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Analysis of functional interactions of phospholipids in the cell nucleus

Analýza funkčních interakcí fosfolipidů v buněčném jádře

Doctoral thesis

Supervisor: Prof. RNDr. Pavel Hozák, DrSc.

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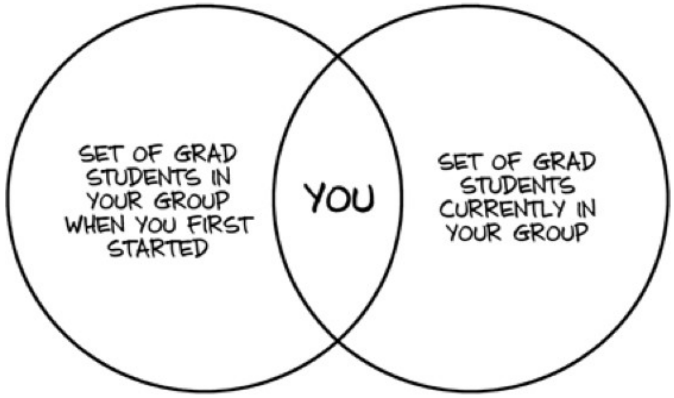
Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 04.06.2020

Podpis .....

Veronika Biddle



HOW TO TELL IT'S TIME TO GRADUATE

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title: "How to tell it's time to graduate" - originally published 11/26/2014

I would like to thank my supervisor Pavel Hozák for giving me the opportunity to work in his research group and for teaching me how to be an independent scientist.

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Abbreviations

Akt	protein kinase B
ATP	adenosine triphosphate
ATR	Ataxia telangiectasia and Rad3-related protein
ATX1	Arabidopsis homolog of trithorax
BAF	BRM- or BRG-associated factors
BASP1	brain acid soluble protein 1
BRG1	Brahma-related gene 1
BRM	Brahma gene
DAG	diacylglycerol
DFC	dense fibrillar component
DNA	deoxyribonucleic acid
EEA1	early endosome antigen 1
FC	fibrillar centre
FYVE	Fab1/YOTB/Vac1/EEA domain
GC	granular component
GFP	green fluorescent protein
Grp1	general receptor for phosphoinostides 1
H1	histone H1
H3	histone H3
H3K4meX	X-methylated lysine 4 at histone H3
H3K9meX	X-methylated lysine 9 at histone H3
HDAC1	histone deacetylase 1
hnRNP U	heterogeneous nuclear ribonucleoprotein U
Hrs	hepatocyte growth factor-regulated tyrosine kinase substrate
IGCs	interchromatin granular clusters
ING2	inhibitor of growth family member 2
IP3	inositol 1,4,5-trisphosphate
IPMK	inositol multiphosphate kinase

LSD1	lysine-specific histone demethylase 1
MIGs	mitotic interchromatin granules
NLIs	nuclear lipid islets
NM1	nuclear myosin 1
NORs	nucleolar organising regions
NuMa	nuclear mitotic apparatus protein
NXF1	nuclear RNA export factor 1
OSH1	oxysterol-binding protein homolog 1
PA	phosphatidic acid
PBR	polybasic region
PH	pleckstrin homology
PHD	plant homeodomain
PHF8	PHD finger protein 8
PI	phosphatidylinositol
PI(3)P	phosphatidylinositol 3-phosphate
PI(3,4)P2	phosphatidylinositol 3,4-bisphosphate
PI(3,4,5)P3	phosphatidylinositol 3,4,5-trisphosphate
PI(3,5)P2	phosphatidylinositol 3,5-bisphosphate
PI(4)P	phosphatidylinositol 4-phosphate
PI(4,5)P2	phosphatidylinositol 4,5-bisphosphate
PI(5)P	phosphatidylinositol 5-phosphate
PI3KC2α	phosphatidylinositol 3-kinase type IIα
PI3KC2β	phosphatidylinositol 3-kinase type IIβ
PI4Kα	phosphatidylinositol 4-kinase α
PI4Kβ	phosphatidylinositol 4-kinase β
PIP4KIIα	phosphatidylinositol 5-phosphate 4-kinase type IIα
PIP4KIIβ	phosphatidylinositol 5-phosphate 4-kinase type IIβ
PIP4KIIγ	phosphatidylinositol 5-phosphate 4-kinase type IIγ
PIP5KIα	phosphatidylinositol 4-phosphate 5-kinase type Iα

PIP5KIγ_i4	phosphatidylinositol 4-phosphate 5-kinase type Iγ_i4
PIs	phosphoinositides
PLC	phospholipase C
PTEN	phosphatase and tensin homolog
RING	really interesting new gene
RNA	ribonucleic acid
RNA pol I/II	RNA polymerase I/II
SC-35	serine/arginine-rich splicing factor 2
SF-1	steroidogenic factor 1
SHIP-1	Src homology 2 domain-containing inositol 5-phoshpatase 1
SHIP-2	Src homology 2 domain-containing inositol 5-phoshpatase 2
Sin3A	paired amphipathic helix protein Sin3a
Star-PAP	nuclear speckle targeted PIP5KIα regulated-poly(A) polymerase
TAF3	TATA box binding protein (TBP)-associated factor 3
TBP	TATA box binding protein
TREX	transcription-export
TTD	tandem tudor domain
UBF	upstream binding factor
UHRF1	ubiquitin-like PHD and RING finger domains-containing protein 1
WT1	Wilms's tumor 1

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Abstract (English)

Phosphoinositides (PIs) are glycerophospholipids with a negative charge. As components of cell membranes, PIs are involved in membrane and cytoskeletal dynamics, cell movement and signalling, and the modulation of ion channels and transporters. Apart from the cytoplasm, phosphoinositides also localise to the cell nucleus. PIs play a role in crucial nuclear processes, such as DNA transcription, pre-rRNA and pre-mRNA processing, cell differentiation, DNA damage response, or apoptosis. Phosphatidylinositol 4-phosphate (PI(4)P) and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) are the most abundant phosphoinositides in the cell. However, their exact localisation and function in the nucleus are largely unknown. Here, we describe their localisation at super-resolution level and their involvement in some nuclear processes.

PI(4)P is present in nuclear lamina, nuclear speckles and nucleoli, and it forms small foci in nucleoplasm. The majority of nuclear PI(4)P localises to the nucleoplasm, whereas almost 16 % is present in nuclear speckles. On the other hand, the majority of nuclear PI(4,5)P2 localises to nuclear speckles, almost 30 % localises to nucleoplasm and the lesser portion to nucleoli. In the nucleoplasm, PI(4,5)P2 forms small foci called nuclear lipid islets (NLIs). Their core is rich in lipids and is surrounded by proteins, RNA and DNA. The NLIs' periphery is associated with RNA polymerase II (RNA pol II) transcription machinery. In addition, nuclear myosin 1 (NM1) localises to the NLIs' periphery. NM1 interacts with PI(4,5)P2 directly and this interaction is required for RNA pol II transcription. The levels of active RNA polymerase II transcription are dependent on the levels of PI(4,5)P2. Therefore, NLIs provide a structural platform promoting the formation of RNA pol II transcription factories.

Mass spectrometry analysis of immunoprecipitated PI(4)P lipid-protein complexes revealed almost 100 nuclear proteins participating in essential nuclear processes such as pre-mRNA processing, transcription or nuclear transport indicating the role of PI(4)P as an important player in the cell nucleus.

Furthermore, we show that lysine-specific histone demethylase 1 (LSD1) interacts with PI(4)P and PI(4,5)P2 directly. LSD1 demethylates H3K4me2 active histone mark and thus represses transcription. The binding of PI(4)P to LSD1 inhibits its activity, on the contrary, the binding of PI(4,5)P2 to LSD1 stimulates its activity in vitro. Phosphorylation of PI(4)P or dephosphorylation of PI(4,5)P2 might also have a quick regulatory effect on LSD1 function in vivo.

