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Funding Source: McNair Scholars Program

Reconstituting the Cvt pathway in *pichia pastoris*

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In yeast, autophagy is primarily a response to nutritional stress in which the cell sacrifices some of its cytoplasm and organelles in order to survive. *Saccharomyces cerevisiae* contains a specialized pathway for the delivery of certain proteins to the vacuole by selective autophagy called the Cytoplasm to vacuole targeting (Cvt) pathway. While autophagy is generally believed to be non-selective, the Cvt pathway is highly specific, transporting only a few selected proteins to the vacuole. The two known cargo proteins of the Cvt pathway are -mannosidase1 (Ams1) and the inactive precursor of aminopeptidase1 (prApe1). The pathway begins in the cytosol when prApe1 oligomerizes into dodecamers, which then aggregate to form the Ape1 complex. We are using the Ape1 aggregate as a model system for studying how protein aggregates are delivered to the vacuole/lysosome by constitutive and selective autophagy. We have introduced Ape1 from *S. cerevisiae* into the yeast *Pichia pastoris* in an attempt to reconstitute the Cvt pathway in this yeast. *P. pastoris* is related to *S. cerevisiae*, but does not contain the Cvt pathway. We are currently studying the requirements for the uptake of Ape1 into the vacuole in *P. pastoris*.

This project was completed to fulfill a Capstone requirement.