Subcellular Processes in Plants and Animal Cells

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First observations of the nucleoplasmic lipid islets: “black holes” in the cell nucleus?

M. Sobol[1], S. Yildirim[1], V. Filimonenko[1], A. Filimonenko[1], P. Hozak[1]

[1]Institute of Molecular Genetics of the ASCR, v. v. i., Biology of the Cell Nucleus, Prague, Czech Republic

sobol@img.cas.cz
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The nucleus is a highly organized cell compartment, where controlled gene expression, DNA replication, and RNA processing occur. These processes are spatially ordered via the nucleoskeleton, which is involved in nuclear compartmentalization and critical for nuclear functioning [1]. In spite of the growing interest and extensive research concerned to the nuclear organization, so far mostly protein complexes have been found as important for spatial nuclear ordering. We describe novel structures containing phosphatidylinositol 4,5-bisphosphate (PIP2) which seem to contribute as well. Based on scarce literature data relating to PIP2 presence in interchromatin granule clusters and in the nucleolus, we carried out ultrastructural mapping of PIP2-containing structures using pre-embedding immunolabeling and 3D electron tomography. We showed that these structures propagate through the nucleolus where they connect individual fibrillar centers (containing enzymes and transcription factors for ribosomal DNA transcription) and the dense fibrillar component (where the transcription and maturation of rRNA takes place). Besides PIP2 detected in interchromatin granule clusters, the PIP2-positive structures stretch into the nucleoplasm where they appear as previously undescribed 70-100 nm roundish “lipid islets” (Figure 1). We mapped the elemental content of these islets using electron energy-loss microscopy. They appear surrounded by chromatin, and carbon mapping showed high density of organic compounds inside the islets indicating that lipids might be the main inner constituents of these structures (Figure 2). To reveal the plausible functions of these PIP2-containing islets, we mapped mutual localization of PIP2 with nuclear proteins involved in transcription, splicing, and higher order chromatin organization using advanced immunogold electron microscopy and super-resolution light microscopy. We show that at the periphery of the islets, PIP2 co-localizes or is located in immediate vicinity with nascent transcripts, pre-lamin A, LAP2α, H3K4me2, and H3K9me2 (Figures 3-5). Direct binding and mobility assays also showed nucleoplasmic interactions between PIP2 and nuclear myosin 1 (NM1), which is a part of chromatin remodelling complex B-WICH [2] and promotes Pol I and Pol II transcription [3, 4]. Furthermore, the recruitment of lamin A into NM1-bound lipo-protein complex via interactions with PIP2 was demonstrated. We also revealed the association of PIP2 with core histones in pull-down experiments, and showed that the mobility of histone H2B depends on PIP2. Since lamin A is involved in chromatin remodelling via binding to DNA directly or to BAF and core histones, we propose that PIP2 might modulate the state of chromatin by interactions with NM1, core histones and lamin A. Taken together, this data allow us to suggest that PIP2 plays an important role in the organization of chromatin architecture and thus in regulation of gene transcription.

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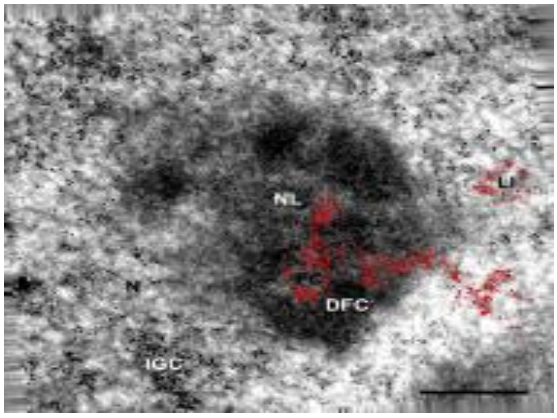

Figure 1. 2D-projection image acquired during single-axis electron tomography of PIP2-containing structures. PIP2 is localized using pre-embedding procedure with 0.8 nm immunogold particles (marked in red). N, nucleus; IGC, interchromatin granule cluster; LI, lipid islet; NL, nucleolus; FC, fibrillar center; DFC, dense fibrillar component. Bar, 100 nm.