Abstrakt (Czech)

Fosfoinositidy jsou glycerofosfolipidy se záporným nábojem. Jako složky buněčných membrán se podílejí na membránové dynamice, buněčném pohybu, signalizaci a na modulaci iontových kanálů a membránových transportérů. Kromě cytoplazmy fosfoinositidy také lokalizují do buněčného jádra. Fosfoinositidy hrají roli v klíčových jaderných procesech, jako je transkripce DNA, zpracování pre-rRNA a pre-mRNA, reakce na poškození DNA, diferenciace buněk nebo apoptóza. Fosfatidylinositol 4-fosfát (PI(4)P) a fosfatidylinositol 4,5-bisfosfát (PI(4,5)P2) jsou nejhojnějšími fosfoinositidy v buňce. Lokalizace a funkce obou fosfoinositidů v buněčnem jádře je do značné míry neznámá. V této práci popisujeme lokalizaci PI(4)P a PI(4,5)P2 na úrovni superrozlišení a jejich zapojení do jaderných procesů.

PI(4)P je přítomen v jaderné lamině, jaderných speckles, jádérkach a tvoří malá ohniska v nukleoplasmě. Většina jaderného PI(4)P se lokalizuje do nukleoplasmy, zatímco jenom 16% je přítomno v jaderných speckles. V případe PI(4,5)P2 je situace opačná, většina je lokalizována v jaderných speckles, 30 % je v nukleoplasmě a menšina v jadérkách. V nukleoplazmě tvoří PI(4,5)P2 malé ložiska nazývané nukleární lipidové ostrůvky (NLIs). Jádro NLIs je bohaté na lipidy a je obklopené proteiny, RNA a DNA. Periferie NLIs je spojena s transkripcí katalyzovanou RNA polymerázou II (RNA pol II). Jaderný myosin 1 (NM1) lokalizuje na periferii NLIs, kde interaguje s PI(4,5)P2. Tato interakce je nezbytná pro transkripci zprostředkovanou RNA pol II. Hladiny aktivní transkripce RNA pol II jsou závislé na hladinách PI(4,5)P2. NLIs poskytují strukturální platformu podporující tvorbu transkripčních továren RNA pol II.

Analýza hmotnostní spektrometrie imunoprecipitovaných PI(4)P lipidovo-proteinových komplexů odhalila téměř 100 jaderných proteinů účastnících se podstatných jaderných procesů, jakými jsou zpracování pre-mRNA, transkripce nebo jaderný transport. Tyto výsledky poukazují na zásadní roli PI(4)P v podstatných jaderných procesech.

V této práci dále ukazujeme, že lysin-specifická histon demetyláza 1 (LSD1) interaguje přímo s PI(4)P a PI(4,5)P2. LSD1 odstraňuje metylovou skupinu z H3K4me2 a potlačuje tak transkripci genů. Vazba PI(4)P na LSD1 inhibuje aktivitu enzymu, avšak aktivita enzymu je stimulována interakcí s PI(4,5)P2 in vitro. Fosforylace PI(4)P nebo defosforylace PI(4,5)P2 představuje možnost rychlého regulačního mechanismu ovlivňujíci funkci LSD1 in vivo.

1. Introduction

Phosphoinositides (PIs) are glycerophospholipids with a negative charge. PIs are amphipathic molecules, they contain hydrophobic fatty acyl tail and hydrophilic inositol head. By phosphorylation and dephosphorylation of the inositol head at 3', 4', and 5' positions, seven different PIs can be formed: three monophosphates - phosphatidylinositol 3-phosphate (PI(3)P), phosphatidylinositol 4-phosphate (PI(4)P), phosphatidylinositol 5-phosphate (PI(5)P); three bisphosphates - phosphatidylinositol 3,4-bisphosphate (PI(3,4)P2), phosphatidylinositol 3,5-bisphosphate (PI(3,5)P2), phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) and one trisphosphate - phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)P3; Figure 1). Their interconversion is tightly regulated by related kinases and phosphatases which phosphorylate or dephosphorylate the inositol ring at specific positions. Even though PIs form only a small portion of overall lipid content in the cell, they participate in various signalling pathways as important signalling molecules (reviewed in [1]).

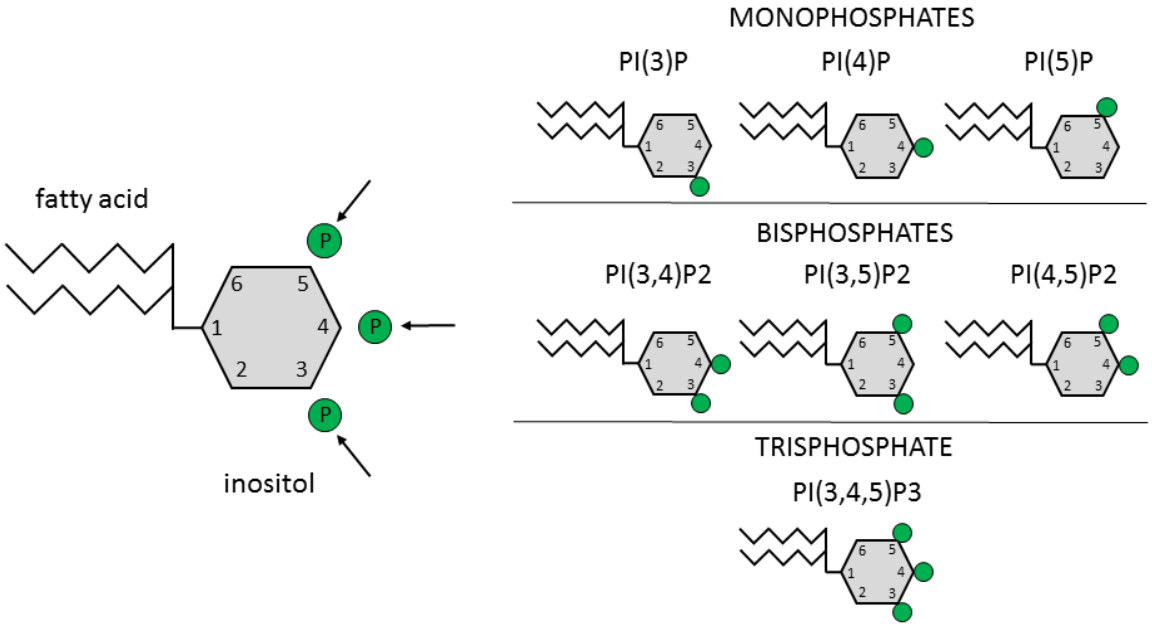

Figure 1: Schematic depiction of phosphoinositide species. Phosphatidylinositol (left) is composed of hydrophobic fatty acid tail and hydrophilic inositol head, which can be phosphorylated at three different positions - 3', 4' and 5', forming seven different phosphoinositde species (right). Green circles illustrate phosphorylated sites of the inositol head.

Phosphoinositides (PIs) are integral parts of membranes in the cell. PIs localise to different cellular membranes and are enriched in specific organelles. Their localisation and production depends on localisation and activity of their synthesising enzymes [2]. PIs are important second messengers in many signaling pathways. They are involved in cytoskeletal dynamics, cell movement, vesicle formation and intracellular trafficking. PIs also play a role in modulation of ion channels and transporters, endocytosis and exocytosis, and cell proliferation (reviewed in [1-3]).

