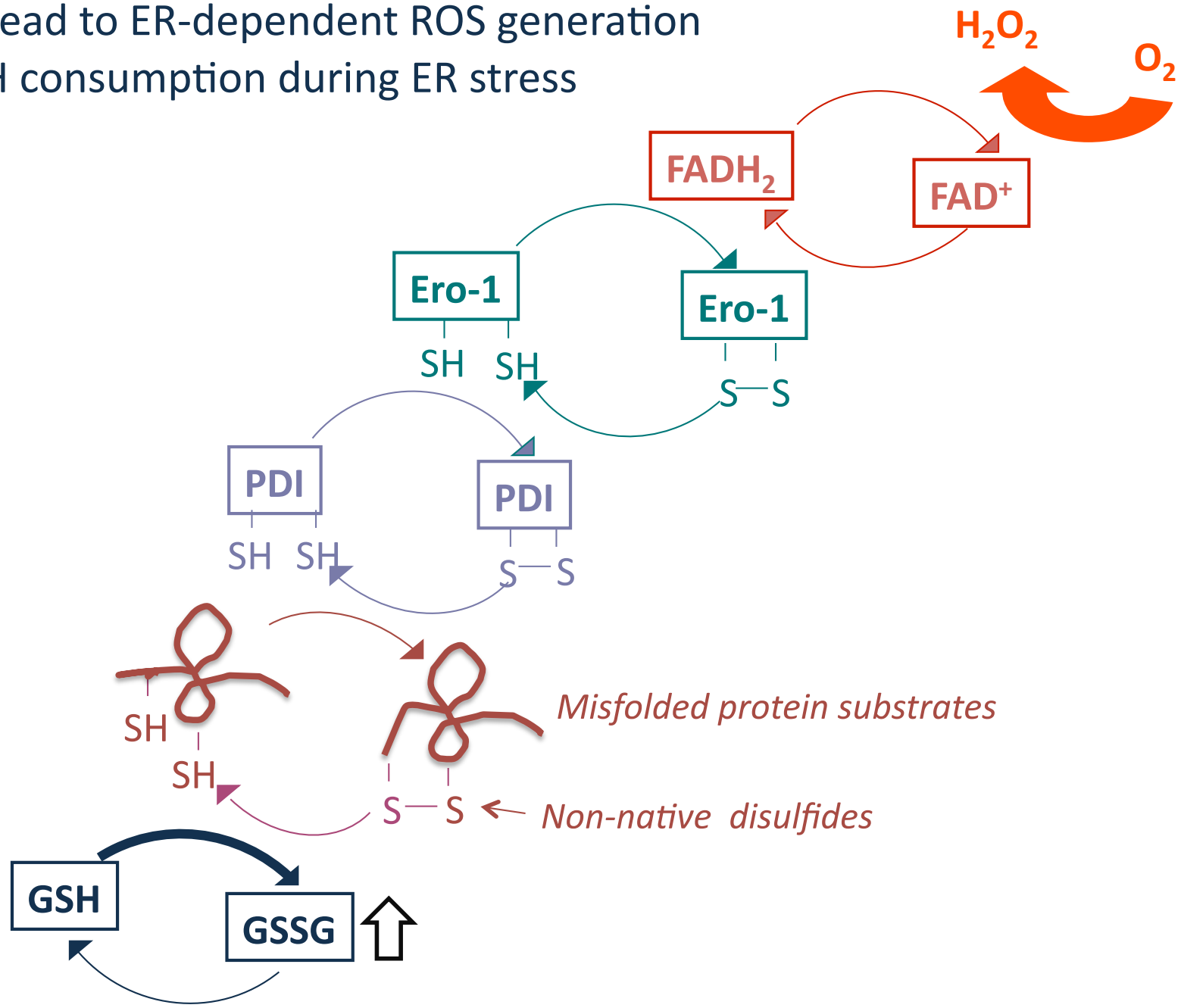


Futile Ero1/PDI/glutathione cycles of attempted folding lead to ER-dependent ROS generation during ER stress: *caveats*

