The role of Nuclear Phosphatidylinositol 4,5-bisphosphate in RNA Polymerase II Transcription
Thesis review from Can Balaban by Dr. Enrique Castaño

Abstract and questions for CAN BALABAN

The thesis is very interesting and relevant to the field of gene regulation and nuclear structure as it focuses on the basic mechanism of action of RNA pol II-associated proteins and complex formation. The first part deals with a novel way to view two different populations of proteins that interact with PIP2 "exposed and hidden". The data obtained here may provide several interesting hypotheses for future work with a number of relevant candidates. The analyzed data shows the involvement of exposed vs hidden PIP2 binding motifs in different nuclear processes. This is unexpected as the 191 'exposed' proteins show gene expression as regulators of Pol II, mRNA splicing, and cell cycle, whereas 324 'hidden' proteins are involved in RNA splicing and transport, cell cycle regulation, and response to heat or viral infection, therefore, could regulate later stages of the gene expression. The different processes mentioned may involve particular complexes with similar physical and chemical characteristics to follow a procession order.

The PIPs binding proteins in the nucleus has begun to take relevance and acceptance during the last couple of decade. Still, more work is needed to fully grasp the functionality of this family of lipids. Here Can has shown many of the players involve primarily in an active nuclear process that links all stages of transcription from epigenetic regulation to the transport of mRNA. Moreover, it is known that some diseases do affect key enzymes that affect the metabolism of PIP2, thereby affecting PIP2 amounts and resulting in diverse effects. This work does provide a further list of candidates to evaluate in such studies.

As such it will be interesting to see in the future the role of many of the candidates found in this work. The study by Can, did continue by analyzing further one selected protein for his thesis. Myosin phosphatase rho-interacting protein (MPRIP), is a protein that shows very interesting activities. The work done on phase separation MPRIP, F-actin interaction, and correlation with

different phosphorylation stages of RNA pol II are shown and have a wide interest in the field of gene regulation and nuclear structure. The results by Can show the region responsible for phase separation and PIP2 binding for MPRIP, as well as a very interesting percentage of the cells showing F actin fibers that are covered in PIP2 when it is overexpressed. During the overexpression process, Can was able to analyze better the phase separation and also the dynamics that occur in two different well-seen forms in the cells. The simple droplet and the more structured fiber resemble several structures that form in the cells. This together with his following data on RNA pol II provide a full picture of how nuclear structures are formed and the dynamics associated with the nuclear process during normal and stress conditions. The set of experiments with FRAP and fluorescence microscopy provide hints on how structures can be formed in the nucleus without the need for membranes. Nuclear structures are a basis for several processes to take place. As they require a coordinated set of molecules close together to carry the process like Transcription, RNA processing, and ribosomal assembly as examples. The processes do take advantage of organizing structures in an entropy-driven mechanism and therefore reducing the chemical energy from the cell to maintain these structures and to direct specific molecules to particular structures.

Furthermore, the work done on correlating different phosphorylated forms of Pol II CTD with MPRIP does provide an insight into how MPRIP is acting together with PIP2 in pol II activity. The results shown in the silencing experiments were a decreased colocalization between Tyr1P-CTD and nuclear PIP2-containing structures upon MPRIP depletion. Since Tyr1 phosphorylation of CTD marks the promoter-proximal pause of RNAPII this may link MPRIP functions with nuclear actin and NM1 at this particular point. Much work has been done on actin and NM1 that direct them to transcription. Here Can has added a more specific point in the transcription process where these proteins may be having an effect.

Together all data provide a model for MPRIP during transcription and its influence of PIP2 on specific phosphorylation sites. This model can be further used for other nuclear processes, from epigenetics, gene regulation, RNA processing, and Cell cycle control where PIP2 binding proteins

may change binding specificities upon posttranslational modifications. With all the work done I can state that the quality of the theses done by the applicant Can Balaban deserves to be granted the PhD.

Questions raised

During the 4 steps of transcription, how do you envision that the physicochemical properties of PIPs may be relevant to the process and how are protein modifications involved?

The proteins that were identified for interactions with PIP2 after partial degradation (Hidden) are likely to interact under normal conditions with PIP2 or under what circumstances would they interact with PIP2? Would those circumstances require posttranslational modifications?

Do you think the evolution of motifs that interact with PIP2 is related between Hidden and exposed domains or is the process the response to their evolution?

Professor Enrique Castaño