Nuclear PIP2 and myosin I: new important players in DNA transcription

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Nuclear myosin I (NM1) is a 120 kDa molecular motor described in the cell nucleus. NM1 was shown to be involved in chromatin remodeling, repositioning of transcriptionally activated regions in the nucleoplasm and also in transcription with RNA pol I. It was shown that Myosin 1C binds to negatively charged phospholipids, specifically to phosphatidylinositol-(4,5)bisphosphate (PIP2) with a very high affinity and this binding tethers myosin I to plasma membrane. Based on this we asked if NM1 also has binding properties to PIP2. We made single-point mutations in the pleckstrin homology (PH) domain of NM1 which was shown to be responsible for PIP2 binding and applied fluorescence recovery after photobleaching (FRAP) and fluorescence correlation spectroscopy (FCS). Mutant NM1 became faster in mobility compared to its wild type NM1 – this shows that NM1 binds to PIP2 in the cell nucleus. We then depleted PIP2 in the nucleus by co-transfecting the cells with inositol 5-phosphatase which would cleave 5-phosphate of PIP2 in the nucleus and with NM1. After PIP2 depletion in nuclei, NM1 became faster showing once more that PIP2 binding reduces NM1 mobility. Phospholipase C delta (PLCδ) is an enzyme which binds to PIP2 via its PH domain and cleaves PIP2 into inositol (1,4,5) triphosphate and DAG. We mutated the PIP2 binding domain of PLCδPH and co-transfected cells with NM1and wild type PLCδPH or mutant PLCδPH. FRAP results showed that NM1 mobility increased when PIP2 was occupied by wild type PLC [illegible] mutant one. All these data indicated that NM1 binds to PIP2 in the cell nucleus, and this was further confirmed by electron microscopy. We then focused on the function of PIP2 in ribosomal gene transcription and showed that PIP2 binds to the transcription machinery. Removal of PIP2 from in vitro transcription assays caused the inhibiton of transcription for ribosomal genes and it was possible to recover the transcription efficiency by adding back PIP2 to the transcription reaction. The data suggest that nucleolar PIP2 might serve as a transcription factor for ribosomal genes, and together with nucleolar myosin I it might form the structural core of nucleoli. Involvement of other structural proteins will be discussed.

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