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B-2. Characterization of the nuclear localization signal of the actin-binding Moesin protein

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The Moesin protein of Drosophila melanogaster is the single representative of the highly conserved Ezrin-Radixin-Moesin (ERM) protein family in the fly. ERM proteins play essential role in cytoskeletal rearrangements, since they act as a crosslinker between cell membrane proteins and the actin cytoskeleton. They all consist of an amino-terminal FERM and a carboxy-terminal actin-binding domain, and a flexible alpha helical region interconnecting the two terminal domains. The activity of ERMs is regulated via conformational change. In their inactive, closed arrangement the FERM and actin-binding domains interact with each other. The binding of Phosphatidylinositol 4,5-bisphosphate (PIP2) by the FERM domain, and subsequent phosphorylation of a Threonine residue at position 559 inhibits the intramolecular interaction, and as a result, the proteins acquire opened, active conformation. The cytosolic roles of ERM proteins are well characterized moreover, our laboratory demonstrated recently that in the nucleus Drosophila Moesin is involved in mRNA export. To gain deeper insight into the nuclear function of the protein, we decided to study its nuclear import in details. Methods: To achieve our goal, we generated DNA constructs expressing mutant forms of Moesin tagged with GFP. The nuclear transport of the mutant proteins was monitored in cultured Drosophila cells where the import of Moesin was induced at the same time by the inhibition of mRNA export. Results: In the first set of experiments we determined the amino acids responsible for the nuclear localization (NLS). Next, the regulation of the NLS motif has been analyzed, and we found that the NLS is in fact bipartite and it is regulated neither by phosphorylation nor through monomeric actin binding. Finally, we determined if the protein enters the nucleus in its active or inactive conformation, monomeric or dimeric form. and in complex with G-actin. Conclusion: Our results reveal the fine details of the tightly regulated, active nuclear import of ERM proteins and enable the manipulation of their nuclear function

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B-3. Deciphering the Role of Nuclear Lipid Islets in RNA Pol II transcription

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Nuclear compartmentalization is crucial for spatiotemporal regulation of key processes such as transcription and splicing. Recently, we have identified an F-actin regulatory protein (MPRIP) in nucleus by using microscopy