1.1. Nuclear metabolism of phosphoinositides

Apart from the cytoplasm, phosphoinositides (PIs) also localise to the cell nucleus. Nuclear metabolism of PIs was reported more than three decades ago. It was shown that isolated nuclear envelopes from rat liver were able to incorporate radioactively labelled phosphorus from adenosine triphosphate (ATP). The products of this incorporation were phosphatidic acid (PA), phosphatidylinositol 4-phospahe (PI(4)P) and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) [4]. A later study showed that even the isolated nuclei without membranes can incorporate radioactively labelled phosphates into PA, PI(4)P and PI(4,5)P2. Moreover, the amount of detected PI(4,5)P2 was much higher in differentiated cells [5]. These data prove that there are phosphoinositide kinase activities within the cell nucleus and the presence of a certain nuclear PIs metabolism which is independent from the one in the cytoplasm.

Following paragraphs summarise the PI-synthesising enzymes which were found to be present in the nucleus (Figure 2).

Several kinases including class II phosphatidylinositol 3-kinases PI3KC2α and PI3KC2β, which phosphorylate phosphatidylinositol (PI) or PI(4)P to form PI(3)P or PI(3,4)P2, were observed in the cell nucleus [6, 7]. Two phosphatidylinositol 4-kinases localise to the nucleus, PI4Kα and PI4Kβ phosphorylate PI to form PI(4)P [8-10]. Nuclear PI(4,5)P2 can be formed by phosphorylation of PI(5)P by type II phosphatidylinositol 5-phosphate 4-kinases PIP4KIIα, PIP4KIIβ and PIP4KIIγ [11-14] or phosphorylation of PI(4)P by type I phosphatidylinositol 4-phosphate 5-kinases PIP5KIα and PIP5KIγ_i4 [15-17]. Kinases PIP5KIα and PIP5KIγ_i4 can also produce PI(3,4,5)P3 from PI(3,4)P2. Nuclear PI(3,4,5)P3 can also be produced by phosphorylating PI(4,5)P2 with class I phosphatidylinositol 3-kinases PI3Kβ and PI3Kγ [18-20], or with inositol multiphosphate kinase (IPMK) [21, 22].

Four phosphatases including phosphatase and tensin homolog (PTEN); [23-25], Src homology 2 domain-containing inositol 5-phoshpatases 1 and 2 (SHIP-1, SHIP-2); [26-28], and type I PI(4,5)P2 4-phosphatase localise to the cell nucleus [29]. Although PTEN can dephosphorylate PI(3,4)P2 and PI(3,4,5)P3 at 3' position of the inositol ring, it was shown to preferentially dephosphorylate PI(3,4)P2 in the nucleus [30]. Type I PI(4,5)P2 4-phosphatase generates PI(5)P from PI(4,5)P2 [29]. Nuclear SHIP-1 and SHIP-2 can produce PI(4)P and PI(3,4)P2 by dephosphorylating PI(4,5)P2 and PI(3,4,5)P3 at 5' position of the inositol ring [26-28]. Phosphorylated SHIP-2 preferentially dephosphorylates PI(4,5)P2 to form PI(4)P in the nucleus [27].

Beside phosphoinositide kinases and phosphatases, several isoforms of phospholipase C (PLC) localise to the cell nucleus. In particular, PLCβ_1-β_4, PLCγ_1, PLCδ_1, PLCδ_4 and PLCζ isoforms were found to be present in the cell nucleus [31-37]. PLC produces second messengers inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) by cleaving PI(4,5)P2 [38].

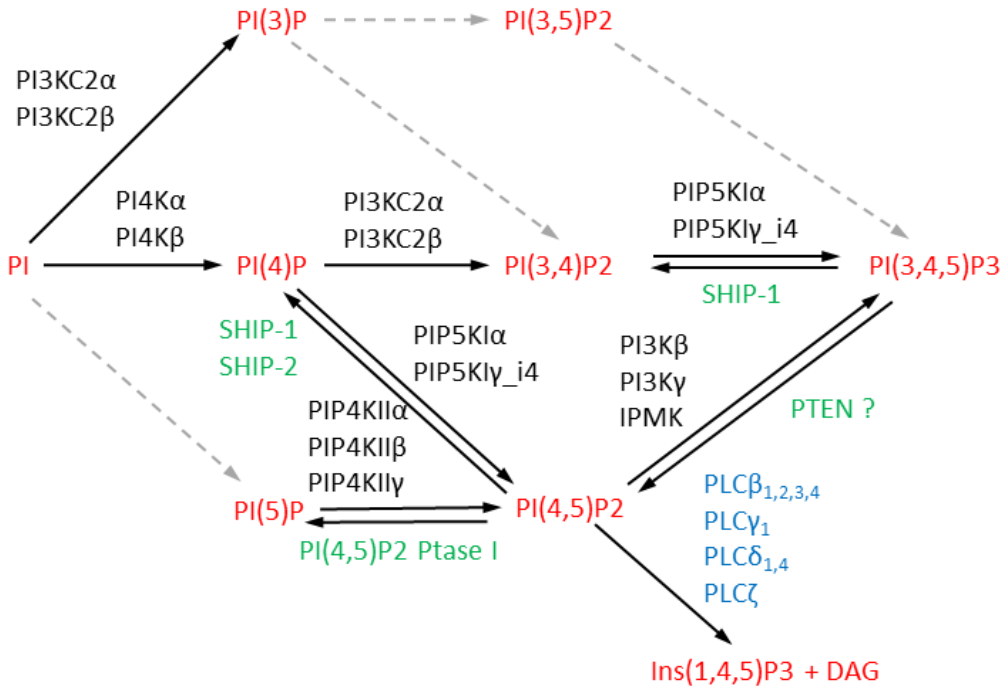

Figure 2: Metabolism of nuclear phosphoinositides (PIs). Enzymes participating in nuclear metabolism of PIs are depicted: PIs kinases (black), PIs phosphatases (green) and phospholipases (blue). Pathways catalysed by enzymes which were confirmed to localise to the nucleus are depicted by black arrows and possible pathways catalysed by yet unknown enzymes are shown by dashed grey arrows.

1.2. Function of phosphoinositides in the nucleus

Only 4% of the lipid content is made by phosphoinositides (PIs) in rat liver nuclei [39]. However, PIs are implicated in essential nuclear processes, such as DNA transcription, pre-rRNA and pre-mRNA processing, cell differentiation, DNA damage response, or apoptosis. The involvement of PIs in these processes is described in following chapters.

1.2.1. RNA polymerase II dependent transcription

Nuclear PIs play a role in DNA transcription by interaction with RNA polymerase II and transcription factors or at the epigenetic level by interaction with histone H1 or chromatin modifying proteins.

PIs are involved in RNA polymerase II transcription by interaction with transcriptional activator steroidogenic factor 1 (SF-1); [40]. SF-1 is involved in the control of production of steroid hormones and a development of gonads and adrenal glands [41]. Fatty acyl chains of PIs are hidden in SF-1's hydrophobic pocket and inositol head is accessible for modifications. PI(4,5)P2 binds SF-1 and is phosphorylated to PI(3,4,5)P3 by inositol polyphosphate multikinase (IPMK). PI(3,4,5)P3 bound to SF-1 is then dephosphorylated by phosphatase and tensin homolog (PTEN) to PI(4,5)P2. Knock-down of IMPK or overexpression of PTEN, as well as the disruption of SF-1 interaction with PIs, leads to the reduction of transcription by SF-1. The exact mechanism of action of PIs on SF-1 is not known, PI(3,4,5)P3 production from PI(4,5)P2 bound to SF-1 enhances its transcriptional activity, most likely by recruiting other nuclear coactivator proteins [40, 42].

