Determining the Role of a Transcription Factor in Protein Degradation

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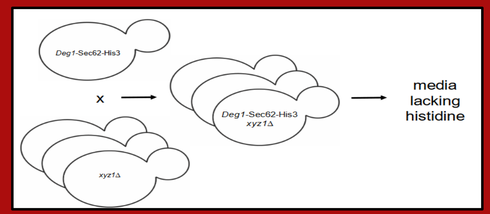
Abstract

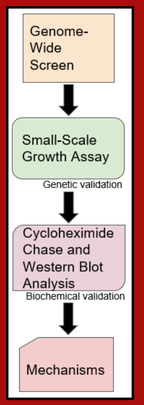
Proteins are essential to life. They perform a variety of functions within the cell, including cell regulation and DNA synthesis. Just as important as protein synthesis is the antiparallel process of protein degradation. A protein must be degraded when it is no longer necessary, is damaged, or behaves aberrantly to prevent organismal harm. Proteins can behave aberrantly by persistently engaging with a protein channel called the translocon, which allows proteins to move across the membrane of the endoplasmic reticulum. In humans, a protein known to clog the translocons is a component of low-density lipoproteins (or "bad cholesterol"). A ubiquitin ligase in yeast known as Hrd1 polyubiquitylates the aberrant protein, tagging it for degradation via the proteasome. The proteasome detects polyubiquitination and degrades tagged proteins, recycling them into shorter fragments. Ubiquitin ligases rarely function alone, and yeast lacking Hrd1 still exhibit residual degradation of translocon-clogging proteins, suggesting the existence of alternative degradation pathways. We performed a genome-wide screen to identify genes that may play a role in protein degradation of translocon-clogging proteins, identifying a potential 150 candidates. Further small-scale reporter assays were performed, confirming the role for 42 genes in protein degradation. Additional biochemical validation using cycloheximide chase showed the requirement of 3 genes, one of which is part of a heterodimeric transcription factor complex involved in lipid synthesis. With the process of protein degradation being conserved in both yeast and humans, validated genes may represent therapeutic targets for patients with elevated levels of cholesterol.

Genome-Wide Screen to identify genes regulating the abundance of translocon-clogging proteins

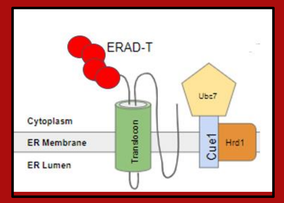
Yeast strains lacking or expressing at reduced levels each of the ~6,000 genes were crossed with yeast expressing the model translocon-clogging protein *Deg1*-Sec62-His3. In the absence of histidine, only mutants with increased abundance of this artificial protein are expected to grow. The yeast were plated on media lacking histidine. ~150 mutants grew in the absence of histidine. These positive results were then validated in small-scale growth assays. Mutants validated in small-scale growth assays were biochemically characterized.

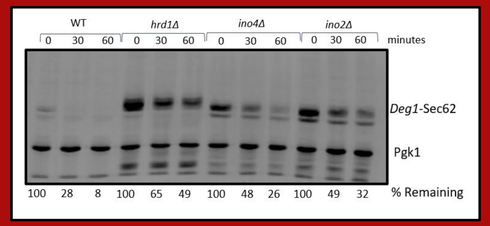


Flowchart of Project



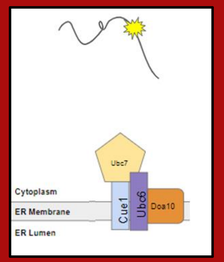
Heterodimeric transcription factors Ino2 and Ino4 contribute to protein degradation at the translocon (ERAD-T)

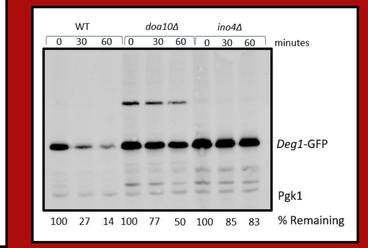




Yeast of the indicated genotypes were transformed with an empty vector plasmid or a plasmid encoding *Deg1*-Sec62 and subject to cycloheximide chase and western blotting to detect *Deg1*-Sec62 and Pgk1.

