“IRE1 Signaling Affects Cell Fate during the Unfolded Protein Response”

Jenn Hou
Burke Group Literature Seminar
November 19th 2016
Protein Synthesis Pathway

1. **Nucleus**: genes are transcribed into mRNA.

2. **Endoplasmic Reticulum**: ribosomes translate mRNA into secretory and transmembrane proteins.

3. **Golgi Apparatus**: proteins are post-translationally modified.

4. **Final Destination**: proteins are secreted from the cell or reside as transmembrane proteins.

What is the Unfolded Protein Response (UPR)?

- Protein misfolding:
  - Glucose deprivation
  - Calcium dysregulation
  - Viral infection
  - Hypoxia

- Aggregation of misfolded proteins leads to ER stress.

- Persistent ER stress turns on the UPR.

- Unmitigated UPR manifests in:
  - Cancer
  - Neurodegenerative disorders
  - Diabetes

www.mun.ca/biology/desmid/brian/BIOL2060/BIOL2060-12/12_08.jpg
UPR is Important for Homeostasis in Secretory Cells

**Neutrophil**

**Secretory Cells**
- Plasma B cells
- Salivary gland cells
- Pancreatic beta cells
- Prominent ER

Images from:
http://drpersian.blogfa.com/9002.aspx
http://intranet.tdmu.edu.ua/data/kafedra/internal/histolog/classes_stud/en/stomat/ptn/1/06%20Blood.%20Lymph.%20Hematopoiesis..files/image011.jpg
Key Mediators of the UPR

The Three Branches of the UPR Exhibit Different Kinetic Behaviors to Protect or Kill Cells

Model for IRE1 Activation

1. IRE1 promoters reversibly associate and make closed dimers.

2. Binding of unfolded proteins to ER luminal domains, drives oligomerization.

3. Trans-autophosphorylation promotes association between dimers.

4. Association into oligomers.

ER Stress Induces IRE1 Mediated Splicing of Xbp-1

Does IRE1 mediated splicing of Xbp-1 persist in the presence of sustained ER stress?


IRE1 Mediated Splicing of Xbp-1 Diminishes after Sustained ER Stress

**Tunicamycin**
Inhibits N-linked glycosylation

**Thapsigargin**
Blocks ER Ca$^{2+}$ Pump

At later time points where IRE1 signaling is turned off, do cells still produce misfolded proteins?


Cells Continue to Produce Misfolded Proteins during Persistent ER Stress

How do the kinetics of the other two branches of the unfolded protein response differ from IRE1 signaling?

The Three Branches of the UPR Exhibit Different Kinetic Behaviors to Protect or Kill Cells

Atf6 Activation Persists during Continual ER Stress

**BiP mRNA** = HSP70 ER chaperone

**Tunicamycin**: Inhibits N-linked glycosylation

**Thapsigargin**: Blocks ER Ca\(^{2+}\) Pump


PERK Activation Does Not Decrease during Continual ER Stress

Chop mRNA = increases expression of proapoptosis genes

Tunicamycin: Inhibits N-linked glycosylation
Thapsigargin: Blocks ER Ca^{2+} Pump

Generation of a Chemically Regulated IRE1 Mutant to Investigate the Downstream Effects of IRE1 Signaling

**ATP-Binding Domain**

Human IRE1: F Q Y I A I E L C A A – 647  
Yeast IRE1: F L Y I A L E L C N L – 750

![ATP and 1NM-PP1](image)

**ATP Analog**

**Human IRE1 (I642G) Mutant:**
- Mutate ATP binding domain
- Mutation disrupts kinase activity
- INM-PP1 allosterically turns on RNAse without the need for ER stress

1NM-PP1 Maintains IRE1 mediated \( Xbp-1 \) mRNA Splicing

1NM-PP1 Maintains IRE1 mediated Xbp-1 mRNA Splicing

Sustained IRE1 Signaling Promotes Cell Viability

Arrows indicate statistical significance.

Retinitis Pigmentosa Leads to Blindness

- Autosomal dominant
- Proline 23 to histidine mutation
- P23H photoreceptors atrophy

Images from:
- http://drstevensoong.com/retinitis-pigmentosa/
- http://institutoholofotes.org.br/retinose-pigmentar/
- http://iovs.arvojournals.org/data/Journals/IOVS/932940/z7g0080656430002.jpeg
Mutant Retinal Cells Display Decreased Cytoprotective BiP and Increased ProApoptotic Chop Levels

Sustained UPR Leads to Retinal Degeneration

Conclusions

• The three branches of the UPR exhibit different kinetics:
  • IRE1 signaling is immediate and transient
  • ATF6 signaling persists
  • PERK signaling is sustained

• Generated an inducible IRE1 signaling mutant that demonstrates constant activity
  • Sustained IRE1 activity promotes cell viability

Critiques:
• Evaluate additional indicators for cell physiology in sustained IRE1 signaling model (cresyl violet staining, and MTT assay) in terms of ER function (post-translational modifications).
• Test if sustained IRE1 activation in the retinitis pigmentosa rat model would protect against retinal degeneration.