- Constitutively increased Ero1 overexpression does not lead to detectable oxidant generation, at least in unstressed cells *(Sevier et al. Cell 2007).*
- In vivo 1:1 stoichiometry of H_2O_2 production via Ero1 is unclear.
- Rate constants of Ero1/PDI/protein/glutathione thiol exchanges may be quite low. Example: 182 $M^{-1}.s^{-1}$ for DsbA vs. GSH *(Hu et al, J Prot Chem 1999).*
- Electron acceptors other than oxygen can potentially support Ero1p oxidation in some instances *(Tu et al, Science 2000).*
- Free FAD levels support Ero1/PDI/protein oxidation *(Papp et al, BBRC 2005)* and strongly affect Ero1p activity (although less so for Ero1α – *Wang et al JBC 2009*). Little is known regarding FAD sufficiency in the ER.

Futile Ero1/PDI/glutathione cycles of attempted folding lead to ER-dependent ROS generation during ER stress: *caveats*

- ER seems underoxidized during yeast UPR. Some ER underoxidation still detected during ER stress due to transfection of cys-free misfolded substrate *(Merksamer et al, Cell 2008).*

- Most studies have used DHE or DCF to address oxidant generation during the UPR: nature of oxidants generated is less than clear.

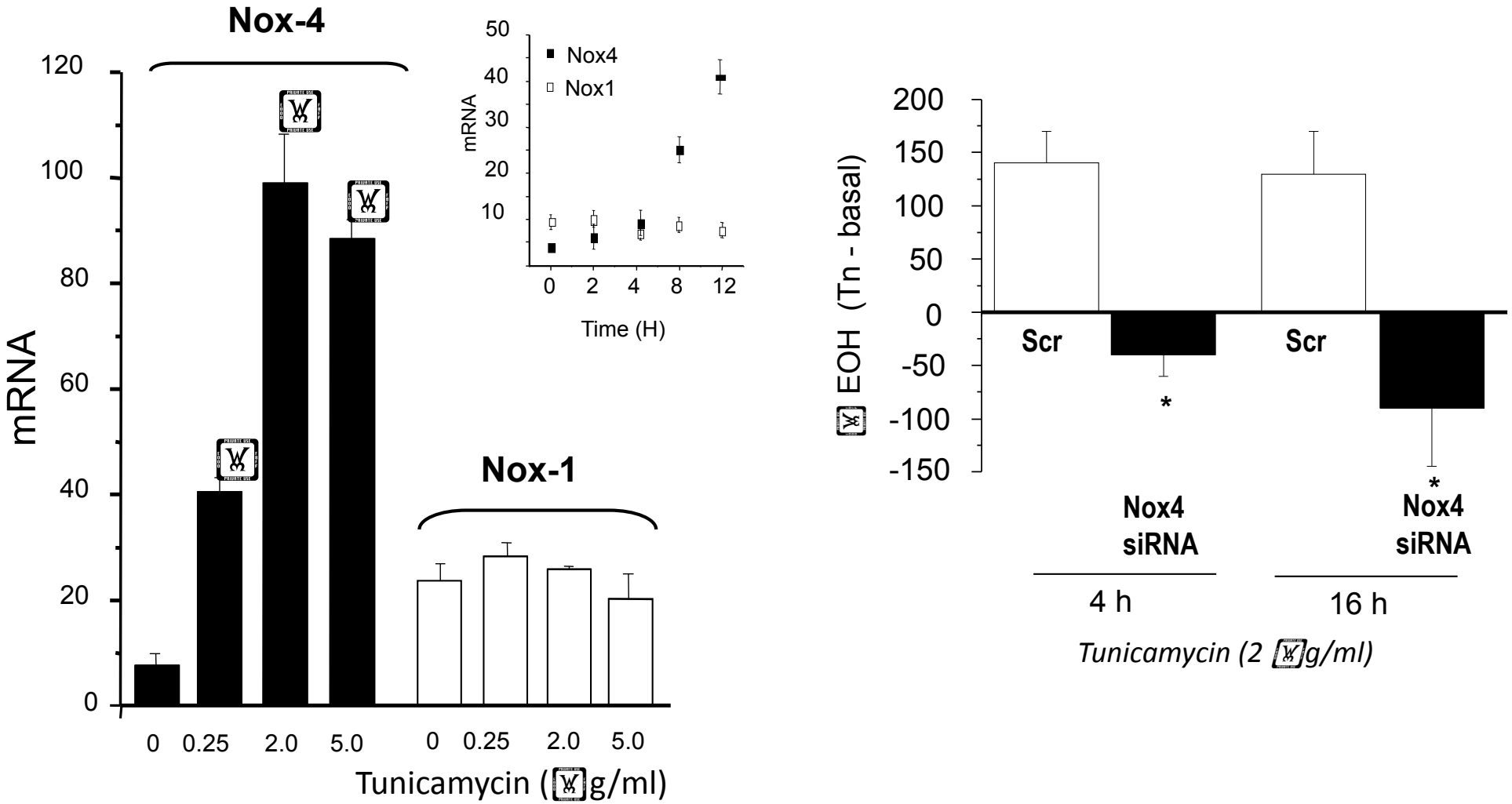
- Thiol antioxidants protect against UPR-induced oxidants *(Haynes et al, Mol Cell 2004; Sevier et al, Cell 2007)*: how? (ROS scavenging by glutathione is slow!).

