SECTION II

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The presence of several enzymatic activities related to the “PI cycle,” such as PLC and PI kinase in nuclear matrix, raised the possibility that a separate nuclear phosphoinositide system exists, but the exact processes that are regulated by the PI lipids and their modifying enzymes have been elusive. The widely studied PI3K has been implicated in a large number of nuclear processes. It has been shown to function in the nuclear speckles where it aids in mRNA processing, mRNA export, ribosome biogenesis, DNA replication and participates in several cell survival signalling (Davis WJ et al., 2015). PIPKIα, in addition to being associated with membrane dynamics, has recently been revealed to possess an exciting role of in the nucleus. The presence of endogenous PIPKIα in nuclear speckles at the sites of pre-mRNA processing has been described earlier (Boronenkov I.V. et al., 1998). Recent studies described the association of a non-canonical poly(A) polymerase, named StarPAP with PIPKIα enzyme in nuclear speckles (Mellman D.L. et al., 2008). StarPAP was found strongly stimulated by PIP2 and responsible for the control of the expression of selected group of genes (Mellman D.L. et al., 2008). Nuclear targeting of phosphoinositide metabolizing enzymes is achieved through various mechanisms. Three putative nuclear localization signal (NLS) polybasic motif in p110β of PI3K explains its nuclear import (Kumar A et al., 2011). In addition to having NLS, others like PIPKIIβ is targeted to the nucleus by a “kinase insert region” (Barlow et al., 2010). PIPKIα neither has an NLS nor any “kinase insert region”, yet has been shown to possess both cytoplasmic and nuclear distribution. While large number of studies warrant for the spatial distribution of PIPKIα in the asynchronous nuclei of mammalian cells, the organization of the enzyme during the various stages of the cell cycle still remains elusive. Based on the above observation, our objective of the study is:
A: To elucidate the molecular mechanism(s) responsible for nuclear translocation of PIP5K in the absence of an NLS and a “Kinase Insert Region”.

1. Biochemical & Microscopic characterization of the prevalent PTMs linked to PIP5K.
2. Site Directed Mutagenic (SDM) analysis of PIP5K to determine the exact sites involved with the PTM determined.

B: To characterize the spatio-functional sub-nuclear localization of the PIPKIα regulated by cell cycle stages of cultured mammalian cells.

1. Elucidation of localizational pattern of PIP5K upon synchronization of cells in G1/S and G2/M phases of the cell cycle.
2. Determine the functional role of PIP5K in the sub-nuclear domains as elucidated in the previous objective.