Characterization of a Novel Mutation in the COPI Vesicle on Binding to Dilysine Motifs

Kimberly Felipe[1], Sara Custer,[2] Elliot J. Androphy[3]

[1]Indiana University School of Medicine, [2,3]Indiana University School of Medicine, Department of Dermatology

Background/Objective: The heptameric COPI coatomer complex is involved in the formation of vesicles and the intracellular trafficking of proteins between the Golgi and Endoplasmic Reticulum as well as throughout the cytoplasm. Members of the COPI complex bind dilysine motifs found in the C-terminal domain of the cargo protein, particularly KKxx or KxKxx. We generated a point mutation in the WD40 domain of the COPI alpha subunit (α-COP). We hypothesized that the E269V mutant α-COP would not co-immunoprecipitate (co-IP) COPI cargo proteins terminating with a dilysine domain of KxKxx (Nucleolin and Stasimon/Tmem41b), but would bind cargo proteins terminating in KKxx (FLAG-Syntaxin17). We predicted that a mutation in the α-COP C-terminus, which impairs interaction with ε–COP, would not affect its ability to co-IP dilysine-containing cargo.

Experimental Design or Project Methods: HEK-293TT cells were transfected with Myc-tagged wild-type, E269V, and triple mutant (3X) α-COP. The E269V α-COP mutant has an amino acid change at position 269 from glutamic acid to valine. The triple α-COP mutant has three amino acid changes that eliminate binding with the ε–COP COPI subunit. Transfected α-COP was immunoprecipitated using magnetic anti-Myc beads. Endogenous Nucleolin was immunoprecipitated using magnetic Protein A beads conjugated to rabbit polyclonal anti-Nucleolin antibody. Western blots of inputs and immunoprecipitates of each experiment were conducted to determine the ability of α-COP to co-IP C-terminal dilysine-containing proteins.

Results: Endogenous Nucleolin and Stasimon co-immunoprecipitated with WT and 3X α-COP, but not E269V α-COP.

Conclusions and Potential Impact: The inability of mutation E269V to co-IP dilysine proteins implies that the WD40 domain of the COPI α-COP protein is required for binding to KxKxx-terminating proteins, as typified by Nucleolin and Stasimon.The C-terminal 3X mutation shows that ε-COP is not necessary for dilysine recognition and implies that α-COP directly binds to this KxKxx motif.