- Increasing ER oxidation is likely to oxidize Ero1 regulatory disulfides, shutting off catalytic activity *(Tavender and Bulleid 2010).*

The ER is a relevant (but not the only) location for Nox4

- Nox 4 in the ER:
  - *Ambasta et al. J Biol Chem 2004*
  - *Martyn et al. Cell Signal 2005*
  - *Serrander L et al. Biochem J 2007*
  - *Chen K et al. J Cell Biol 2008*
  - *Helmcke I et al. Antiox Redox Signal 2009*
  - *Lee CF et al. Circ Res 2010*
  - *Wu R-F et al. Mol Cell Biol 2010*
- Nox4 in focal adhesions:
  - *Hilenski L et al. ATVB 2004*
  - *Lyle AN et al. Circ Res 2009*
- Nox4 in mitochondria:
  - *Block K et al. PNAS 2009*
  - *Ago T et al. Circ Res 2010*
  - *Grahan K et al. Cancer Biol Ther 2010*
  - *Kuroda J et al. PNAS 2010*
- *Less clear: plasma membrane, nucleus*

Nox4 induction accounts for ER stress-induced ROS generation in vascular smooth muscle cells

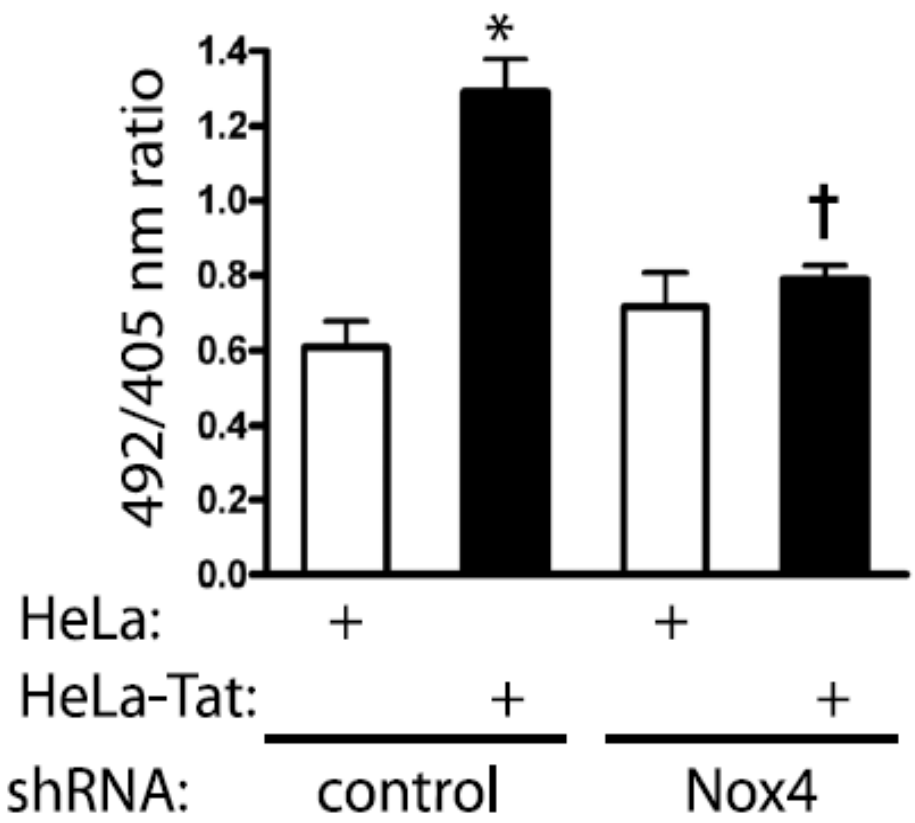


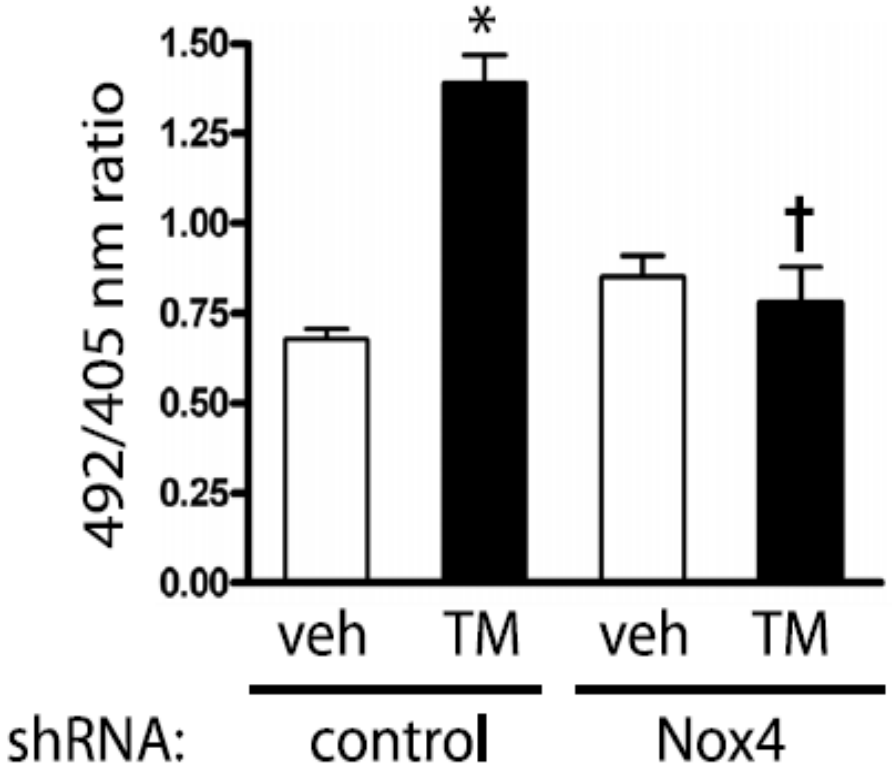
Santos et al, ARS 2009 - Similar results published by Pedruzzi et al, MCB 2004

Nox4-derived H_2O_2 mediates endoplasmic reticulum signaling

Ru-Feng Wu, Zhenyi Ma, Zhe Liu, and Lance S. Terada* From the Department of Internal Medicine, Division of Pulmonary and Critical Care, University of Texas Southwestern Medical Center, .

