

Identification of novel interactors of *SEC6* via genetic suppressor screen using a *Saccharomyces cerevisiae* genomic DNA library

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Polarized protein secretion is a fundamental process for all organisms, from yeast to higher eukaryotes. The secretory pathway in eukaryotes includes many steps mediated by hundreds of essential proteins. Our interest lies on Sec6, which is an 88kDa protein subunit of the tethering complex named exocyst, which is known to play diverse roles in recognition, tethering, and SNARE-mediated fusion of secretory vesicles. Previous work on Sec6 done by Munson and Songer (2009) explored phenotypes of two novel *SEC6* mutant proteins, whose conserved surface amino acids had been altered. Both *sec6-49* and *sec6-54* mutant alleles displayed severe temperature sensitive growth and secretion defects. Interestingly, at non-permissive conditions trafficking of secretory vesicles to the plasma membrane was unimpaired, but none of the exocyst subunits were correctly polarized at sites of secretion. Biochemical analyses examining the state of exocyst assembly in both mutant backgrounds showed that the complex is intact at non-permissive conditions. We hypothesize that Sec6 has an important anchoring function for the exocyst, and that mislocalization of exocyst stems from compromising Sec6's interaction with an unknown factor(s). Our research aims to identify and validate novel interactors of Sec6 that are critical for anchoring the exocyst complex at the plasma membrane. We utilized a classic genetic high copy suppressor screen to identify genes that suppress the temperature sensitive (ts) phenotype of *sec6-49* cells upon overexpression. The plasmids from cells that survived temperature shift after transformation were purified, and genomic inserts were confirmed via restriction digests. Currently, sequencing via primers flanking the genomic insert has been initiated to identify the location of the genomic inserts and candidate genes. Overall, the isolation and characterization of novel interactors will shed light on mechanistic details of Sec6 function, which is critical for understanding mechanistic details of quality control in the secretory pathway.