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A multicopy suppressor screen in yeast to look for negative regulators of Ser13 phosphorylation-based trafficking to the pre-vacuolar compartment

Resident membrane proteins of the yeast trans-Golgi network (TGN) frequently cycle between the TGN and both the early and late (prevacuolar) endosomal compartments. The model yeast TGN protein, A-ALP, which contains the cytosolic domain of the TGN protein, depeptidyl aminopeptidase A (DPAP A), and the transmembrane and lumenal domains of the vacuolar membrane protein, alkaline phosphatase, have been studied. The cytosolic domain of A-ALP has been shown to be phosphorylated in vivo and in vitro. Of the 25 potentially phosphorylatable residues, only one, Ser13, was observed to influence trafficking between the TGN and endosomes. It has been suggested that phosphorylation of Ser13 is required for trafficking of A-ALP from the TGN to the pre-vacuolar compartment, which implies that phosphorylation of Ser13 may act as a switch for association of A-ALP with vesicular trafficking machinery. It is also important to note that once A-ALP reaches the vacuole by way of the PVC, it is cleaved in the lumenal domain, which allows us to follow the trafficking of the protein. We designed a screen to overexpress negative regulators, such as phosphatase, so that A-ALP will exhibit slower trafficking to the pre-vacuolar compartment. Yeast that seemed to have slower A-ALP trafficking to the PVC were identified by an ALP Overlay Assay. These positives are now being screened by western blot in order to verify that these cells exhibit slower trafficking to the PVC and eventually the vacuole. This can be determined by a western blot by examining the amount of processing of A-ALP in the vacuole. Those that are processed less give the indication of slower trafficking to the PVC, which is possibly the result of high phosphatase activity.