Abstract (in English)

The phosphatidylinositols are a subclass of glycerophospholipids with their inositol head group linked to the diacylglycerol backbone. The differential phosphorylation of the inositol head group yields seven different phosphoinositide phosphates (PIPs) which can be mono-, bis,- or tris-phosphorylated. The roles of the cytoplasmic PIPs have been extensively studied in vesicular trafficking, ion channels, generating second messengers and, membrane and cytoskeletal dynamics. While their cytoplasmic functions are very well described, the molecular mechanism of their nuclear functions are still poorly understood.

From the nuclear PIPs, the Phosphatidylinositol 4,5-bisphosphate (PIP2) is the most abundant phosphoinositide in the cell nucleus and it participates to the nuclear architecture by regulating processes such as chromatin remodeling, DNA-damage response and gene expression. In the cell nucleus, it localizes mostly to nuclear speckles where it interacts with the splicing machinery. In nucleolus, PIP2 is involved in the RNA Polymerase I machinery to regulate rDNA transcription. Recently, we have defined a nucleoplasmic pool of PIP2 which is observed in 40 to 100nm foci. The nascent transcripts of RNA Polymerase II (RNAPII) were visualized at their periphery and RNA was shown to be essential for their integrity. In this study, we sought to examine the role of PIP2 in the RNAPII transcription machinery.

The RNAPII transcription cycle is generally divided into four steps; Initiation, promoter-proximal pausing, elongation, and termination. The specific post-translational modifications of the C-terminal domain (CTD) of RNAPII correlates with the stages of the RNAPII transcription and coins the term “CTD code”. The phosphorylation of the Serine5 residues of this domain associates with the initiation condensates which are formed through liquid–liquid phase separation (LLPS). The Tyrosine1 phosphorylation of the CTD occurs during the promoter-proximal pausing and marks the release of the RNAPII from the initiation condensate. This process is essential to trigger the release of RNAPII, as the subsequent Serine2 phosphorylation of the CTD abolishes the affinity of RNAPII for the initiation condensate.

With this study, we have identified the nuclear interactome of PIP2 and defined the processes that are associated with PIP2-effectors. The Myosin Phosphatase Rho-Interacting Protein (MPRIP) was revealed as a promising effector to mediate PIP2-associated RNAPII transcription. Accordingly, we have identified MPRIP in the same complex with RNAPII and NMI. By using super-resolution microscopy, we have localized

MPRIP at nuclear PIP2-containing structures. In the overexpression experiments, MPRIP shows LLPS characteristics in nucleus and exhibits a novel phase behavior that points to a regulatory function regarding nuclear actin polymerization. Furthermore, we show that the depletion of MPRIP promotes the formation of the initiation condensates and increases the association of Ser5P-CTD (Serine 5 phosphorylated CTD) with PIP2. It is then revealed that MPRIP mediates the association between Tyr1P-CTD and PIP2. Finally, we show that MPRIP determines the localization of the Tyr1P-CTD to nuclear speckles and NLIs. Overall, our data shed light on the role of PIP2 in RNAPII transcription through identifying a novel transcription regulator that defines the association between Tyr1P-CTD and PIP2.