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M-1. Characterisation of lamin A/C interaction with phosphoinositides

Rositsa Maystorova[1], Sara Lopes[1], Lenka Jarolímová[1], Margarita Sobol[1], Vlada Filimonenko[1], Pavel Hozák[1]

[1] Department of Biology of the Cell Nucleus, Institute of Molecular Genetics of the Czech Academy of Sciences, v.v.i., Vídeňská 1083, 142 00, Prague, Czech Republic

rositsa2.maystorova@gmail.com

Lamins are type V intermediate filaments found in the nuclei of most eukaryotes where they are essential for molecular events, such as chromatin organization, transcription, DNA replication and damage response, and mechanotransduction. In vertebrates, three lamin genes (A, B1 and B2) give rise to various isoforms that undergo complex post-translational modifications. These modifications are proposed to regulate the localization and interactions of lamins with their binding partners. Lamin A exhibits striking variations in the set and expression levels of its isoforms in different tissues and at different developmental stages (Maraldi *et al,* 2018). The role of lamins at the nuclear periphery has been studied in more detail, but much less is known about their assembly state, dynamics and interactions in the interior. Recent studies suggest a role of the nucleoplasmic pool in chromatin organization, motion and in its accessibility by regulating epigenetic modifier complexes. Depletion of the nucleoplasmic lamin A is correlated with disease phenotypes like Hutchinson-Gilford progeria (Naetar *et al,* 2017). Preliminary data from immunoprecipitation and pull-down experiments from nuclear lysates carried out at the Laboratory of Biology of the Cell Nucleus show lamin A in a complex with nuclear myosin I and a phosphoinositide phosphatidylinositol 4,5-bisphosphate, PIP2. Phosphoinositides play important roles in cell signalling events, and recently have been reported in the nuclear interior where they are implicated in transcriptional regulation (Sobol *et al)*. Many nuclear proteins have been shown to associate with phosphoinositides and mediate their interaction via lysine/arginine-rich areas in their sequences (K/R-rich motifs) (Jungmichel *et al* 2014). Aim:To confirm and understand which domain of lamin A directly binds to PIP2. Methods: The lamin A gene was screened for putative K/R-rich motifs in Sublime software. Conventional cloning, recombinant protein expression and purification in bacteria were carried out. The binding will be probed via protein-lipid overlay assays. Results:Four lamin A truncation mutants bearing clusters of those regions were generated and will be tested for direct binding to PIP2 and other phosphoinositides *in vitro*. Elucidating the binding domain of lamin A to PIP2 would allow us to prepare lamin mutants unable to bind PIP2 and address the question of the biological relevance of the interaction in the context of the lamin A/NMI/PIP2 complex.

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