PI(4,5)P2 was also shown to interact with RNA polymerase II. In fluorescence microscopy, PI(4,5)P2 co-localises with the largest subunit of RNA polymerase II phosphorylated in its C-terminal domain. Phosphorylated RNA polymerase II, but not unphosphorylated RNA polymerase II, also co-immunoprecipitates with PI(4,5)P2. These data suggest that PI(4,5)P2 might be involved in RNA polymerase II-mediated transcription. The direct role of PI(4,5)P2 in transcription is not known [43].

1.2.1.1. Epigenetic regulation of RNA polymerase II transcription

PI(4,5)P2 is involved in the regulation of RNA polymerase II transcription through the specific interaction with histone H1. PI(4,5)P2 binds to the H1 C-terminal part. H1 acts as a transcriptional repressor and its interaction with PI(4,5)P2 reverses H1's inhibitory effect on transcription by RNA polymerase II. Protein kinase C phosphorylates histone H1 which causes the disruption of H1-PI(4,5)P2 interaction and then transcriptional repression [44].

Another way in which PI(4,5)P2 regulates the gene expression is through the interaction with BAF chromatin remodelling complex. BAF directly interacts with PI(4,5)P2 in micelles and liposomes [45]. PI(4,5)P2 mediates the interaction of BAF complex with actin subunit within the complex. PI(4,5)P2 enhances the interaction of BAF complex and chromatin during lymphocyte activation [46].

PI(4,5)P2 is involved in transcriptional repression through the interaction with a transcriptional corepressor - brain acid soluble protein 1 (BASP1). PI(4,5)P2 interacts with myristoylated BASP1 and they are recruited to the promoter of target genes of Wilms's tumor 1 (WT1) transcriptional regulator. The interaction of PI(4,5)P2 and BASP1 is required for the recruitment of histone deacetylase 1 (HDAC1) to the promoter regions which causes a reduction in histone acetylation and then transcriptional repression [47].

PI(5)P is involved in gene transcriptional regulation during myoblast differentiation. It interacts with TATA box binding protein (TBP)-associated factor 3 (TAF3) through its polybasic region (PBR) within plant homeodomain (PHD) finger. TAF3 is a component of RNA polymerase II pre-initiation complex [48]. Its PHD finger maintains the interaction with H3K4me3, a mark of active transcription. Changes in levels of PI(5)P, mediated by PIP4KIIβ, influence the affinity of TAF3 to H3K4me3 and thus gene expression of myogenic genes during myoblast differentiation [49]; (Figure 3). PI(5)P is also bound by PBR of ubiquitin-like PHD and really interesting new gene (RING) finger domain-containing protein 1 (UHRF1) and influences its function. UHRF1 plays a key role in targeting DNA methyltransferase 1 to replication forks in order to preserve the DNA methylation pattern. UHRF1 has various domains of which the PHD and the tandem tudor domain (TTD) are responsible for recognising histone modifications. When PI(5)P is bound to PBR, UHRF1-TTD domain recognises H3K9me3. When PI(5)P is not present, PBR blocks the interaction of TTD and

H3K9me3 and UHRF1-PHD domain recognises unmodified H3. These binding properties are facilitated by conformational changes of UHRF1 protein caused by binding of PI(5)P to PBR region. PI(5)P acts as a allosteric modulator of UHRF1 activity [50].

In plants, the activity of (Arabidopsis homolog of trithorax) ATX1 is negatively regulated by PI(5)P. ATX1 methylates lysines 4 on histones H3. PI(5)P interacts with PHD finger domain within ATX1 protein [51]. Elevated levels of PI(5)P cause a translocation of ATX1 from the nucleus to the cytoplasm and a reduction of H3K4me3 levels and thus transcription inhibition [52].

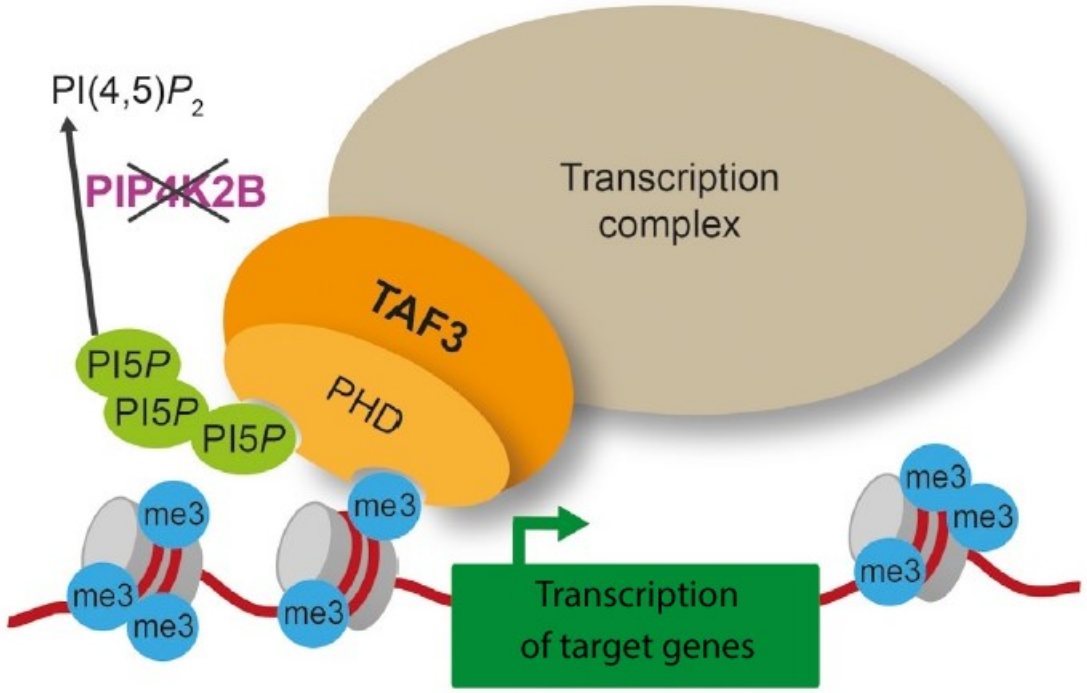

Figure 3: PI(5)P changes the gene expression through the interaction with TAF3. PI(5)P interacts with PHD domain of TAF3, which is responsible for binding to H3K4me3. PIP4KIIβ-mediated changes of PI(5)P levels, influence the affinity of TAF3 to H3K4me3 and thus expression of target genes. TAF3 - TATA box binding protein-associated factor 3, PHD - plant homeodomain finger. Taken and modified from Stijf-Bultsma et al., 2015 [49].

1.2.2. RNA polymerase I dependent transcription

PI(4,5)P2 is required for transcription by RNA polymerase I (RNA pol I). PI(4,5)P2 depletion from the nuclear extract causes significant reduction in the level of RNA

polymerase I transcription in vitro. Addition of PI(4,5)P2 restores the level of RNA pol I transcription. PI(4,5)P2 is a component of RNA pol I machinery; it interacts with RNA pol I and a transcription factor UBF (upstream binding factor). PI(4,5)P2 binds UBF directly which causes conformational changes and this interaction enhances the binding of UBF to rDNA. These observations suggest the involvement of PI(4,5)P2 in forming the transcription initiation complex and RNA Pol I transcriptional activation [53].

PI(4,5)P2 also regulates RNA polymerase I at the epigenetic level by interaction with histone lysine demethylase PHD finger protein 8 (PHF8). PI(4,5)P2 binds directly to K/R-rich motif within PHF8 protein sequence and influences its demethylase function. PHF8 demethylates H3K9me1/2 at rDNA promoter and thus activates rDNA transcription [54]. The disruption of the PI(4,5)P2-PHF8 interaction led to the increased pre-rRNA transcription. PI(4,5)P2 maintains the level of rDNA transcription by inhibiting the H3K9me2 demethylase activity of PHF8 [55].

