P494

Role of RKTG, a novel Golgi localized receptor, in Gbetagamma regulated transport of secretory proteins from the Golgi to the Plasma Membrane.

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Recent studies have revealed a role for G protein betagamma signaling at the Golgi via protein kinase D (PKD) regulating fission of plasma membrane (PM) destined secretory vesicles from the trans Golgi network (TGN). The mechanism by which the Gbetagamma subunits are recruited to and activated at the Golgi in this process remains elusive. Recent studies reveal that a newly characterized Golgi localized, membrane protein belonging to the Progestin and AdipoQ Receptor (PAQR) family, RKTG (Raf Kinase Trapping to the Golgi), interacts with the Gbeta subunit and sequesters Gbetagamma to the Golgi thereby regulating the functions of Gbetagamma. Here we show that over expression of RKTG causes vesiculation of the Golgi, while a Gbeta binding mutant of RKTG does not cause vesiculation. Also, the C-terminal fragment of GRK2 (GRK2ct), which interacts with, and inhibits Gbetagamma signaling, and Gallein, a small molecule inhibitor of Gbetagamma, were both able to inhibit Golgi vesiculation in the presence of RKTG. Furthermore, a dominant negative form of PKD (PKD-DN) and a pharmacological inhibitor of PKD, Go6976, also inhibited RKTG mediated vesiculation of the Golgi. Collectively, these results reveal a novel role for the newly characterized, Golgi localized RKTG receptor, in regulating Gbetagamma at the Golgi, thus controlling Golgi to PM protein transport via the Gbetagamma-PKD signaling pathway.

Signaling Scaffolds and Complexes

P495

Interaction of JLP with Plk1 recruits FoxK1 to interact and form a ternary complex.

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JLP (JNK associated Leucine zipper protein) is a scaffolding protein, which has been shown to interact with and activate JNK/p38MAPK pathway. Its interaction with various signaling proteins is associated with coordinated regulation of cellular process such as endocytosis, motility, neurite outgrowth, cell proliferation and apoptosis. Here we identified a mitotic Serine/Threonine kinase, Polo like kinase 1 (Plk1), as a novel interaction partner of JLP through a mass spectrometry based approach. We show that

the N-terminal domain of JLP interacts with the polo-box domain (PBD) of Plk1 in a phosphorylation-dependent manner. Our results indicate that, JLP is phospho-primed on Thr 351 residue on its N-terminus, which is recognized by the PBD of Plk1 leading to phosphorylation of JLP at additional sites. Moreover, treatment of cells with the Plk1 inhibitor, BI2536 affects the interaction demonstrating the importance of Plk1 kinase activity in this process. Since JLP is a scaffolding protein that recruits proteins to mediate specific cell signaling events, we hypothesized that the interaction of JLP with Plk1 might result in the recruitment of other proteins to this complex. To test this hypothesis, we carried out SILAC labeling of proteins in mitotic cells in the presence or absence of BI2536. Through mass-spectrometry we identified the transcription factor, FoxK1 as a Plk1-dependent JLP-interacting protein. Furthermore, we show that JLP, Plk1 and FoxK1 form a ternary complex, which occurs only during mitosis. Knockdown of Plk1 and not JLP, affected the interaction between JLP and FoxK1 indicating that the formation of the ternary complex is dependent on Plk1. FoxK1 has been previously characterized as a transcriptional repressor of cyclin dependent kinase inhibitor, p21/WAF1. We observed that knockdown of JLP in U2OS cells results in increased protein levels of FoxK1 and a reduction of p21 expression. Moreover, immunofluorescence studies in asynchronous cells showed that FoxK1 is excluded from the nucleus during mitosis. Based on our observations, we propose that formation of the ternary complex between JLP, Plk1 and FoxK1 regulates the stability and/or localization of FoxK1.

P496

The XB130/Tks5 Adaptor Protein Interaction Regulates Src-mediated Signal Transduction.

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Adaptor proteins are signal transduction organizers that coordinate the formation of molecular complexes and translocation of molecules to relay signals from the extracellular environment to intracellular targets. The adaptor protein, XB130 regulates cell growth, survival and migration. Yeast-2 hybrid screening revealed that XB130 interacts with another adaptor protein, Tks5, which is highly involved in cell migration. We hypothesize that XB130 and Tks5 may act in conjunction to recruit and translocate proteins to initiate signal transduction cascades, leading to regulation of cell growth, survival or motility. Using co-immunofluorescence microscopy, XB130 and Tks5 were detected in the cytoplasm and peri-nuclear region of BEAS-2B human bronchial epithelial cells. Moreover, co-immunoprecipitation showed that XB130 and Tks5 interact endogenously and form a complex with Src-tyrosine kinase. Tks5 interacts with XB130 by binding to polyproline rich motifs in the N-terminal of XB130 and conversely, XB130 binds to the fifth SH3 domain of Tks5, as detected by immunoprecipitation of overexpressed XB130 deletion mutants and GST fusion protein pulldown of XB130 single amino acid substitution mutants or Tks5 SH3 domains. Cell motility studies showed that siRNA downregulation of XB130 inhibits lateral cell migration in a wound healing assay, whereas, downregulation of Tks5 inhibits cell invasion in a gelatin degradation assay. However, cell growth and survival studies showed that downregulation of