Mol. Cell. Biol. doi:10.1128/MCB.01445-09





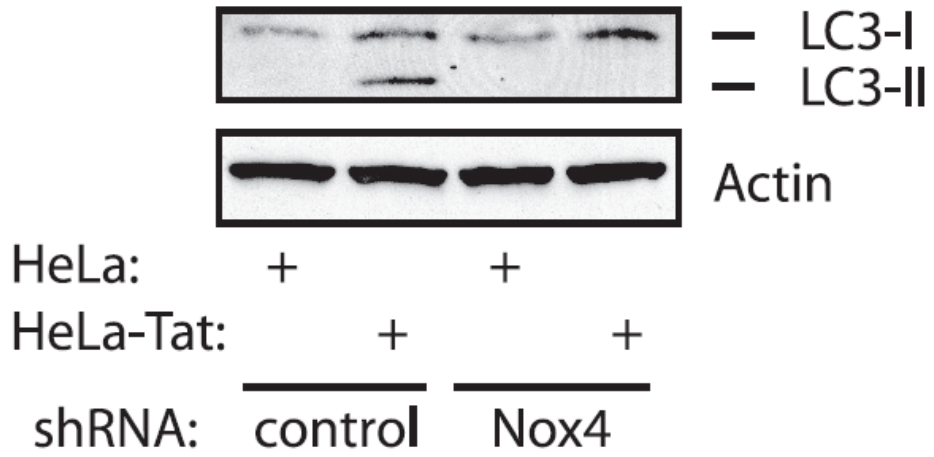
Physiological effects of ROS in the UPR

UPR SIGNALING

APOPTOSIS

- Several references *(reviewed in Santos CX et al, ARS 2009)*

AUTOPHAGY *(Wu R-F et al, Mol Cell Biol 2010)*



Protein disulfide isomerase (PDI) interacts with NADPH oxidase

- PDI co-localizes and/or co-ippt with NADPH oxidase complex subunits in VSMC (p22phox, Nox1, Nox4 and Nox constructs) [1]
- PDI loss-of-function (neutralizing Ab, antisense oligo, siRNA) abrogates ROS production due to angiotensin II in VSMC [1,2]
- PDI overexpression in VSMC induces spontaneous, agonist-independent NADPH oxidase activation and Nox1 expression [2]
- PDI co-localizes with Nox subunits in macrophages and PDI silencing inhibits *L. chagasi* phagocytosis [3]
- PDI converges with neutrophil NADPH oxidase [4]

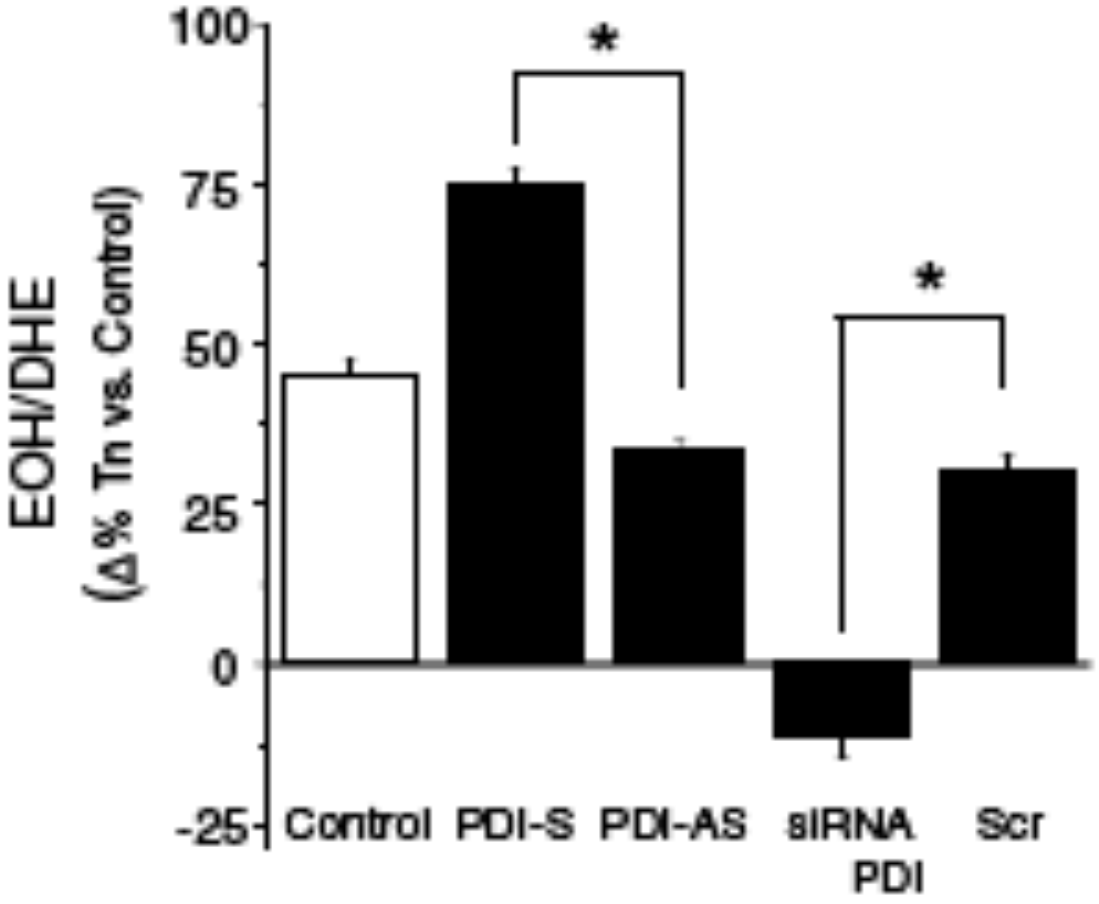
1 . Janiszewski M et al, J Biol Chem 2005