1.2.3. RNA processing

Phosphoinositides play a role in pre-rRNA and pre-mRNA processing. PI(4,5)P2 localises to interchromatin granular clusters (IGCs) which contain proteins involved in DNA transcription and pre-mRNA processing. In fluorescence microscopy, PI(4,5)P2 co-localises with a splicing factor SC-35, which localises to IGCs. These data suggest that PI(4,5)P2 might be involved in pre-mRNA splicing. Immunodepletion of PI(4,5)P2 from nuclear extract inhibited the splicing reaction in vitro. Addition of PI(4,5)P2 and its binding partners into the splicing reaction can partially restore the splicing activity. However, the direct role of PI(4,5)P2 in splicing is not known [43].

PI(4,5)P2 also plays a role in polyadenylation of mRNA. It stimulates the activity of nuclear speckle targeted PIP5KIα regulated-poly(A) polymerase (Star-PAP), which results in longer poly(A) tails. Other phosphoinositides do not have the same effect. As a response to oxidative stress, Star-PAP and PIP5KIα complex is formed where PI(4,5)P2, produced by PIP5KIα, affects the processivity of Star-PAP and polyadenylation of its targeted mRNAs [16].

Moreover, PIs are involved in the nuclear mRNA export. PI(4,5)P2 and PI(3,4,5)P3 directly interact with nuclear export factor Aly through its N-terminus rich in lysines and arginines [56]. Aly is a component of transcription-export (TREX) complex which is

recruited to spliced mRNA in a transcription-independent manner [57]. Phosphorylation of Aly by Akt kinase is important for binding to PI(3,4,5)P3. Inhibition of kinase activity decreased the Aly-PI(3,4,5)P3 binding. The disruption of the interaction between PI(3,4,5)P3 and Aly leads to attenuated mRNA export and cell proliferation [56]. Moreover, Wickramasinghe et al. showed the phosphorylation of PI(4,5)P2 to PI(3,4,5)P3 by IPMK regulates the selection of gene transcripts involved in DNA repair process (e.g. RAD51 recombinase) for the nuclear export (Figure 4). IPMK depletion causes the accumulation of mRNAs and PI(4,5)P2 in nuclear speckles. The recognition of target mRNAs by Aly is decreased after IPMK depletion, but addition of PI(3,4,5)P3 partially restores the binding of Aly to mRNAs [58].

PI(4,5)P2 is also involved in early stages of pre-rRNA processing in the nucleolus. PI(4,5)P2 interacts with protein fibrillarin, which plays a role in modification and processing of pre-rRNA during ribosomal assembly [59]. The direct interaction of PI(4,5)P2 and fibrillarin causes its conformational changes and modulates its binding to rRNA in vitro [53].

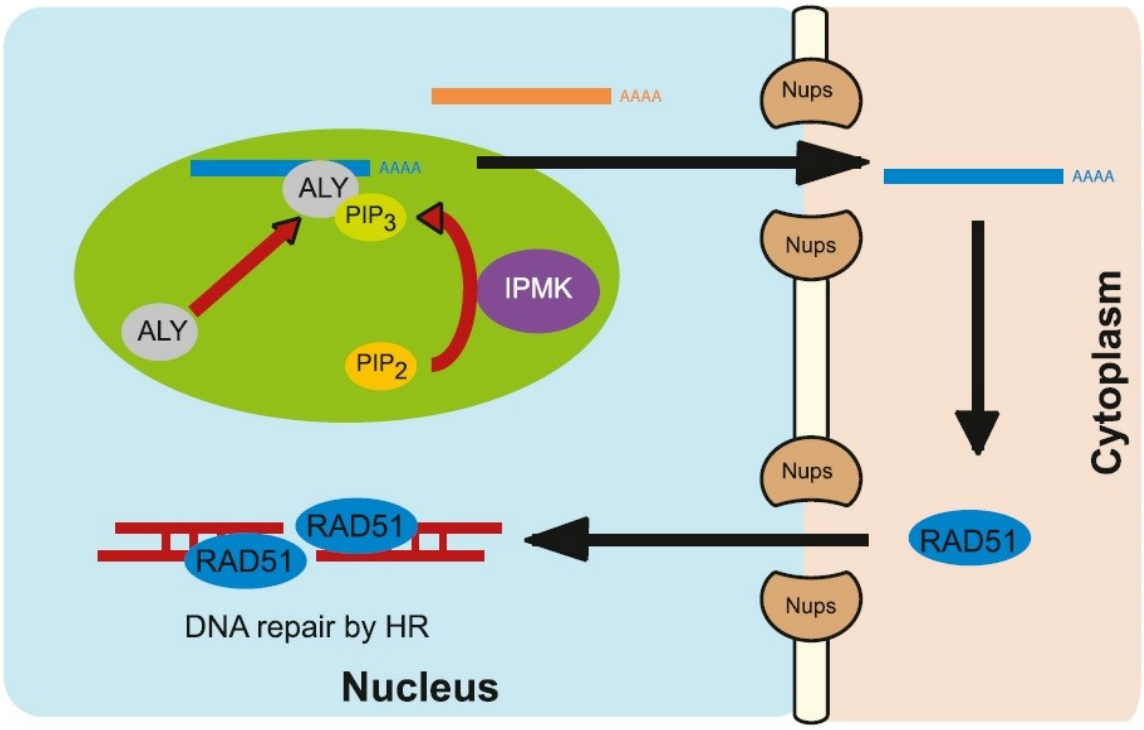

Figure 4: Regulation of nuclear mRNA export by PIs. The phosphorylation of PIP2 to PIP3 by IPMK regulates the selection of gene transcripts involved in DNA repair process (e.g. RAD51 recombinase) for the nuclear export. PIP2 - phosphatidylinositol 4,5-bisphosphate, PIP3 - phosphatidylinositol 3,4,5-trisphosphate, IPMK - inositol polyphosphate multikinase, Nups - nucleoporins, HR - homologous recombination. Taken from Wickramasinghe et al., 2013 [58].

1.2.4. DNA damage response

Phosphoinositides are also involved in response to the DNA damage. The DNA damage induced by irradiation or chemical modification increases levels of PIs in the cell nucleus. PIs are rapidly accumulated at DNA damage sites. PI(4,5)P2 and PI(3,4,5)P3 are important for the recruitment of Ataxia telangiectasia and Rad3-related protein (ATR) to DNA damage sites [60]. ATR is a serine/threonine kinase which phosphorylates a number of substrates as a response to the DNA damage, which causes the inhibition of DNA replication and the stimulation of DNA repair process [61]. ATR is most likely recruited to the DNA damage sites due to an actin polymerisation mediated by PIs [60].

PI(5)P also plays an important role in the DNA damage response. It interacts with inhibitor of growth family member 2 protein (ING2) through its PHD finger domain,

which can then bind to H3K4me3 at promoter regions of DNA damage-responsive genes. ING2 is a part of a chromatin modification complex composed of HDAC1 and transcriptional corepressor Sin3A [62]. The DNA damage associated gene repression is impaired when ING2-PI(5)P interaction is obstructed [63]. The levels of PI(5)P are increased upon UV irradiation. This is due to the inhibition of PIP4KIIβ, which generates PI(4,5)P2 from PI(5)P. PIP4KIIβ is phosphorylated by p38 kinase upon UV irradiation and its activity is inhibited [64]. Overexpression of PIP4KIIβ leads to a decreased level of ING2 signal in the cell nucleus and an increased level of ING2 signal in the cytoplasm. This reduction of PI(5)P level causes de-association of ING2 from chromatin. Binding of PI(5)P to ING2 is important for association of ING2 with chromatin, and is required for p53 activation and p53-mediated apoptosis [65]. Similarly, the elevated PI(5)P levels can also be caused by type I PI(4,5)P2 phosphatase, which generates PI(5)P from PI(4,5)P2. This enzyme is translocated to the nucleus upon DNA damage induction, the elevated PI(5)P levels stimulate the p53-mediated apoptosis by interacting with ING2 [66].

