Rebecca Rone

Major: Biological Engineering
University: University of Missouri-Columbia
Faculty Mentor: Dr. Per Stromhaug
Mentor Department: Biological Sciences
Funded by: Life Sciences Undergraduate Research Opportunity Program

Making the Cvt pathway/autophagy in vitro

Rebecca Rone and Per Stromhaug

Autophagy, Greek for “self eating”, occurs in all eukaryotic cells to remove damaged or unwanted organelles or to provide a source of nutrients during starvation. In autophagy, a double membrane surrounds a cluster of contents, damaged organelles, bulk cytoplasm, and aminopeptidase I (Ape1), forming an autophagic vesicle to be sent to the vacuole. A unique Cvt pathway occurring in *Saccharomyces cerevisiae*, includes a membrane encapsulation of only Ape1 to be transported directly to the vacuole. Most of the proteins needed for autophagy and the Cvt pathway have been identified, but their roles have yet to be determined. In the Cvt pathway, the Ape1 aggregates to form a dodecamer before forming a Cvt complex by combining with the protein Atg19. The Cvt complex affixes to the autophagic membrane presumably with the aid of Atg11 and Atg8. An array of proteins, Atg9-Atg2 complex, Atg1, and Pi3-kinase complex, help complete the formation of the autophagic vesicle encompassing Ape1. The autophagic vesicle then fuses with the vacuole releasing the Ape1 into the lumen of the vacuole. Until now, only whole cells have been used to examine autophagy. Due to the complexity of the whole cell, the functions of the all proteins needed for autophagy have not been determined. Our main goal is to be able to construct the Cvt pathway in vitro. We have begun inserting the *APE1* gene from *S. cerevisiae* into another strain of yeast, *Pichia pastoris*, where the Cvt pathway does not occur. Once the proteins are expressed in *P. pastoris*, we will study the interaction of Ape1 with other Cvt proteins. From *P. pastori* we will extract Cvt proteins for examining in a test tube. While *in vitro*, it will be possible to determine the molecular function each protein contributes to autophagy. A better understanding of the process of autophagy will be beneficial to understanding and treatment of many diseases such as cancer, liver disease, muscular disorder, neurodegeneration and bacteria infections.