INTRODUCTION

Secretory proteins are co-translationally imported into the endoplasmic reticulum (ER) of the cell, where they will be folded, modified, and sent to the Golgi apparatus. The balance between the protein load coming into the ER and the ER’s capacity to fold incoming proteins is critical. If the balance is upset, ER stress can occur, resulting in the accumulation of unfolded and misfolded proteins inside the ER. The cell initiates the Unfolded Protein Response (UPR) to combat ER stress (Walter & Ron, 2011); however, the UPR will signal for apoptosis if the cell cannot recover (Szegedi, Logue, Gorman, & Samali, 2006). ER stress is implicated in many physiological conditions, such as neurodegenerative diseases, Type II diabetes, and heart disease (Kaufman, 2002).

Ire1, an ER transmembrane nuclease, plays an important role in the UPR. Ire1’s cytosolic nuclease domain cleaves the mRNA for X-box binding protein 1 during ER stress. Once cleaved, the mRNA end fragments are ligated together to form a spliced product (Calfon et al., 2002; Yoshida, Matsui, Yamamoto, Okada, & Mori, 2001). Spliced Xbp1 can be translated into an active transcription factor that can turn on a host of genes that increase the folding capacity of the ER and the capacity of the secretory pathway (Harding et al., 2003; Travers et al., 2000). In addition, Ire1 cleaves other mRNAs localized to the ER membrane, leading to their subsequent degradation and thereby reducing the load on the ER in a pathway termed regulated Ire1-dependent decay (RIDD; J. Hollien & Weissman, 2006; Julie Hollien et al., 2009).

We have identified differences in mRNA targets in the RIDD pathway between Drosophila Ire1 and mammalian Ire1 (summarized in Table 1). Mammalian Ire1 has only a handful of RIDD targets, and sequence based requirements have to be met in order for degradation to take place (Moore & Hollien, 2015). On the other hand, Drosophila Ire1 has a broad specificity in targets, with localization to the ER membrane being both necessary and sufficient for degradation (Gaddam, Stevens, & Hollien, 2013). In my research, I have sought to answer the question of how the difference in RIDD target specificity between Drosophila and mammalian cells is mechanismically achieved.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>RIDD target features</th>
<th>RIDD specificity</th>
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<tbody>
<tr>
<td>Mammalian</td>
<td>Specific sequence and structural motif</td>
<td>Only a few targets; high specificity</td>
</tr>
<tr>
<td>Drosophila</td>
<td>Localization to the ER membrane</td>
<td>Many targets; broad specificity</td>
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*Table 1* Comparison of RIDD in mammalian and *Drosophila* cells
RESULTS AND DISCUSSION

We compared the *Drosophila* Ire1 sequence to representative mammalian Ire1 sequences, and found that while the sequence is mostly conserved, *Drosophila* has about 100 extra amino acids at its C terminus (Figure 1). We hypothesized that this C terminal domain is responsible for the difference in RIDD activity.

![Comparison of Ire1 sequences](alignment.png)

*Figure 1 Comparison of Ire1 sequences*
Alignment of C terminal ends (starting at amino acid 843) of human Ire1 (hIre1) and *Drosophila* Ire1 (dIre1).

To test this hypothesis, we made a panel of plasmids encoding variants of Ire1 under copper-inducible promoters. The panel included wild type *Drosophila* Ire1 (dIre1 wt), human Ire1 (hIre1 wt), a nuclease-dead mutant of *Drosophila* Ire1 (dIre1 nd), and a truncated version of *Drosophila* Ire1 ending at amino acid 958, which removes the additional amino acids at the C terminus not found in mammalian Ire1 (dIre1 ΔCTD).

We co-transfected the Ire1 variants in *Drosophila* S2 cells with known RIDD mRNA reporters. We then treated with copper to induce transcription of the Ire1 variant plasmids, and thereby overexpress the protein in the cell. Under these conditions, Ire1 can dimerize and activate as it would under ER stress. We purified total RNA from these cells and quantified relative RNA levels of the reporters by qPCR. mRNA levels of dSparc, a *Drosophila* RIDD target, are not significantly changed upon expression of dIre1 ΔCTD (Figure 2A). dAct 5C was used as a negative control, as actin should not be a RIDD target in any case. mBlos1 mRNA, both a *Drosophila* and mammalian target, is reduced in the presence of dIre1 ΔCTD, suggesting it is a target (Figure 2C). mBlos1S, the negative control, is a stabilized form of mBlos1 that is not targeted by RIDD (Moore & Hollien, 2015).

My results suggest that without its C terminal domain, *Drosophila* Ire1 is no longer capable of degrading such a wide range of targets, and reverts to more specific targets similar to the mammalian version. This work sheds light on how *Drosophila* Ire1 may be able to have different RIDD activity than mammalian Ire1. We are currently working to corroborate these results by stably transfecting our Ire1 constructs into mammalian Ire1 knockout cells and monitoring the degradation of various RIDD targets in these cells.
Figure 2 Deletion of Drosophila Ire1’s C terminal domain (CTD) results in a change in RIDD specificity

A-B) Ire1 constructs were placed under a copper-inducible promoter and transiently co-transfected into Drosophila S2 cells with actin-promoted dSparc or dAct5C. We induced expression with Cu for 7 hours, collected RNA, and monitored dSparc or dAct5C mRNA levels by qPCR. Reporter mRNA levels were normalized to mRNA levels of Rpl19. Three biological replicates are shown, with matching symbols designating experiments done side by side. Bars represent average of the three replicates. * designates significant difference from dIre1 nd with p<0.05

C-D) Ire1 constructs were placed under a copper-inducible promoter and transiently co-transfected into Drosophila S2 cells with actin-promoted mBlos1 or mBlos1S. We induced expression with Cu for 7 hours, collected RNA, and monitored mBlos1 or mBlos1S mRNA levels by qPCR. Reporter mRNA levels were normalized to mRNA levels of Rpl19. Three biological replicates are shown, with matching symbols designating experiments done side by side. Bars represent average of the three replicates. * designates significant difference from dIre1 nd with p<0.05

REFERENCES