2. Aims

The involvement of phosphoinositides (PIs) in the cell signalling and diseases make them very important players in the organism. It is therefore crucial to study the basic processes they are involved in. PI(4)P and PI(4,5)P2 are the most abundant PIs in the cell. Still, we lack a knowledge about their precise localisation and function. The function of nuclear PI(4)P is completely unknown. Thus, we will address following questions:

1. **Which tools are applicable for detection of nuclear phoshoinositides?**
2. **What is the distribution of phosphoinositides within the nucleus, in particular PI(4)P and PI(4,5)P2?**
3. **What are the binding partners of PI(4)P and PI(4,5)P2 in the cell nucleus?**
4. **How is PI(4,5)P2 involved in DNA transcription?**
5. **What is the function of PI(4)P in the cell nucleus?**

3. Research papers

Individual papers are attached at the end of the thesis.

Publication 1
Tools for visualization of phosphoinositides in the cell nucleus

Kalasova I, Fáberová V, Kalendová A, Yildirim S, Uličná L, Venit T and Hozák P

Histochem Cell Biol. 2016 Apr; 145(4):485-96. doi: 10.1007/s00418-016-1409-8.

IF: 2.553 (2016)

V. F. performed experiments (DNA cloning, protein expression and purification, fluorescence microscopy).

Publication 2
Nuclear phosphatidylinositol 4,5-bisphosphate islets contribute to efficient RNA polymerase II-dependent transcription

Sobol M, Krausová A, Yildirim S, Kalasová I, Fáberová V, Vrkoslav V, Philimonenko V, Marášek P, Pastorek L, Čapek M, Lubovská Z, Uličná L, Tsuji T, Lísa M, Cvačka J, Fujimoto T and Hozák P

J Cell Sci. 2018 Apr 13; 131(8). pii: jcs211094. doi: 10.1242/jcs.211094.

IF: 4.517 (2018)

V. F. performed experiments (immunoprecipitation, sample preparation for lipid mass spectrometry).

Publication 3
Super-resolution localisation of nuclear PI(4)P and identification of its interacting proteome

Fáberová V, Kalasová I, Krausová A and Hozák P

Cells. 2020; 9(5): 1191. doi:10.3390/cells9051191.

IF: 5.656 (2018)

V.F. designed and performed most of the experiments (fluorescence microscopy, cellular fractionation, immunoprecipitation, pull-down assays, western blotting, data analysis) wrote and revised the manuscript.

Publication 4
Chromatin-associated PI(4)P regulates lysine-specific histone demethylase 1

Kalasová I, Kalendová A, Fáberová V, Marášek P, Uličná L, Vacík T and Hozák P

Manuscript.

V.F. performed experiments (cellular fractionation, pull-down assays, DNA cloning, protein expression and purification, demethylation reactions).

4. Discussion

4.1. Visualisation tools for nuclear phosphoinositides

More than 30 years ago, phosphoinositides (PIs) were found present in the cell nucleus [4, 5]. Until now, there have been observed numerous functions of PIs in nuclear processes, such as epigenetic regulation of DNA transcription, pre-mRNA processing and DNA damage (reviewed in [67, 68]). However, we still lack knowledge about their detail localisation, metabolism and function in the cell nucleus. For the purpose of studying their function, reliable tools for in vitro and in vivo visualisation of PIs in the cell nucleus are required.

We tested commercially available antibodies and phosphoinositde-binding domains as tools for their visualisation. By overexpression of PIs-binding domains, we were not able to detect nuclear pools of PIs. Overexpressed PLCδ-PH, Tubby, Grp1-PH, Akt-PH, OSH1-PH failed to recognise nuclear PI(4,5)P2, PI(3,4)P2, PI(3,4,5)P3 and PI(4)P [69]. Mutant domains resembled the signal of wild-type domains. This observation might be a consequence of overexpression of GFP-tagged domains, which are enriched unspecifically in the nucleus after the overexpression due to their ability to diffuse into the nucleus because of their size [70]. Only overexpressed EEA1-FYVE domain recognised PI(3)P signal in the nucleolus, which was lost in the mutant domain [69]. These data correspond with previous observation of PI(3)P enriched in dense fibrillar component of nucleoli of human and baby hamster kidney fibroblasts. PI(3)P was detected by double FYVE domain of the receptor tyrosine kinase substrate Hrs [71]. The function of PI(3)P in the nucleus is unknown.

The overexpression of PIs-binding domains failed to localise PIs in the nucleus, therefore we purified PLCδ-PH, Tubby and OSH1-PH domains tagged with eGFP and incubated them with fixed and permeabilised cells as analogous to antibodies. Purified PLCδ-PH and Tubby domains detected PI(4,5)P2 in nucleoli, nuclear speckles and nucleoplasm. The same pattern was also observed by anti-PI(4,5)P2 antibody. Beside PI(4,5)P2, the antibody also recognises PI(3,4,5)P3 on protein-lipid overlay assay. However, when PI(3,4,5)P3 was pre-incubated with the antibody, PI(4,5)P2 signal decreased only partially, whereas the pre-incubation with PI(4,5)P2 abrogated the PI(4,5)P2 signal fully [69]. The levels of PI(3,4,5)P3 in the cell are about twenty times lower than the levels of PI(4,5)P2 [70], therefore we believe that the anti-PI(4,5)P2

antibody recognises the nuclear pool of PI(4,5)P2. Purified OSH1-PH domain detected PI(4)P in nuclear speckles and nucleoplasm. A similar pattern was also observed by anti-PI(4)P antibody. The antibody specifically recognises PI(4)P on protein-lipid overlay assay and it was also blocked only by PI(4)P in antibody blocking assay [69].

In conclusion, anti-PI(4,5)P2 and purified GFP-tagged PLCδ-PH domain are suitable for detection of nuclear PI(4,5)P2 and anti-PI(4)P and purified GFP-tagged OSH1-PH domain are suitable for detection of nuclear PI(4)P. Using appropriate controls, these tools are applicable for visualisation of phosphoinositides in the cell nucleus.

4.2. PI(4)P and PI(4,5)P2 localisation and function within nuclear sub-compartments is different

PI(4)P and PI(4,5)P2 are the two most abundant phosphoinositides in the cell [2], therefore we focused on studying their localisation by super-resolution and electron microscopy with tested antibodies and PI-binding domains. PI(4)P and PI(4,5)P2 are localised to the same nuclear sub-compartments, however their distribution within the sub-compartments is different.

