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## **Creating a temperature sensitive mutant of Atg21 using intein**

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Autophagy is the highly regulated process by which a cell sequesters pieces of itself into vesicles called autophagosomes to recycle old components or to respond to nutritional stress. In addition, *S. cerevisiae* uses a specialized form of autophagy called the Cvt pathway to deliver the protease Aminopeptidase I (Ape1) to the vacuole. The Cvt pathway utilizes the same proteins and molecular mechanisms as autophagy. Atg 21 belongs to a novel family of phosphoinositide binding proteins and is essential for the Cvt pathway but not for nitrogen starvation-induced autophagy in *S. cerevisiae*. Based on previous studies combined with the location of Atg21 it has been proposed that the protein might be involved in generating new autophagic membrane or recruiting existing membrane to the site of sequestering vesicle formation but the exact function has yet to be determined. To further research the function of Atg21 a temperature sensitive inducible expression system was designed using Intein. Intein is a protein segment that can excise itself from a protein and rejoin the remaining portion (Extein) of the protein with a peptide bond. Intein mediated protein splicing occurs after mRNA translation. The presence of one of three conserved residues at the N-terminus (Ser, Thr, or Cys) and one of two dipeptides at the C-terminus (His-Asn or His-Gln) are essential for the splice junctions. Six cysteine residues (two near the N-terminus, two in the conserved domain, and two near the C-terminus) in Atg21 were selected as insertion sites for Intein. A temperature sensitive intein (TS-1/TS-19) was inserted at each different site on Atg21. Wild Type Intein was inserted in each site as a control for TS-1/TS-19 containing Atg21 cells. The expression of these Intein containing Atg21 cells has been verified by Western blotting and further experiments will now be conducted to verify the function of Atg21.