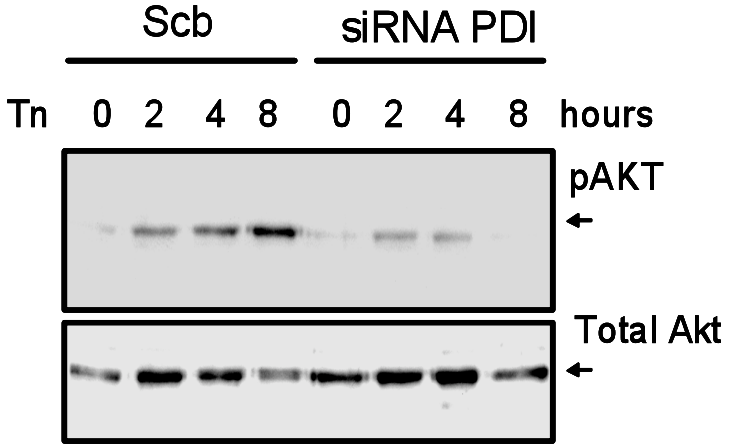
2 . Fernandes et al, Arch Biochem Biophys 2009

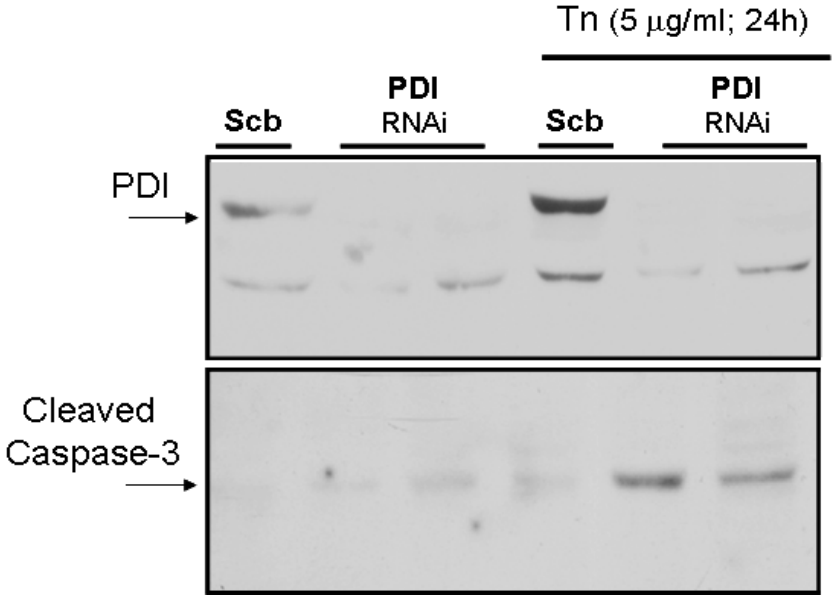
3 . Santos CX et al, J Leukocyte Biol 2009

