Briana Sprick, Biology

University: University of Missouri-Columbia
Year in School: Senior
Hometown: Kansas City, Missouri
Faculty Mentor: Dr. Steven Nothwehr, Biological Sciences
Funding Source: Life Sciences Undergraduate Research Opportunity Program

Effect of mutations on Ste13p protein trafficking in yeast

Briana T. Sprick, Ricardo Restrepo, and Steven F. Nothwehr

Retrieval of Ste13, a Golgi integral membrane protein in yeast, from the early endosome to the trans-Golgi network (TGN) is mediated by binding of the protein to the clathrin adaptor AP-1, which interacts with the first 12 residues of the Ste13 cytosolic domain. Deletion of this region of Ste13 to prevent AP-1 association significantly accelerates the rate of trafficking from the TGN to the pre-vacuolar compartment (PVC). In order to pinpoint which specific residues are critical for AP-1 binding, we created mutants with various deletions in the first twelve residues of the cytosolic domain of A-ALP, a model protein comprised of the cytosolic region of Ste13 fused to alkaline phosphatase to assess their processing rates. The mutations are: deletion of amino acids 2-11, deletion of amino acids 4-11, deletion of amino acids 6-11, deletion of amino acids 8-11, and deletion of amino acids 10-11. Processing kinetics were assessed using [35-S]methionine pulse-chase experiments. Experiments thus far demonstrate that deleting only residues 10 and 11 has the same effect as deleting the entire 2-11 region. This suggests either that the first 12 residues of the cytosolic domain must remain entirely intact for the protein to bind to AP-1, or that residues 10 and 11 are especially critical for AP-1 binding.