Endoplasmic Reticulum Associated Degradation (ERAD), Autophagy, and Protein Conformational Diseases: Lessons from Model Organisms and Therapeutic Strategies

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PancreasFest, 2017
Proteins come in many shapes and sizes
Unfortunately, it’s really really hard to fold proteins in the cell
1. The protein folding funnel
2. The cell is a crowded place
3. Each cell-type possess a distinct protein folding environment (and different protein composition)
4. Subcompartments in eukaryotic cells are chemically distinct
The secretory pathway
How are aberrant polypeptides degraded within the secretory pathway?
ER Associated Degradation (ERAD)

Removal of aberrant proteins from the secretory pathway

- How are ERAD substrates selected?
- Are chaperones required?
- What is the protease?

ca. 1996
Solution: Develop an \textit{in vitro} ERAD assay
Solution: Develop an *in vitro* ERAD assay

ER-derived microsomes
Solution: Develop an *in vitro* ERAD assay
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Solution: Develop an *in vitro* ERAD assay

**Diagram:**
- Yeast cytosol
- ATP

**Diagram Details:**
- Membrane structure
- Protein complexes
- ATP molecules

Diagram depicts the interaction of cytosolic components with membrane-bound systems, illustrating the ERAD assay setup.
Solution: Develop an *in vitro* ERAD assay.
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yeast cytosol
ATP

McCracken and Brodsky, 1996
Solution: Develop an *in vitro* ERAD assay

yeast *pre1pre2* cytosol

ATP

Werner *et al.*, 1996
Solution: Develop an *in vitro* ERAD assay
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Lee *et al.*., 2004
Solution: Develop an *in vitro* ERAD assay

19S (PA700)

ATP
Solution: Develop an *in vitro* ERAD assay
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Lee *et al.*, 2004
Solution: Develop an *in vitro* ERAD assay

- BiP
- Scj1-Jem1
- Calnexin

References:
- McCracken and Brodsky, 1996
- Brodsky *et al.*, 1999
- Nishikawa *et al.*, 2001
- Kabani *et al.*, 2003
- Vembar *et al.*, 2010
Solution: Develop an *in vitro* ERAD assay

Hsp70} BiP
Hsp40s} Scj1-Jem1
Calnexin
Solution: Develop an *in vitro* ERAD assay

- ATP
- BiP
- Scj1-Jem1
- Calnexin
Disease-associated ERAD substrates expressed in yeast…

**Substrates**

- **AT-Z, Torsin**
  - Cytoplasm
  - ER
  - Werner et al., 1996
  - Brodsky et al., 1999
  - Palmer et al., 2003
  - Kruse et al., 2006
  - Scott et al., 2007
  - Gelling et al., 2012
  - Zacchi et al., 2014

- **ApoB**
  - Cytoplasm
  - ER
  - Gusarova et al., 2001 & 2003
  - Hrizo et al., 2007
  - Grubb et al., 2012

- **ENaC**
  - Cytoplasm
  - ER
  - α, β, γ
  - Kashlan et al., 2007
  - Buck et al., 2010, 2013, 2015, 2017

- **CFTR**
  - Cytoplasm
  - ER
  - Zhang et al., 2001
  - Sullivan et al., 2003
  - Youker et al., 2004
  - Ahner et al., 2007
  - Hutt et al., 2012
  - Buck et al., 2015

- **Kir2.1 & ROMK**
  - Cytoplasm
  - ER
  - x 4
  - Kolb et al., 2014
  - Buck et al., 2015

- **NCC**
  - Cytoplasm
  - ER
  - Needham et al., 2011
  - Donnelly et al., 2013
Alpha-one antitrypsin “Z” is also targeted for autophagy

In the absence of autophagy, and under high AT-Z expression levels, protein polymers accumulate

Kruse et al., 2006
Disease-associated ERAD substrates expressed in yeast...

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Cytoplasm</th>
<th>ER</th>
</tr>
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<tbody>
<tr>
<td>AT-Z, Torsin</td>
<td>cytoplasm</td>
<td>ER</td>
</tr>
<tr>
<td>ApoB</td>
<td>cytoplasm</td>
<td>ER</td>
</tr>
<tr>
<td>ENaC</td>
<td>cytoplasm</td>
<td>ER</td>
</tr>
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<td>CFTR</td>
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<td>ER</td>
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<tr>
<td>Kir2.1 &amp; ROMK</td>
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<tr>
<td>NCC</td>
<td>cytoplasm</td>
<td>ER</td>
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- Kashlan et al., 2007
- Buck et al., 2010, 2013, 2015, 2017
- Zhang et al., 2001
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- Youker et al., 2004
- Ahner et al., 2007
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- Buck et al., 2015
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- Needham et al., 2011
- Donnelly et al., 2013
Can the ERAD pathway be “drugged” to treat protein conformational diseases?