4 . Lopes LR and collaborators, unpublished observations

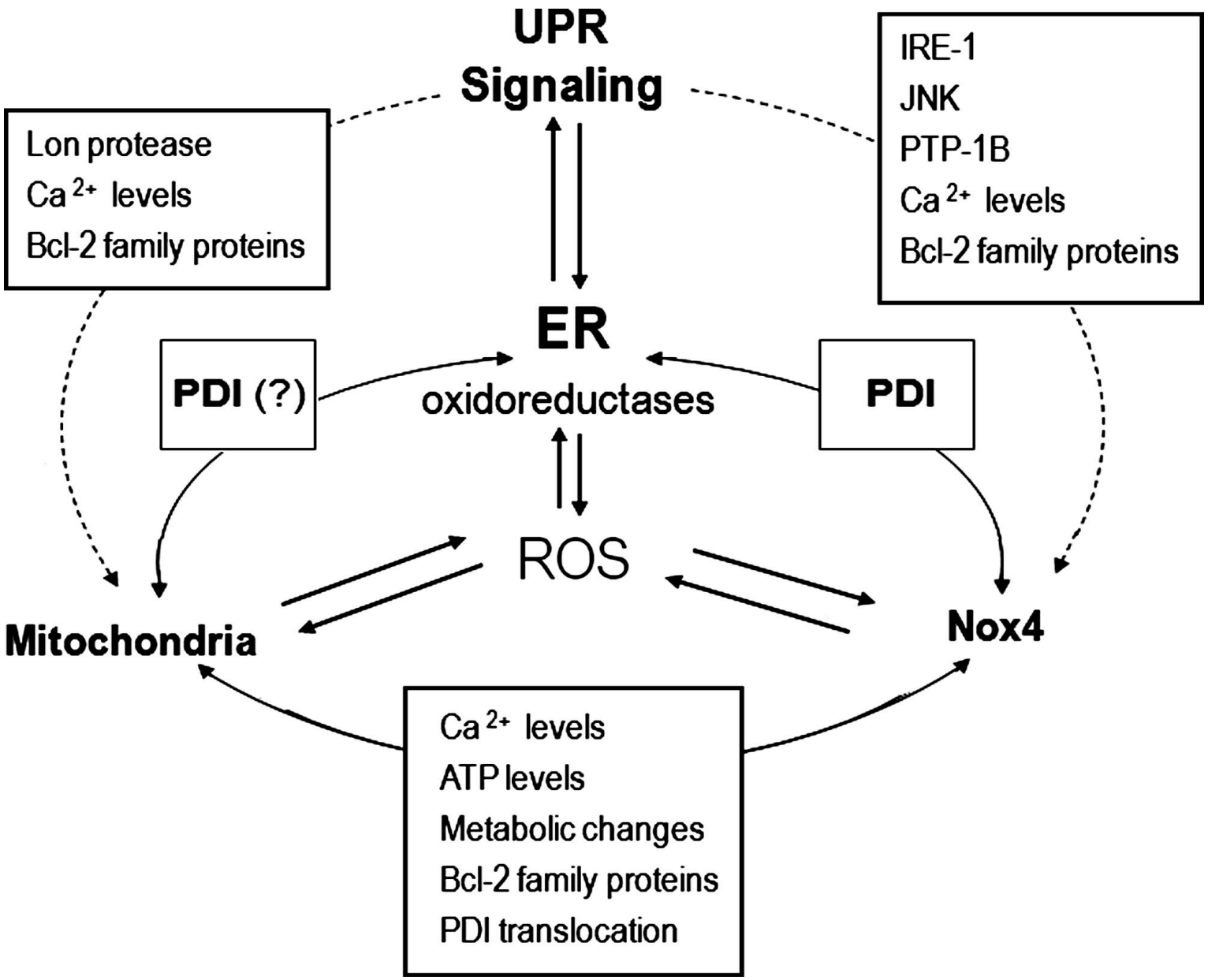
PDI supports ROS generation and cell survival signals during ER stress in vascular smooth muscle cells







