Targeting the Role of Tyrosine in Amot Protein-Lipid Binding Events

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Angiomotins (Amots) are a family of adaptor proteins that have been shown to control cell proliferation and differentiation. Amots can *selectively* bind with high affinity to phosphoinositol containing membranes through the Amot coiled-coil homology (ACCH) domain. This binding event is linked to endocytosis, changes in cellular polarity, and apical membrane sequestration of nuclear transcription factors associated with development of cancerous phenotypes. Although the lipid selectivity of the protein has been well characterized, the residues involved in the ACCH domain binding these membranes have not been fully described. Understanding the structure-function relationship may provide pathways to modulate protein sorting and downstream signaling events inducing cellular differentiation, cancer cell proliferation, and migration. The fluorescent properties of the ACCH domain were previously used to characterize the binding event. However, the relative proximity of the five native tyrosines to the membrane may have led to differences in perceived lipid binding affinities based on fluorescence resonance energy transfer with fluorescently tagged lipids. A variety of short peptides correlating to the amino acid sequence of Amot surrounding these tyrosines were assayed and observed in different membrane mimicking environments. This was done to determine if each tyrosine had the ability to bury into the hydrophobic region of the membrane mimicked by the carbon chain lengths (alcohol study), or simply interacted with the hydrophilic head groups of the lipid (liposome study). In addition, the full length Amot80 ACCH domains (wild-type and tyrosine-to-phenylalanine mutants) were screened for trends in the varying environments. Interactions were characterized by shifts in maximum wavelengths for absorbance, excitation and emission peaks. A characterization of these shifts with respect to what is seen with the various tyrosine and phenalanine mutants may further our understanding of whether each tyrosine is buried within the protein or interacts with the head groups of the membrane.