

CHARACTERIZATION OF *PICHIA PASTORIS* VAC8 PROTEIN ASSOCIATION WITH THE VACUOLAR MEMBRANE. Brian T. Boehmer, Katie Hoeflerlin, Michelle R. Fry*, Bradley University, Department of Chemistry and Biochemistry, Peoria, IL 61625, mfry@bradley.edu

In *Pichia pastoris*, Vac8p has been shown to be critical for vacuolar inheritance, protein trafficking in the cytoplasm-to-vacuole pathway, and autophagy. PpVac8p is an armadillo repeat protein that contains putative fatty acylation consensus sites at the amino terminus. We had previously generated mutated forms of PpVac8p that lacked one or both fatty acylation consensus sites and demonstrated that they exhibited loss of function in microautophagy. In these studies, we compared the location and membrane interactions of wild-type and amino-terminal modified forms of PpVac8p to better understand the structure and function of PpVac8p. We investigated the subcellular location of PpVac8p in accordance with its involvement in the late molecular events of microautophagy using subcellular fractionation and Western blot analysis. These studies demonstrated that wild type Vac8p cosediments largely with 13,000 x g pellet which includes the vacuole, while forms of Vac8p lacking one or both fatty acylation consensus sites localized in both the 13,000 x g pellet and 13,000 x g supernatant. Secondly, we used differential extraction to determine the type(s) of interactions displayed between PpVac8p and the vacuolar membrane. The PpVac8p is partially released from the vacuolar membrane by sodium carbonate, but is highly resistant to removal by NaCl and urea. Surprisingly, PpVac8p remained associated with the 13,000 x g pellet upon treatment with 1% Triton X 100 but seems to either form detergent-resistant aggregated structures of higher molecular mass or is structurally altered in the presence of Triton X 100 to retard mobility on electrophoresis.