Integration of distinct ROS sources during ER stress



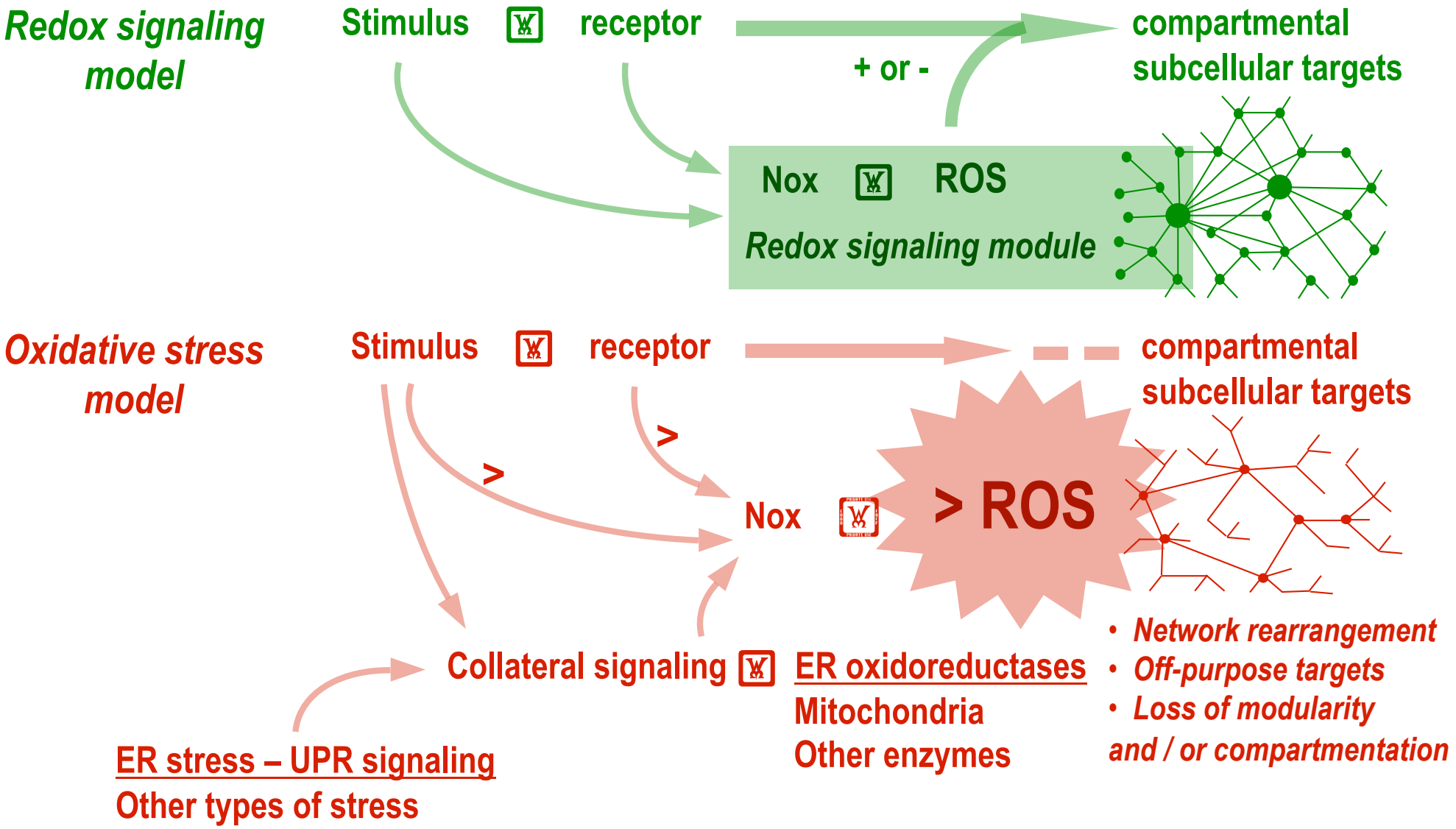
Santos et al, ARS 2009

Summary

- The ER is a quantitatively relevant source of ROS.
- Ero1 and PDI are crucially involved in ROS generation in the context of disulfide introduction and isomerization in nascent proteins, although other pathways for disulfide formation may also be important in upper eukaryotes.
- ROS generation is an integral component of the UPR and mediates adaptive and apoptotic signaling.
- Ero1/PDI cycles, Nox4 and mitochondria may contribute to generate ROS in the UPR – mechanisms are yet unclear and may be cell type and context-specific.
- Interaction between PDI and NADPH oxidase, as well as other pathways, integrate ER (dys)function and ROS during the UPR.

ER stress and ER oxidoreductases are relevant players in redox pathophysiology:

Implications in the understanding of oxidative stress as modular redox signaling dysruption



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