We showed that PI(4)P specifically localises to nuclear lamina, nucleolus, nuclear speckles and small nucleoplasmic foci [72]. PI(4,5)P2 localises to nucleolus, nuclear speckles and small nucleoplasmic foci as well [73]. Small portions of PI(4)P and PI(4,5)P2 can be detected within nucleoli. Through the use of transmission electron microscopy, we detected PI(4)P predominantly in dense fibrillar component (DFC) and smaller portion also localises to a granular component (GC) of nucleoli [72]. On the contrary, nucleolar PI(4,5)P2 signal is denser in fibrillar centre (FC) and DFC [74]. The nucleoli are known sites of ribosome biogenesis. Proteins involved in rDNA transcription are stored in FCs which are surrounded by DFCs. Transcription of rDNA occurs at the border of FC/DFC and early rRNA processing also occurs in DFC, while components involved in later rRNA processing are localised to GC [75, 76]. It was shown that PI(4,5)P2 associates with RNA polymerase I (RNA pol I) transcription machinery and it is important for promoting the transcription by RNA pol I [53]. Our data propose that PI(4,5)P2 might be dephosphorylated to PI(4)P during the transcription of rDNA or during the early stages of rRNA processing happening in DFC. PI(4)P might also play a role during the later stages of ribosomal assembly due to its

localisation to GC. PI(4)P might also be generated in the nucleolus from phosphatidylinositol by phosphatidylinositol 4-kinase PI4Kα which was shown to localise to the nucleolus [9, 10].

Moreover, PI(4)P and PI(4,5)P2 localise to nuclear speckles. PI(4,5)P2 signal is denser in nuclear speckles than the PI(4)P signal. Close to 16 % of total nuclear PI(4)P localises to nuclear speckles, whereas nearly 70 % of total nuclear PI(4,5)P2 localises to nuclear speckles [72, 73]. PI(4)P localises more at the edges of nuclear speckles, where the active transcription of several genes occur [77-79]. PI(4,5)P2 localises inside of speckles rather than at their edges. In addition, enzymes which synthesise PI(4)P and PI(4,5)P2 also localise to the nuclear speckles. Particularly, phosphatidylinositol 4-kinase PI4Kβ which generates PI(4)P from PI [80], phosphatidylinositol 4-phospate 5-kinases PIP5Kiα and PIP5KIγ_i4 [15-17], which phosphorylate PI(4)P to PI(4,5)P2 and SHIP-2 phosphatase which dephosphorylates PI(4,5)P2 to PI(4)P [27]. These data clearly suggest that there is an active interconversion of PI(4)P and PI(4,5)P2 happening in nuclear speckles, which might be coupled with regulation of the activity of certain proteins by phosphorylation and dephosphorylation of bound PI(4)P/PI(4,5)P2. Furthermore, phosphatidylinositol 3-kinase PI3KC2α is present in the nuclear speckles which indicates the presence of PI(3)P, PI(3,4)P2 and PI(3,4,5)P3 in nuclear speckles as well [6]. Nuclear speckles can serve as a place for the metabolism and the storage of nuclear PIs. Additionally, PIs might be involved in transcription and splicing via interaction with nuclear speckle associated proteins.

During cell division, PI(4)P and PI(4,5)P2 are dispersed in the cytoplasm [72, 74]. Moreover, PI(4,5)P2 was shown to localise to nucleolar organising regions (NORs) together with UBF and RNA polymerase I [74], and also to mitotic interchromatin granules (MIGs) together with nuclear speckle associated proteins [43].On the other hand, PI(4)P is not localised in NORs, MIGs or any other structures which supports their functional difference in the cell nucleus [72].

Furthermore, we found that 28 % of overall nuclear PI(4,5)P2 localises to nucleoplasm and it forms foci which are 40-100 nm in diameter, we called them nuclear lipid islets (NLIs). They are composed of lipids and are surrounded by proteins, DNA and RNA. DNA is not an integral part of NLIs, because NLIs' staining was resistant to DNase treatment. On the other hand, NLIs' staining was lost after RNase treatment,

which shows that RNA is the integral part of NLIs. Indeed, we showed that approximately 70 % of NLIs co-localised with RNA located on their surface [73].

By immunofluorescence and immunoprecipitation experiments, we showed that NLIs are associated with nascent transcripts, RNA polymerase II (RNA pol II) and general transcription factors. The reduction of PI(4,5)P2 level led to decreased level of RNA pol II transcription, which suggests that RNA pol II transcription requires intact NLIs [73]. We propose that NLIs provide a structural platform promoting the formation of RNA pol II transcription complexes.

Other lipid structures, nuclear lipid droplets (nLDs), were previously identified in the nucleus. They have hydrophobic core consist of triacyglycerols and cholesterol, and hydrophilic surface consist of polar lipids and proteins [81]. Although nLDs and NLIs are organised similarly, nLDs are approximately ten times bigger than NLIs and are usually connected with extended inner nuclear membrane [82].

As PI(4,5)P2 is an essential component of NLIs in the nucleoplasm, we tested whether PI(4)P is the component of NLIs as well. By electron microscopy, we visualised both phosphoinositides and we did not observe the significant co-localisation of PI(4)P and PI(4,5)P2 in NLIs. In addition, the foci formed by PI(4)P are much smaller than the foci formed by PI(4,5)P2 and they are apparently part of different nuclear protein-lipid complexes [72].

All our observed data point to distinct nuclear functions of PI(4)P and PI(4,5)P2. PI(4)P has a unique role in regulation of nuclear processes, it is not just a precursor of PI(4,5)P2.

4.3. Binding partners of PI(4)P and PI(4,5)P2 and their function

To elucidate the role of PI(4)P in nuclear processes, we identified its potential nuclear protein binding partners by immunoprecipitation with the anti-PI(4)P antibody followed by mass spectrometry analysis of nuclear extracts. As PI(4,5)P2 binding partners are better-known, we included anti-PI(4,5)P2 immunoprecipitation for MS analysis as well to compare PI(4)P and PI(4,5)P2 protein interactors.

Nearly 100 nuclear proteins were identified, which are involved in pre-mRNA and pre-rRNA processing, transcriptional regulation, transport, ribosomal assembly, DNA replication and repair. It corresponds with observed localisation of PI(4)P in the cell nucleus. Among the detected nuclear interactors, there were twelve proteins enriched

only in PI(4)P fraction, compared to control and PI(4,5)P2 fraction (Table S1, blue); [72]. The proteins are determined as fundamental components of various nuclear processes, like DNA replication, transcription and pre-mRNA splicing [83]. Although, these 12 proteins were found only in PI(4)P fraction, their interaction with PI(4)P needs to be verified by additional approaches. The majority of the identified interactors were found in both PI(4)P and PI(4,5)P2 immunoprecipitates [72]. Considering that PI(4)P is a precursor for PI(4,5)P2, or vice versa [67], they can have similar interaction partners. It was shown that phosphorylation of PIs, directly bound to proteins, can change the protein conformation and thus binding affinities of the proteins to their binding partners in a functional complex [42, 58].

The interaction of identified heterogeneous nuclear ribonucleoprotein U (hnRNP U), nuclear RNA export factor 1 (NXF1) and nuclear mitotic apparatus protein (NuMa) with PI(4)P and PI(4,5)P2 was verified by antibodies and PI-coated beads on Western blot [72]. The proteins showed different binding patterns to both PIs which could be an outcome of different antibodies' affinity to their corresponding epitopes and PI-coated beads to their baits. NuMa was previously described as a phosphoinositide-interacting protein [84]. HnRNP U is involved in initiation of transcription by RNA polymerase II. Together with actin, hnRNP U interacts with phosphorylated C-terminal domain of RNA polymerase II and decreased levels of hnRNP U abolished incorporation of BrUTP into nascent RNA [85]. We showed that the reduction of PI(4,5)P2 level led to decreased level of RNA polymerase II transcription. Potentially, PI(4)P, PI(4,5)P2 and hnRNP U might cooperate in the initiation of transcription. NXF1 plays a role in the mRNA export from the cell nucleus as a part of TREX complex [86]. Another component of TREX complex, Aly, was found to interact with PIs. The interaction of Aly and PI(3,4,5)P3 is crucial for functional mRNA export [56]. These results indicate that PIs might be essential parts of mRNA transport complexes.

