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

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Amot proteins have been shown to control cell proliferation and differentiation and can *selectively* bind with high affinity to phosphoinositol containing membranes. This binding event is linked to endocytosis, changes in cellular polarity, and apical membrane sequestration of nuclear transcription factors associated with development of cancer phenotypes. Although the lipid selectivity of the protein has been well characterized, the mechanisms involved in the Amot coiled-coil homology domain (ACCH) binding these membranes are not yet known. The fluorescence properties of the ACCH domain were used to characterize the binding event, however it became clear each of the five native tyrosines proximity to membrane might differ based on fluorescence resonance energy transfer experiments 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 to determine if each tyrosine had the ability to bury into the hydrophobic region of the membrane (alcohol study), or simply interacted with the hydrophilic head groups (liposome study). Interactions were characterized by shifts in absorbance, excitation and emission scans peaks. A characterization of these shifts with respect to what is seen with the various tyrosine-phenalanine mutants will further our understanding of whether each tyrosine is buried within the protein or interacts with the membrane.

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