Ste24 Substrate Specificity in Translocon Quality Control

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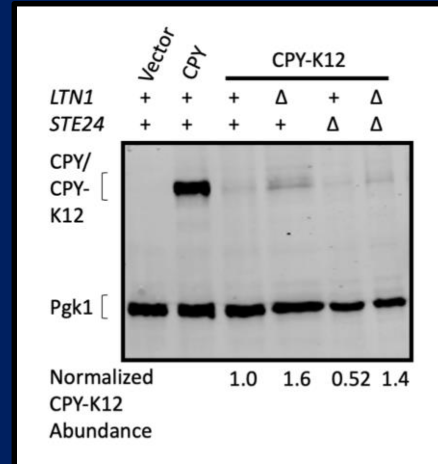
Abstract

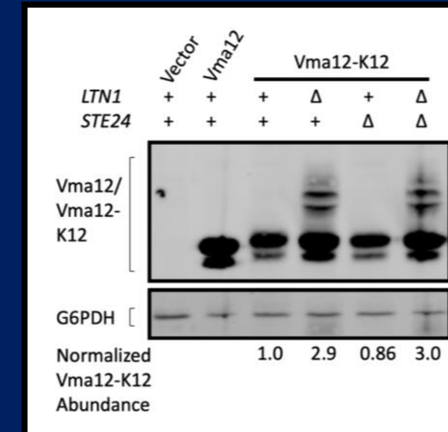
Translocons are molecular channels that allow proteins to cross membranes in a process called translocation. This enables proteins to reach their appropriate cellular locations to perform their specific functions. If translocons become clogged, they cannot be used as passages for other proteins. Unresolved translocon clogging may result in diseases, such as type 2 diabetes. There are two modes of translocation, posttranslational translocation (PTT) and cotranslational translocation (CTT). Errors can occur in both processes, resulting in clogged translocons. Ste24 is a conserved enzyme that degrades clogging proteins that enter the translocon via PTT. It is unknown if Ste24 also degrades proteins that use CTT. We tested the hypothesis that Ste24 degrades clogged proteins that undergo CTT, using a *Saccharomyces cerevisiae* model system. The abundance of proteins engineered to clog translocons during CTT was analyzed via western blot analysis in yeast containing or lacking *STE24*. We found Ste24 does not promote degradation of proteins that undergo CTT. These data contribute to the knowledge of how cells maintain functional translocons, suggesting translocon quality control enzymes exhibit strong specificity in the types of translocon-clogging proteins they degrade. Our results may have implications for understanding and treating diseases associated with translocon dysfunction.

Ste24 is not required for CPY-K12 degradation



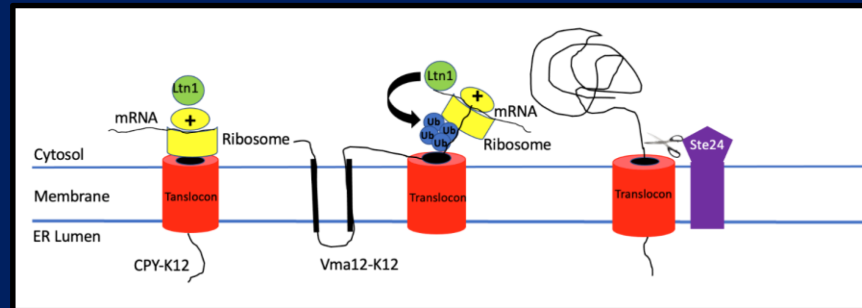
Yeast of the indicated genotypes were transformed with an empty vector or plasmid encoding soluble proteins CPY or CPY-K12. CPY and CPY-K12 were detected using western blot analysis. Pgk1 serves as a loading control.

Ste24 is not required for Vma12-K12 degradation



Yeast of the indicated genotypes were transformed with an empty vector or plasmid encoding integral membrane proteins Vma12 or Vma12-K12. Vma12 and Vma12-K12 were detected using western blot analysis. G6PDH serves as a loading control.

CPY-K12 and Vma12-K12 are model translationally stalled ER-targeted proteins



CPY and Vma12 are model ER-targeted proteins. The addition of a sequence of 12 lysine residues (K12) at the proteins' C-termini results in highly positive poly-lysine tails (indicated by "+"). Once a poly-lysine tail of an ER –targeted protein reaches the highly negative ribosome exit tunnel, translation stalls at the translocon, in part due to electrostatic interactions. The ubiquitin ligase Ltn1 contributes to the degradation of such proteins. Ste24 promotes degradation of non-ribosome-associated translocon-clogging proteins. This work demonstrates that Ste24 does not contribute to translationally stalled translocon-associated proteins.

Conclusion

- Ste24 does not contribute to protein degradation of model, engineered ribosome-stalled, translocon-engaged proteins.

Future Directions

- Identify the physiological/non-engineered substrates of Ste24 and Ltn1
- Characterize substrate recognition mechanisms utilized by Ste24 and Ltn1

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