Abstract for general public

Circadian metabolomic studies have shown that a large portion of metabolites changes in abundance with time of day. The most rhythmic metabolites that were observed are lipids, among them phosphatidylinositols (PtdIns). PtdIns can be modified by kinase and phosphatase to produce seven distinct phosphoinositides (PIs). Despite their low abundance, PIs control numerous aspects of cellular signaling. Indeed, they are involved in recruitment and activation of many proteins and pathways that control cell's life and death. Moreover, and depending on stimuli, PIs levels and distribution show a wide range of variation. Whether this distribution follows a specific circadian pattern under basal condition, or in response to various stimuli, is still unknown. Moreover, how this dynamic affects various aspects of daily cellular signaling and physiology has not been a subject of investigation. Phosphoinositides phosphatase targets and removes specific phosphates groups from PIs. The inositol 5'-phosphatase SHIP2 is one of the enzymes involved in PIs metabolism. SHIP2 regulates cellular response to growth factor, endocytosis, cell differentiation and tumorigenesis. Several observations suggest a direct link between PIs signaling and the circadian clock. Moreover, preliminary data in our lab shows that depletion or inhibition of SHIP2 alters clock genes expression and oscillations. Therefore, this protein will be used as a model to investigate time of day dependent changes in metabolism and interconversion of PIs in regulation of the physiological process above. This project stands on three main objectives. **In the first,** we plan to study in more detail how posttranslational modification and subcellular distribution of SHIP2 regulate various aspects of circadian changes in transcription. RNA-seq and bioinformatics will be combined to uncover transcriptional and post-transcriptional processes in which this protein is directly involved. *In vitro* mutagenesis will be used to study how lipid phosphatase activity and adaptor function of SHIP2 modulate nuclear subcellular structure such as nuclear speckles and chromatin modifications. Cellular fractionation combined with standard mass spectrometry will be used to examine the role of SHIP2 in nuclear speckles organization and dynamics. Moreover, chromatin organization such as histone posttranslational modifications will be addressed using chromatin immunoprecipitation and high throughput sequencing. Together, these experiments will address the exact function of SHIP2 in transcriptional regulation of the molecular clock. **In the second**, we will study the role of SHIP2 in growth factor mediated signal transduction. We will use *in vitr*o mutagenesis to examine how growth factors mediated SHIP2 phosphorylation affect its lipid 5-phosphatase activity using genetically encoded fluorescent probes and cell imaging. The impact of these modifications on the adaptor function of SHIP2 will be addressed using co-immunoprecipitation and standard mass spectrometry. Next, global phosphoproteome analysis will be carried out to uncover the activity of various signaling pathways in which this protein is directly involved. Biosensors and circadian reporters will be combined with live cell imaging to address the exact function of SHIP2 in cell growth and differentiation. Functional assays will also be conducted to evaluate different SHIP2 domains and its partners in regulation of these processes. **In the third,** we will focus on the role of SHIP2 and its various forms in cancer cell signaling. *In vitro* mutagenesis, stable knockdown and overexpression strategies will be used to examine the role of SHIP2 in cancer cell growth, migration, and anchorage independent growth. Time of day dependent changes in these processes will also be addressed. Role and activity of signaling pathways uncovered in aim1&2 will be specifically addressed here. Their functional contribution to cancer growth and therapy resistance will be carried out using tumor xenograft models as an *in vivo* system. As we are the first to examine the role of phosphoinositides in regulation of various aspects of circadian physiology, the results of this project will provide future combinatorial therapeutic opportunities to treat diseases such as cancer.