We also showed that nuclear myosin 1 (NM1) localises to the NLIs' periphery. NM1 interacts with PI(4,5)P2 directly through its pleckstrin homology (PH) domain [73]. NM1 is involved in initiation of RNA polymerase II transcription [87]. Knock-down of NM1 decreased the level of RNA pol II transcription. Subsequent overexpression of NM1 rescued the RNA pol II transcription level, however the overexpression of NM1 mutated in PI(4,5)P2-binding site failed to rescue the transcription [73]. These data suggest that PI(4,5)P2-NM1 interaction is required for RNA pol II transcription. NM1

recruits chromatin remodelling complexes to gene promoters [88], and PI(4,5)P2 interaction with NM1 might be crucial for this regulation.

Lysine-specific histone demethylase 1 (LSD1) was also identified as PI-interacting protein [89]. We showed that LSD1 interacted with multiple PIs directly, but the strongest interaction was with PI(4)P. LSD1 demethylates H3K4me2 and H3K4me1 histone active marks and thus represses transcription [90]. We studied how PIs can influence LSD1 function. The binding of PI(4)P to LSD1 inhibited its activity, but on the other hand, the binding of PI(4,5)P2 to LSD1 stimulated its activity in vitro (Kalasova, manuscript). Phosphorylation of PI(4)P or dephosphorylation of PI(4,5)P2 might also have a quick regulatory effect on LSD1 function in vivo. It was shown that phosphorylation of PIs can cause conformational changes of their binding partners and thus the binding to their protein interactors and function [53]. LSD1 does not have any DNA- or histone-binding domains, therefore it is dependent on the interaction with other proteins within chromatin modifying complexes and PIs might regulate these interactions and thus the function of LSD1 in gene expression.

5. Summary and conclusions

5.1. PI-binding domains are suitable for detection of PIs in the cell nucleus

Purified GFP-tagged PLCδ-PH and Tubby domains detect PI(4,5)P2 in nucleoli, nucleoplasm and nuclear speckles. Using purified GFP-tagged OSH1-PH, PI(4)P was detected in nucleoplasm and nuclear speckles for the first time.

5.2. PI(4)P localises to the cell nucleus

PI(4)P is present in the nuclear lamina, the nuclear speckles, the nucleoli and it forms small foci in the nucleoplasm. The foci are 50 nm in size and are enriched on chromatin. A small portion of PI(4)P localises to the nucleoli, it is predominantly enriched in DFC and less enriched in GC. Almost 16 % of nuclear PI(4)P is present in the nuclear speckles and the rest localises to the nucleoplasm.

5.3. PI(4)P is in complex with nuclear proteins involved in gene expression

Mass spectrometry analysis of immunoprecipitated PI(4)P lipid-protein complexes revealed almost 100 nuclear proteins enriched in the PI(4)P fraction. The proteins participate in essential nuclear processes such as pre-mRNA processing, transcription or nuclear transport indicating the role of PI(4)P as an important player in the cell nucleus.

5.4. Localisation of PI(4)P is different than the localisation of PI(4,5)P2

Both PI(4)P and PI(4,5)P2 localise to the nuclear speckles, the nucleoli and the nucleoplasm, but their distribution within the sub-compartments is different. In the nucleolus, PI(4,5)P2 is predominantly enriched in FC and DFC and PI(4)P is predominantly enriched in DFC and less in GC. In the nucleoplasm, PI(4)P forms smaller foci than PI(4,5)P2 and is not detected in nuclear lipid islets.

5.5. Nuclear lipid islets are structural platforms for formation of RNA polymerase II transcription complexes

Nuclear lipid islets (NLIs) are nucleoplasmic structures 40-100 nm in size. Their core is rich in lipids and it is surrounded with proteins, RNA and DNA. The NLIs' periphery is associated with RNA polymerase II transcription machinery. The levels of active RNA polymerase II transcription are dependent on the levels of PI(4,5)P2. NLIs

provide a structural platform promoting the formation of RNA pol II transcription complexes.

5.6. PI(4)P and PI(4,5)P2 regulate the function of LSD1

Histone demethylase LSD1 demethylates H3K4me1/2 histone marks and binds phosphoinositides directly. In vitro, when PI(4)P is bound to LSD1, its activity is inhibited. On the other hand, when PI(4,5)P2 is bound to LSD1, its demethylase activity is stimulated.

6. Future prospects

Our data indicate that PI(4)P and PI(4,5)P2 are important for DNA transcription. PI(4,5)P2 regulates transcription by interacting with RNA polymerase II and general transcription factors. Moreover, together with PI(4)P, they regulate transcription also at the epigenetic level by regulating the activity of histone demethylase. Accordingly, we would like to address the following interesting questions:

6.1. How is LSD1 regulated by PIs?

Phosphoinositides regulate activity of proteins as their allosteric modulators by changing their conformation or as signalling molecules by recruiting their interactors to the protein-lipid complex. We showed that PI(4)P inhibits and PI(4,5)P2 stimulates the H3K4me2 demethylase activity of LSD1. However, it is uncertain how PIs regulate the LSD1 function. To study the mechanism of this regulation is necessary as LSD1 is important in cancer and neurodegenerative diseases. The inhibition of LSD1 abolishes the growth of acute myeloid leukemia cells [91], therefore it is a potential target for treatment of this disease.

6.2. Do NLIs regulate global transcription or transcription of specific genes?

NLIs are associated with nascent transcripts, RNA polymerase II and general transcription factors. NLIs provide a structural platform promoting the formation of RNA pol II transcription factories. However, it is not clear which genes are transcribed at the periphery of NLIs. It would be interesting to address whether particular sets of genes are transcribed at the NLIs' periphery and how NLIs regulate their expression.

6.3. What is the function of PI(4)P-hnRNP U complex?

Nuclear PI(4)P interacts with a number of nuclear proteins involved in RNA processing, transcriptional regulation, nuclear transport and other. We showed that hnRNP U interacts with PI(4)P and PI(4,5)P2. The majority of nuclear PI(4)P localises to the nucleoplasm and is enriched on chromatin. It was shown that hnRNP U regulates chromatin compaction by oligomerisation and interaction with chromatin-associated RNAs [92]. Therefore, it would be interesting to address how PI(4)P influences the function of hnRNP U and whether PI(4)P-hnRNP U interaction is essential for genome integrity.

6.4. What is a turnover of PIs during the cell cycle?

Most of the PIs localise to the cell nucleus as well as their metabolising enzymes. In interphase, the majority of PI(4)P and PI(4,5)P2 localise to nucleoplasm and nuclear speckles, and during mitosis, PI(4)P and PI(4,5)P2 undergo a redistribution in the cell [43, 74]. However, it is not known whether the levels of PIs also change during the cell cycle. Therefore, the analysis of the levels of all PIs during all stages of the cell cycle is needed. It would be also interesting to analyse portions of individual PIs in nuclear membrane, nuclear speckles, nucleoli and nucleoplasm by nuclear fractionation followed by lipid mass spectrometry, which might point towards their function in the cell nucleus. For future studies, it is crucial to detect and manipulate the levels of PIs in the cell nucleus.

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8. Attachments

Publication 1
Tools for visualization of phosphoinositides in the cell nucleus

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Publication 2
Nuclear phosphatidylinositol 4,5-bisphosphate islets contribute to efficient RNA polymerase II-dependent transcription

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Publication 3
Super-resolution localisation of nuclear PI(4)P and identification of its interacting proteome

Fáberová V, Kalasová I, Krausová A and Hozák P

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Publication 4
Chromatin-associated PI(4)P regulates lysine-specific histone demethylase 1

Kalasová I, Kalendová A, Fáberová V, Marášek P, Uličná L, Vacík T and Hozák P

Manuscript.