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Reconstituting the Cvt pathway: An approach to unraveling autophagy
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Autophagy, literally self-cannibalism, is a highly regulated catabolic process that is important in the maintenance of all eukaryotic cells. In humans, both excess and insufficient autophagy is linked to many diseases including cancer, Huntington’s and Parkinson’s diseases. Autophagy is the process in which defective and unwanted organelles along with cytoplasm are taken into an autophagosome and transported to the vacuole or lysosome to be degraded and used to build new macromolecules. Although most of the proteins involved in the autophagic pathway are known, their specific functions remain unknown. In order to better understand the molecular mechanisms of autophagy, we are using a specialized autophagic pathway found only in the yeast *Saccharomyces cerevisiae*: the Cytoplasm to vacuole targeting (Cvt) pathway. The Cvt pathway is used to deliver the inactive precursor of aminopeptidase1 (prApe1), to the vacuole. The pathway begins in the cytosol when prApe1 aggregates into dodecamers, which then form the Ape1 complex. This is followed by Atg19 binding to the Ape1 complex. Atg19 interacts with Atg11 (and possibly Atg8), which recruits autophagic membrane. These proteins along with a few others complete the Cvt vesicle, which then fuses with vacuolar membrane allowing Ape1 to be released into the lumen of the vacuole. We are attempting two different approaches to understand the Cvt pathway. In our first approach we are trying to reconstitute the Cvt pathway in *Pichia pastoris*, a related yeast strain in which the pathway does not occur. So far we have been successful in expressing *S. cerevisiae’s ape1* and *atg19* genes in *P. pastoris*. In these strains the Ape1 complex aggregates and Atg19 co-localizes. We are still working on expressing *atg11* in *P. pastoris*. Our second approach is to form the Cvt vesicle in vitro. In *S. cerevisiae* stable Ape1 complexes can be observed microscopically. Ape1 is also stable when co-localized with Atg19 as seen in the microscope. We are currently trying to purify the Ape1 complex, which is essential for using as a scaffold for in vitro formation of the Cvt vesicle.