Protein Quality Control in *Candida albicans*

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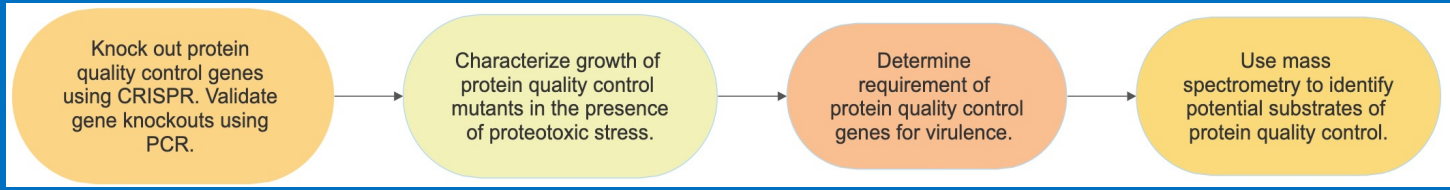
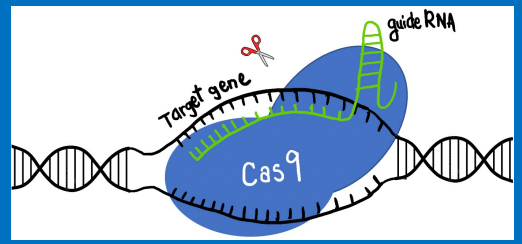
Abstract

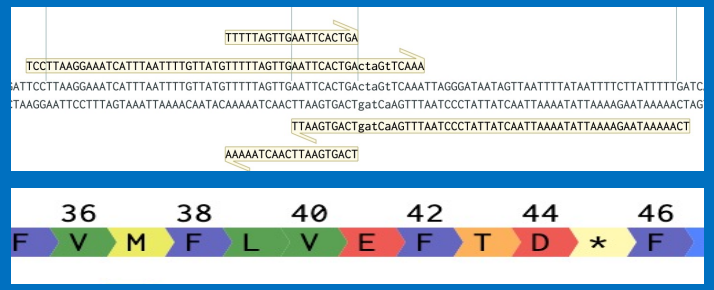
Candida albicans is an opportunistic fungal pathogen that resides as normal flora in the human gastrointestinal tract and mouth. However, upon a patient's immunosuppression due to a preexisting condition or an organ transplant, the yeast can colonize nearly every tissue and organ, causing life-threatening infections. Few treatment options exist for *Candida* infections; therefore, it is essential to understand the molecular mechanisms that contribute to its virulence. We are characterizing the roles of four genes involved in protein quality control, which is uncharacterized in *C. albicans*. Using CRISPR, we are generating homozygous deletions of *HRD1, DOA10, UBC7,* and *STE24*. These knockout strains are being characterized and compared to wild type yeast using growth assays to determine how well they grow in the presence of compounds predicted to increase the abundance of misfolded proteins. A virulence assay will then be performed utilizing a characterized insect infection model, *Galleria mellonella* wax moth larvae. Survival of larvae injected with each knockout strain will be compared to that of larvae injected with the highly virulent wild type strain. These experiments represent a novel investigation of protein quality control in *C. albicans* and have the potential to reveal new therapeutic targets for fungal infections.

Flowchart of Project



CRISPR in *C. albicans*

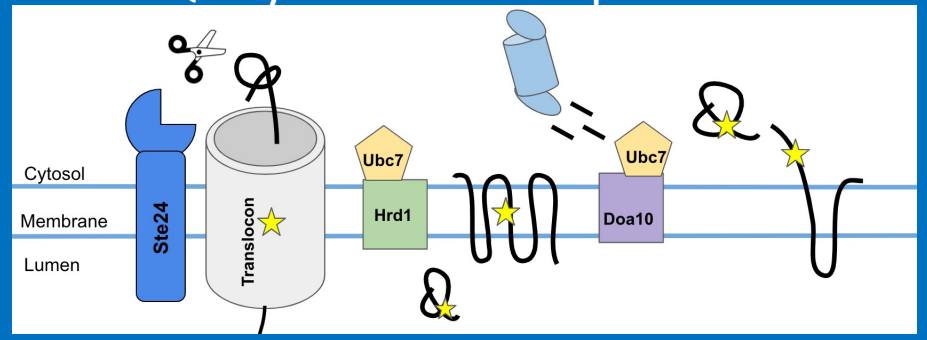




Future Directions

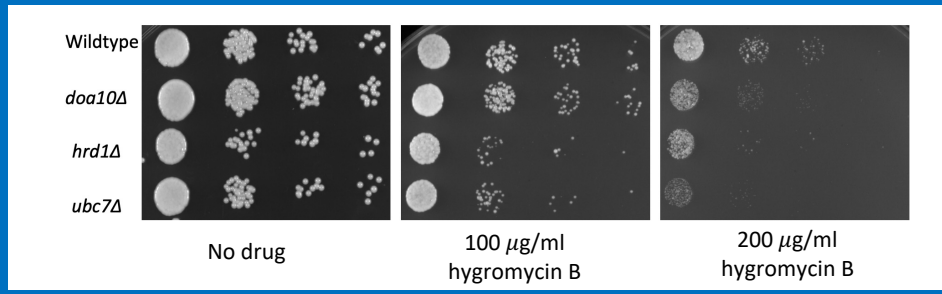
- Knock out *STE24*
- Characterize protein quality control mutants' ability to withstand a variety of stress conditions
- Determine if protein quality control genes are required for virulence, suggesting use as antifungal drug targets

Protein Quality Control at the Endoplasmic Reticulum



Hrd1, Doa10, Ubc7, and Ste24 are protein quality control enzymes at the ER membrane. Hrd1 and Doa10 are ubiquitin ligases, Ubc7 is a ubiquitin-conjugating enzyme, and Ste24 is a zinc metalloprotease. The star represents a degron (an element that is recognized by protein quality control enzymes) at each location in or near the ER.

DOA10, HRD1, and *UBC7* confer resistance to proteotoxic stress



Strains with homozygous deletions of indicated genes were spotted onto rich media without drug or with increasing concentrations of hygromycin B (which is predicted to increase abundance of aberrant proteins) and incubated overnight at 30°C.

Conclusions

- *DOA10, HRD1,* and *UBC7* have been successfully knocked out in *C. albicans*
- Loss of *DOA10, HRD1,* and *UBC7* sensitizes cells to proteotoxic stress

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