How are misfolded proteins in the secretory pathway subject to more than one “quality control” checkpoint?

What are the biochemical/biophysical features that lead to the selection of an ERAD substrate?

How does the ERAD pathway intersect with the unfolded protein response (UPR) pathway and autophagy?
The Big Questions…

Can the ERAD pathway be “drugged” to treat protein conformational diseases?

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Cystic Fibrosis

- Most common fatal inherited disease in the Caucasian population (~30,000 people affected in the US)

- Caused by a mutation in the Cystic Fibrosis Transmembrane conductance Regulator (CFTR)

- In ~90% of CF patients, transport of CFTR to the apical plasma membrane is disrupted

- Up to ~80% of the wild type protein is degraded

- ~100% of the F508del protein is degraded

Zhang and Chen, 2016
The disease-causing CFTR ΔF508 allele is rescued by low temperature and is active.
The disease-causing CFTR ΔF508 allele is partially rescued by a new drug combination

Brodsky and Frizzell, 2015
An *in vitro* assay that detects CFTR ubiquitination in human cell membranes

- HEK293 cells expressing CFTR
- ER-derived microsomes
- incubate at r.t.
- ATP, cytosol, 125I-Ub
- immunoprecipitate with anti-HA
- phosphorimager and western blot
Can the assay be employed to develop small molecule therapeutics?


J. Goeckeler-Fried
Subcritical amounts of PYR-41 potentiate VX809/C18 short circuit current in Ussing chambers (CFBE/ΔF508 monolayers)
PYR-41 analogs exhibit a range of activities

Pfizer: PYR-41 Analogs

CFTR Ubiquitination (HEK293-derived microsomes)

R. Denny, J. Goeckeler-Fried and A. Chiang
The Big Questions…

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How does the ERAD pathway intersect with the unfolded protein response (UPR) pathway and autophagy?
The UPR triggers ERAD, and defects in ERAD induce the UPR.
Critical links between the UPR and pancreatic disease

The UPR is induced in several pancreatitis models (both acute and chronic)

Phenylbutyrate decreases ER stress and pancreatitis severity in some models

Knock-down of a downstream, pro-apoptotic target of the UPR, CHOP, also decreases the severity of pancreatitis

IRE1, a UPR signal transduction protein and regulatory of ER physiology, is required for amylase secretion

Mice deficient for XBP1, which transduces the IRE1 signal, have lower levels of an ERAD-requiring enzyme when fed an alcohol supplemented diet
Are there ways to temper the UPR and/or increase ERAD?

Waldron, Pandol, Lugea, and Groblewski, 2015
Are there ways to temper the UPR and/or increase ERAD?

The BiP (Hsp70) Chaperone Plays a Key Role During Protein Folding and UPR Induction

Waldron, Pandol, Lugea, and Groblewski, 2015
Are there ways to temper the UPR and/or increase ERAD?

Wisen et al., 2010
Are there ways to temper the UPR and/or modulate ERAD?

Increased Hsp70 activity augments the folding of F508del CFTR

Wisen et al., 2010
A growing number of ERAD substrates are linked to human diseases (~70)

<table>
<thead>
<tr>
<th>Chromosomal Locus</th>
<th>Gene</th>
<th>Protein</th>
<th>Disease</th>
<th>Reference Nos.</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>2p24-25</td>
<td>APOB</td>
<td>Apolipoprotein B (Apo B)</td>
<td>A-beta lipoproteinemia</td>
<td>(92, 297, 298, 598)</td>
<td>S</td>
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<tr>
<td>Xq22</td>
<td>GLA</td>
<td>Alpha-galactosidase A (alpha Gal A)</td>
<td>Fabry disease</td>
<td>(179, 559)</td>
<td>S</td>
</tr>
<tr>
<td>7q36.1</td>
<td>KCNH2</td>
<td>Human ether-a-go-go-related gene (HERG) voltage-gated potassium channel</td>
<td>Congenital long QT syndrome</td>
<td>(145, 153, 519, 599, 560)</td>
<td>S</td>
</tr>
<tr>
<td>19p13.3</td>
<td>LDLR</td>
<td>Low-density lipoprotein receptor (LDLR)</td>
<td>Familial hypercholesterolemia</td>
<td>(228, 286, 285)</td>
<td>S</td>
</tr>
<tr>
<td>2q13-14</td>
<td>PROC</td>
<td>Protein C</td>
<td>Protein C deficiency</td>
<td>(240, 241, 352, 494)</td>
<td>S</td>
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<tr>
<td>2q11.2</td>
<td>PROS1</td>
<td>Protein S</td>
<td>Protein S deficiency</td>
<td>(502)</td>
<td>S</td>
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<tr>
<td>1p36.23-25.1</td>
<td>SERPINC1</td>
<td>Antithrombin</td>
<td>Type I antithrombin deficiency</td>
<td>(489, 493)</td>
<td>S</td>
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<tr>
<td>17q13</td>
<td>SERPINF2</td>
<td>Alpha 2-antiplasmin (A2AP)</td>
<td>Alpha 2-plasmin inhibitor deficiency</td>
<td>(81, 352)</td>
<td>S</td>
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<tr>
<td>12q13.3</td>
<td>WF</td>
<td>von Willebrand factor (WF)</td>
<td>von Willebrand’s disease type IA</td>
<td>(17, 18, 48, 518)</td>
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**Digestive**

