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An *in vitro* binding assay to identify amino acid residues in Ste13p necessary for Ste13p/Amp1p interaction

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Certain proteins in eukaryotic cells, such as the yeast *Saccharomyces cerevisiae*, are compartmentalized into intracellular organelles through trafficking among the trans-golgi network (TGN), the early endosome (EE) and the late endosome (LE). Some of these proteins are then transported to the vacuole. Ste13p, an enzyme that processes the mating pheromone α-factor in yeast, moves through from the TGN to the EE and LE and back again. The retrieval of Ste13p from the EE is facilitated by Apm1p, a component of the AP-1 adaptor complex. It has been shown that Apm1p interacts with residues 1-12 of the amino terminus of Ste13p when the residues are fused to glutatione-s-transferase (GST). My project aims to determine which of these residues are essential for this interaction. In order to do this I created constructs with different deletions in this region of the gene for Ste13 in the context of a Ste13-GST fusion construct. I transformed these constructs into E. coli, expressed the Ste13-GST protein, and attached these proteins to gluthatione agarose beads. I will use these beads to perform pull down assays with purified Apm1p tagged with 6xHis. If the Apm1p is pulled down by the Ste13-GST beads but not by the GST alone, this will indicate that it associates with Ste13p. Mutant derivatives of Ste13-GST will be tested in the same fashion. Through analysis of the different mutants, we will be able to determine which amino acids are critical for this association, and by extension, critical for Ste13p retrieval from the EE back to the TGN.