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Making the Cvt pathway/ autophagy *in vitro*

Autophagy occurs in all eukaryotic cells to remove damaged or unwanted organelles or to provide a source of nutrients during starvation. Alterations in autophagy are linked to several human diseases including cancer, Parkinson's and Alzheimer's disease. While most of the proteins required for autophagy now have been identified, the molecular mechanism of how autophagic vacuoles form remains elusive. Our research aims to better understand the proteins functioning in autophagy through the unique Cytoplasm to vacuole targeting (Cvt) pathway *in vitro.* The Cvt pathway occurring in *Saccharomyces cerevisiae*, includes a membrane encapsulation of only Ape1 to be transported to the vacuole. Ape1 aggregates in cytosol to form a dodecamer before forming a Cvt complex by combining with Atg19. The Cvt complex affixes to the autophagic membrane presumably with the aid of Atg11 and Atg8. An array of proteins helps complete the formation of the autophagic vesicle. The autophagic vesicle then fuses with the vacuole releasing Ape1 into the lumen of the vacuole. We have inserted the *APE1* gene from *S. cerevisiae* into the yeast strain *Pichia pastoris,* where the Cvt pathway does not occur. The results display an aggregation of Ape1 but not a transfer to the vacuole. We have begun expressing Atg19 in *P. pastoris* with Ape1 to study the formation of the Cvt Complex. The results show binding of Atg19 to Ape1, but no transport to the vacuole. Once the Cvt pathway is induced in *P. pastoris*, we will extract Cvt proteins for examining in a test tube.