P25 – Protein kinases and phosphatases

P25.1
Diacylglycerol kinase alpha produced phosphatidic acid controls myosin light chain kinase localization and activity in HGF stimulated epithelial cells.

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Diacylglycerol kinases (DGKs) regulate lipid signaling by phosphorylating diacylglycerol to phosphatidic acid (PA). Membrane recruitment and activation of DGK-alpha (DGKA) is required for cell proliferation, migration and matrix invasion induced by growth factor, chemokines and oncogenes in epithelial and endothelial cells.
Through a proteomic approach we identified 76 proteins associated to tyrosine phosphorylated DGKA in Kaposi sarcoma cells. Among these proteins we selected non-muscle myosin light chain kinase (MLCK), for further characterization. MLCK is a regulator of acto-myosin driven contraction, which phosphorylates myosin light chain (MLC). DGKA co-immunoprecipitates with both long (non-muscle) and short (smooth muscle) MLCK isoforms, consistently with the interaction observed with the proteomic strategy.
Inhere we show that in epithelial cells:
(i) HGF-induced activation of DGKA produces PA at the nascent ruffle;
(ii) DGKA-mediated PA generation is necessary and sufficient to recruit MLCK at ruffling site were MLCK promotes ruffle extension;
(iii) expression of a constitutively active and membrane-bound DGKA mutant induces ruffles formation by recruiting MLCK at plasma membrane and promoting MLCK-mediated MLC phosphorylation;
(iv) expression of constitutively active MLCK is sufficient to lead to ruffle assembly independently from DGKA activity.
Altogether those data indicate that PA produced by HGF-induced activation of DGKA, is a novel regulator of the localization and the activity of MLCK required for ruffle formation in epithelial cells.

P25.2
Novel interaction between K-Ras and hnRNP A2/B1 regulated . by K-Ras activation and phosphorylation at Serine 181

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Objectives: Analysis of novel K-RasG12V interactors according to Ser181 phosphorylation
Methods: Proteomics identification of HA-K-RasG12V co-immunoprecipitating proteins by MALDI-TOF and validation of some hits from the ion spectra by western Blotting
Confocal microscopy and cell fractionation to determine colocalization of both proteins in cell compartments.
Results: hnRNP A2/B1 was identified as a novel interactor of HA-K-RasG12V by MALDI-TOF and confirmed by western blot. HnRNP A2/B1 was found to localize in cell membrane and endomembranes in spatiotemporal manner by cell fractionation as well as immunolocalization. Confocal microscopy confirmed the colocalization of hnRNP A2/B1 and K-RasG12V in plasma membrane.
Differential interaction of hnRNP A2/B1 with K-Ras depending on activation status and Ser181-phosphorilation was assessed, given our previous results showing that this modification induce increased Ras activation. Here we demonstrate *in vivo* and in both directions that both endogenous and transfected hnRNP A2/B1 interact preferentially with HA-K-Ras wild type rather than with the constitutively active HA-K-RasG12V. Moreover, this interaction was favored in the pseudophosphorylated form HA-K-RasG12VS181D and, what is more, it was impaired with non-phosphorylabe mutant HA-K-RasG12VS181A. Concomitantly, membrane recruitment of endogenous hnRNP A2/B1 was diminished in HA-K-RasG12VS181A.
Looking for cellular condition where this interaction could be significant, we found that sorbitol-induced osmotic stress increased both membrane recruitment and interaction of hnRNP A2/B1 specially in cells transfected with HA-K-RasG12VS181D.
Conclusions: We describe a novel interaction in plasma membrane.

P25.3
Protein kinase Snf1: transcriptional regulator for cell cycle genes

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The Serine/Threonine protein kinase Snf1 of *Saccharomyces cerevisiae* is a member of the SNF1/AMPK (Sucrose Non-Fermenting 1/AMP-activated protein kinase) family, one of the central components in the nutrient sensing and regulation of the carbon metabolism in eukaryotes.
Glucose depletion or growth on non-fermentable carbon sources lead to the activation of Snf1 which induces the expression of 400 glucose-repressed genes controlling different transcriptional regulators or influencing chromatin remodeling. This kinase is also involved in several other cellular processes such as stress resistance, sporulation, invasive growth and ageing.
Recently, we proposed a new function for Snf1 as cell cycle regulator since we showed that in 2% glucose growing cells Snf1 is required to guarantee the correct expression of CLB5 gene and the G1-to-S phase transition. We also demonstrated the existence of a physical interaction between Snf1 and Swi6, the regulatory subunit of the SBF and MBF transcriptional complexes which regulate the expression of G1 and S phase cyclins, respectively.
Here we focus on Snf1/Swi6 interaction showing that it is independent either on the activatory subunit of Snf1 (Snf4) and on the DNA-binding proteins Mbp1 and Swi4 which interact with Swi6 in SBF and MBF complexes. Although Swi6 is involved in the regulation of the expression of G1 cyclins, the lack of the Swi6/Snf1 interaction does not influence CLN2 expression, since CLN2 transcription is not altered in a snf1Δ strain, at least during exponential phase, suggesting the existence of a specific role of Snf1 in the regulation of the expression of CLB5.