<table>
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<tr>
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<th>Class</th>
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</thead>
<tbody>
<tr>
<td>10q24</td>
<td>ABC2</td>
<td>Multidrug resistance protein 2 (MRP2)</td>
<td>Dubin-Johnson syndrome</td>
<td>(187, 245)</td>
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<tr>
<td>2q24</td>
<td>ABCB11</td>
<td>Bile salt export pump (BSEP)</td>
<td>Progressive familial intrahepatic cholestasis type II (PFIC II)</td>
<td>(192, 335, 524)</td>
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<tr>
<td>13q14.3</td>
<td>ATP7A</td>
<td>Copper-transporting ATPase</td>
<td>Wilson disease</td>
<td>(95, 185, 214, 506)</td>
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<tr>
<td>19q13.2-22</td>
<td>ATP8B1</td>
<td>FIC1 amniophospholipid-transporting ATPase</td>
<td>Progressive familial intrahepatic cholestasis type III (PFIC III)</td>
<td>(134, 374, 506)</td>
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<tr>
<td>4q21.3</td>
<td>FZD4</td>
<td>Fibrogen</td>
<td>Hereditary hyperphosphatemia</td>
<td>(50, 277, 555, 596)</td>
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<tr>
<td>6p21.3</td>
<td>HFE</td>
<td>Hemochromatosis</td>
<td>Hereditary hemochromatosis</td>
<td>(53, 517)</td>
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<tr>
<td>10q11.2</td>
<td>HET</td>
<td>Retinoic acid receptor</td>
<td>Hirschsprung disease</td>
<td>(253, 254)</td>
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**Endocrine**

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<tr>
<td>11p15.1</td>
<td>ABC2</td>
<td>Solute cerebellar neuron receptor</td>
<td>Persistent hyperinsulinemic hypoglycemia of infancy (PHHI)</td>
<td>(481, 561, 562)</td>
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<tr>
<td>3q13</td>
<td>CASR</td>
<td>Calcium-sensing receptor (CaSR)</td>
<td>Familial hypocalciuric hypercalcemia (FHHC)</td>
<td>(203, 210, 380)</td>
<td>S</td>
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<tr>
<td>3q13</td>
<td>CASR</td>
<td>Calcium-sensing receptor (CaSR)</td>
<td>Neonatal severe hyperparathyroidism (NSHPT)</td>
<td>(203, 210, 380)</td>
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<tr>
<td>11p15.5</td>
<td>INS</td>
<td>Proinsulin</td>
<td>Neonatal diabetes (type 1)</td>
<td>(16, 18, 371)</td>
<td>S</td>
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<tr>
<td>19p13.3-13.2</td>
<td>INS</td>
<td>Insulin receptor</td>
<td>Insulin resistance syndrome (type 2 diabetes)</td>
<td>(12, 219, 333, 596)</td>
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<tr>
<td>3p22-21.1</td>
<td>PTH1R</td>
<td>Parathyroid hormone receptor (PTH-R)</td>
<td>Parathyroid hormone resistance</td>
<td>(19)</td>
<td>S</td>
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</table>

**Immune**

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<tr>
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<tbody>
<tr>
<td>19p13.3</td>
<td>ELS</td>
<td>Neutrophil elastase</td>
<td>Severe congenital neutropenia (SCN)</td>
<td>(347)</td>
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<tr>
<td>17q23.1</td>
<td>MPO</td>
<td>Myeloperoxidase (MPO)</td>
<td>Hereditary myeloperoxidase deficiency</td>
<td>(99)</td>
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</table>

**Integumentary**

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>4q21.21</td>
<td>ANTXR2</td>
<td>Anthrax toxin receptor 2</td>
<td>Hyaline fibromatosis syndrome</td>
<td>(101)</td>
<td>S</td>
</tr>
</tbody>
</table>
The Brodsky Lab

Teresa Buck, Ph.D.
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Alan Weissman (NCI)
Peter Wipf (University of Pittsburgh)