

ROLES OF COMPLEXES OF NUCLEAR MYOSIN I AND LIPIDS IN THE CELL NUCLEUS

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KEY WORDS: nuclear myosin, PIP2, cell nucleus, transcription

Nuclear myosin 1 (NM1) is one of the myosins that were recently found in the cell nucleus. We demonstrated earlier that together with nuclear actin, both proteins are associated with rRNA genes and with RNA polymerase I during transcription. Chromatin immunoprecipitation revealed the association of NM1 and actin with rDNA and abortive transcription assays showed that actin functions in elongating Pol I complex. Depletion of NM1 or actin inhibits Pol I transcription in vivo and in vitro, while overexpression of NM1 augments pre-rRNA synthesis. NM1 associates with Pol I via the transcription initiation factor TIF-IA and this association requires phosphorylation of TIF-IA at serine 649. Thus, actin and NM1 are apparently required for efficient transcription of rDNA, and possible models will be discussed. As NM1 has been shown to be necessary for transcription and chromatin remodeling, it is important to reveal the modes of its action in the nucleus.

NM1 is identical to its splicing variant Myosin 1C with the exception that NM1 has 16-residue N-terminal extension. It was shown that Myosin 1C binds to negatively charged phospholipids specifically to phosphatidylinositol(4,5)bisphosphate (PIP2) with a very high affinity (Hokanson et al. 2006) and this binding tethers NM1 to plasma membrane. Based on these findings we asked the question if NM1 also has binding properties to PIP2. In order to investigate this question we made single point mutations in the pleckstrin homology (PH) domain of NM1 where was shown to be responsible for PIP2 binding by Hokanson et al. and applied fluorescence recovery after photobleaching (FRAP) and fluorescence correlation spectroscopy (FCS). Mutant NM1 became faster in mobility compared to its wild type NM1 showing that NM1 bound to PIP2 and this binding slowed down the NM1 mobility. Then we 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. When PIP2 was depleted by inositol 5-phosphatase NM1 became faster showing once more that PIP2 binding reduced NM1 mobility. Phospholipase C delta (PLC δ) is an enzyme 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 PLC δ PH and co-transfected cells with NM1 and wild type PLC δ PH or mutant PLC δ PH. FRAP results showed that NM1 mobility increased when PIP2 was occupied by wild type PLC δ compared to mutant one. All these data indicated that NM1 binds to PIP2 in the cell nucleus, and this was further confirmed by electron microscopy. The functional meaning of these complexes will be discussed.

ACKNOWLEDGEMENTS: This work was supported by the Grant Agency of Czech Republic (reg. no. 204/07/1592), grant LC545 and LC06063 of the MSMT, and by the institutional grant no. AV0Z50390512. SY was supported by the student program of the Grant Agency of the Czech Republic (reg. No. 204/09/H084).