Ino4 contributes to protein degradation in the cytosol of soluble proteins (ERAD-C)



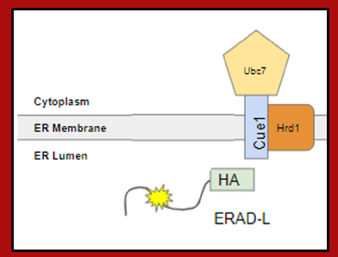


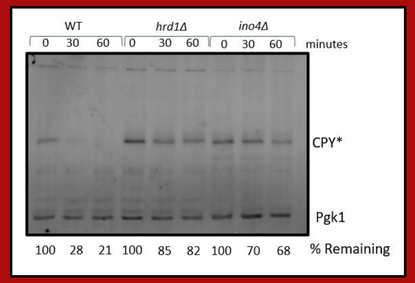
Yeast of the indicated genotypes were transformed with an empty vector plasmid or a plasmid encoding *Deg1*-GFP and subject to cycloheximide chase and western blotting to detect *Deg1*-GFP and Pgk1.

Endoplasmic Reticulum Associated Degradation (ERAD) Pathways

ERAD at the cytosol (ERAD-C), ERAD at the lumen (ERAD-L), ERAD at the translocon (ERAD-T), ERAD at the membrane (ERAD-M), ERAD at the ribosome (ERAD-RA)

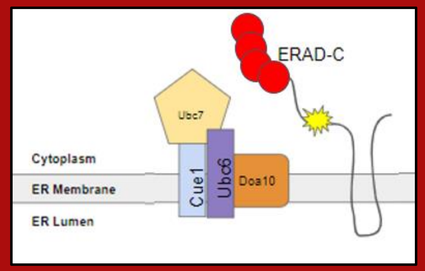
Ino4 contributes to protein degradation in the lumen (ERAD-L)

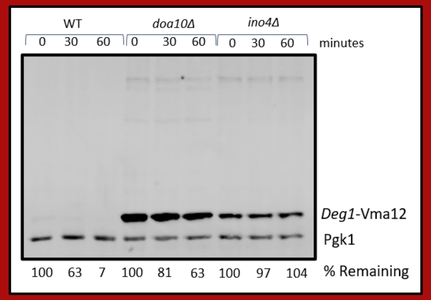




Yeast of the indicated genotypes were transformed with an empty vector plasmid or a plasmid encoding CPY* and subject to cycloheximide chase and western blotting to detect CPY* and Pgk1.

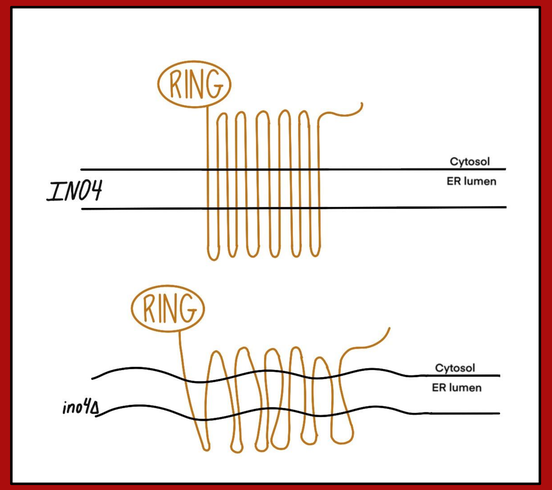
Ino4 contributes to protein degradation in the cytosol of transmembrane bound proteins (ERAD-C)





Yeast of the indicated genotypes were transformed with an empty vector plasmid or a plasmid encoding *Deg1*-Vma12 and subject to cycloheximide chase and western blotting to detect *Deg1*-Vma12 and Pgk1.

Proposed Mechanism for Ino2 and Ino4



Conclusions

- ~150 genes were identified in the genome-wide screen
- 42 genes have been validated in the small-scale growth assay
- Biochemical analysis failed to implicate many genes validated in small-scale growth assays, but 4 genes were identified, including Ino4.
- *INO2/INO4* plays a role in ERAD-T, ERAD-C soluble, ERAD-C transmembrane, and ERAD-L protein degradation.

Future Directions

- Continue to perform biochemical validation to determine the role of Ino4 in other pathways (ERAD-M and ERAD-RA).
- Determine if Ino4 works in the same or in a parallel pathway to Hrd1.
- Determine which parts of the lipid biosynthesis process (regulated by Ino2/Ino4) are important for degradation.
- Validated gene products may represent therapeutic targets for patients with elevated cholesterol.

Funding

- NIH AREA grant GM 111713 (EMR)
- IAS Senior Research Grant (EMR)
- Ball State University Advance Faculty Research Award (EMR)
- BSU Department of Biology Undergraduate Research Grant (ST)
- Ball State University Honors College Fellowship (ST)
- Ball State University ASPiRE Student Research grants (CI, AK, ST)
- BSU Chapter of Sigma Xi Research Award (SW).