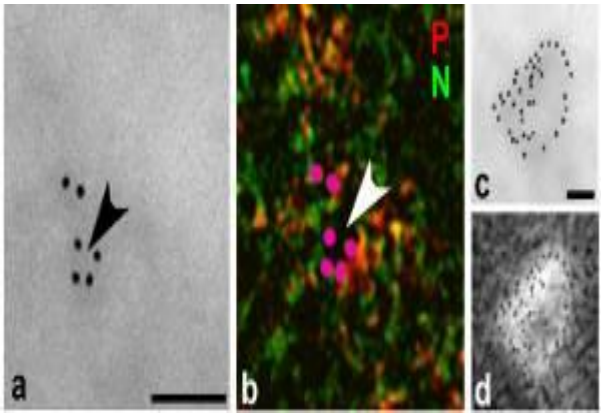

Figure 2. Elemental mapping of phosphorus (P), nitrogen (N), and carbon (C) in lipid islets. a, bright-field TEM image of a lipid islet in the nucleoplasm, visualized by PIP2 immunogold labeling. b, color-coded overlay of elemental maps of P (red) and N (green); the gold particles are marked with magenta. Arrowhead shows to the inner part of the lipid islet. A bright-field TEM image (c) and a carbon map (d) of a different lipid islet. Bars, 100 nm.

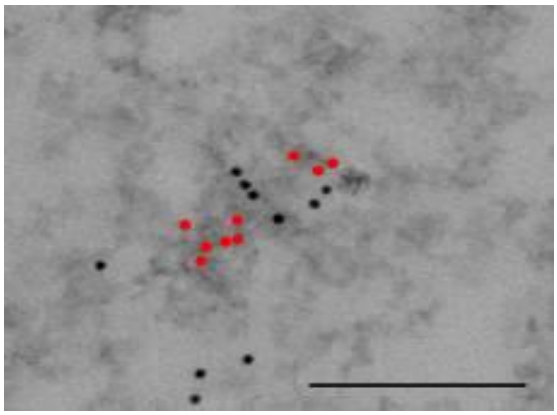

Figure 3. Electron microscopy co-localization of PIP2 with rRNA transcripts (marked in red) after BrUTP incorporation. Bar, 200 nm.

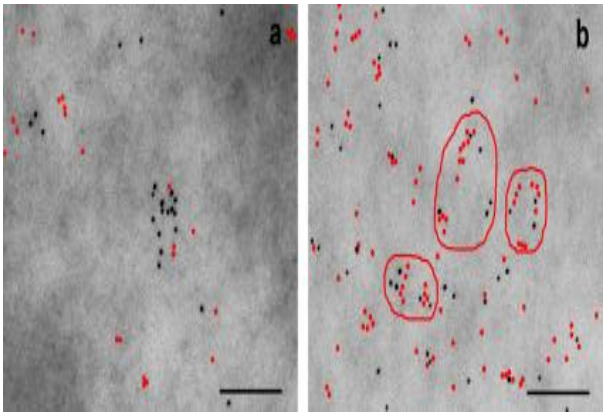

Figure 4. Co-localization of PIP2 with either pre-lamin A (a, marked in red) or LAP2α (b, marked in red). PIP2 is detected with two-dimensional on-section labeling; pre-lamin A and LAP2α are detected with three-dimensional pre-embedding labeling. Lipid islets are outlined in red (b). Bar, 200 nm.

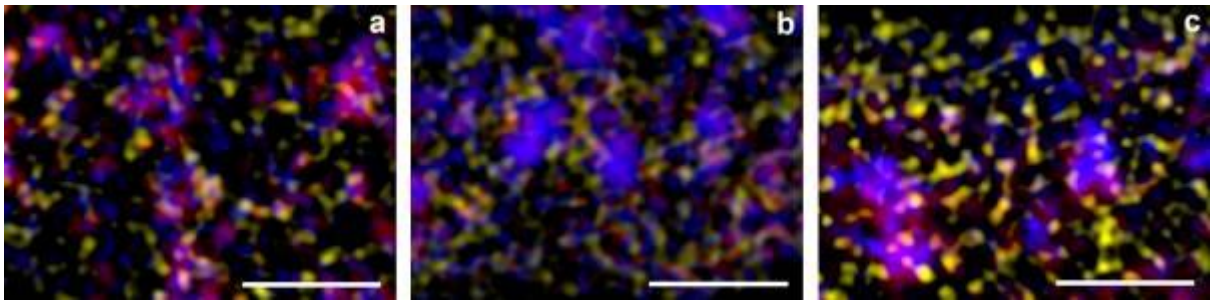

Figure 5. Triple immuno-localization of PIP2 (blue) with Sm (red) and either pre-lamin A (a, yellow), H3K4me2 (b, yellow), or H3K9me2 (c, yellow) detected using super-resolution structured illumination microscopy. Bar, 2 µm.