Phosphatidylinositol 5 Phosphate

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Phosphatidylinositol (PI)-related signaling plays a pivotal role in many cellular aspects, including survival, cell proliferation, differentiation, DNA damage, and trafficking. PI is the core of a network of proteins represented by kinases, phosphatases, and lipases which are able to add, remove or hydrolyze PI, leading to different phosphoinositide products. Among the seven known phosphoinositides, phosphatidylinositol 5 phosphate (PI5P) was the last to be discovered. PI5P presence in cells is very low compared to other PIs, but is has been reported to control many cellular outcomes, including cell proliferation, gene expression and chromatin remodeling.

PI5P PIKFyve myotubularin PI5P4K/PIP4K phosphatases nucleus

1. Introduction

1.1. Phosphatidylinositol Signaling

Multiple cellular functions, including survival, proliferation, differentiation, DNA damage response, and gene transcription can be modulated by a specific network of kinases, phosphatases, and lipases able to modulate the lipid second messenger phosphatidylinositol [1][2][3][4][5]. Phosphatidylinositol (PI) is composed of two different modules: a hydrophilic inositol head group bound through a phosphodiester bond to a glycerol and two fatty acids tails that represent the hydrophobic part of the molecule. The fatty acids tails are prevalently represented by stearic and arachidonic acids but other acyl chains are known to be present [6]. Modifications of the inositol ring due to addition or removal of phosphate groups, together with hydrolysis of the phosphodiester bond by phospholipases C, are the most common changes leading to the production of second messengers involved in many cellular aspects. The first demonstration of lipids as second messengers was indicated by different works, which independently elucidated the process through which PI(4,5)P_2 is cleaved by phospholipases C to diacyglycerol (DAG) and inositol 3-phosphate (IP3). These, in turn, contribute to protein kinases C (PKC) activation and calcium (Ca^{2+}) release from the endoplasmic reticulum [1][2][3][4][5][7]. Subsequently, further studies have led to the discovery of many other PI-related pathways, including those involving several forms of phosphotransferases like PIKinases and PIPKinases [8] or phosphatases like phosphatase and tension homologue deleted on chromosome 10 (PTEN) and SH2-domain containing inositol phosphatase 2 (SHIP2) [9][10].

1.2. Nuclear Lipid Signalling: Focus on Nuclear Phosphoinositides

PI signaling was first described at the plasma membrane level, which involves PI anchored to cell membranes through DAG molecules [1][2][3][4][5]. Interestingly, it soon became clear that PIs and PI-related enzymes could be present in different cellular compartments, including cytoplasmic organellar membranes and nuclei. Nuclear PI

fraction was expected to exist due to the presence of the nuclear membrane, a bilayer formed by lipids and proteins connected to the endoplasmic reticulum (ER) [11][12]. However, different reports have indicated that nuclei almost completely depleted of nuclear envelope are still characterized by the presence of many PIs and PI-related proteins like phospholipases C, phosphatidylinositol phosphate kinases (PIPKs) and diacylglycerol kinases (DGKs) [13][14][15][16][17][18][19]. Strikingly, under different stimuli, these enzymes are able to change the nuclear pool of PIs [14][16][20][21]. This evidence unequivocally demonstrated the existence of PI signaling completely localized within the nuclear compartment of cells.

Seven different phosphoderivatives of PI are known to exist and these are modulated by an intricate network of enzymes whose related pathways often intertwine. Despite the rarity of these second messengers when compared with other lipids like phosphatidylcholine or phosphatidylserine (which represent around the 12–20% of the cellular lipid pool) [1][2][3][4][5], they have been described as modulating many cellular functions acting in different cellular compartments.

2. Phosphatidylinositol 5 Phosphate: A Rare But Essential Lipid

2.1. PI5P Discovery

Out of the different phosphoinositides, PI5P represents only 0.5% of the PI pool present in the cells. However, its function as a second messenger has been widely investigated. Its levels strongly fluctuate due to external stimuli such as TCR activation, insulin treatment, oxidative stress, and pathogen cellular invasion [22]. PI5P was the last PI to be discovered. In the late 1980s, experiments regarding the purification of proteins involved in PI signaling indicated the existence of two related subfamilies of phosphatidylinositol phosphate kinases (PIPK) named type I and type II PIP5K. They were thought to both be able to phosphorylate PI4P, leading to $PI(4,5P)_2$ production. However, in 1997, Rameh and colleagues, studying the substrate specificity of these two PIPK classes, found that type II enzymes were able to specifically phosphorylate another lipid substrate to lead to $PI(4,5)P_2$, which turned out to be PI5P. As synthetic purified lipid substrates were not available at the time, the issue with PI5P detection was due to the contamination of PI4P bovine brain preparations used for experiments involving PI5P. This delayed the detection of the real function of type II PIPK, which from that point was renamed PI5P4K/PIP4K [23] (see later).

2.2. Changes in PI5P Levels Regulate Many Cellular Functions

As already stated, the pool of cellular PI5P can be regulated by many external factors and stimuli. For instance, upon TCR stimulation, quick (two minute) and transient accumulation of PI5P in Uh78 cells occurs. PI5P is in turn bound by DOK proteins, leading to IL-2 promoter activity in T cells [24]. In addition, induction of platelet aggregation by thrombin treatment has been partially connected to a three-fold increase in PI5P levels in cells [25]. Other reports have described insulin treatment as able to increase levels of PI5P in 3T3-L1 adipocytes, CHO cells stably expressing insulin receptors, and skeletal muscle cells [26]. Interestingly, insulin-dependent PI5P accumulation has been connected with GLUT4 internalization-enhancing glucose uptake from the extracellular environment [27]. On

the other hand, increased levels of PI5P obtained by insulin or infection by *Shigella flexeneri* $PI(4,5)P_2$ 4-phosphatase IpgD lead to actin remodeling and endosome formation through TIAM1 [28]. *S. flexeneri* infection-related changes of PI5P have also been proposed to internalize and degrade cell surface levels of ICAM-1, inhibiting neutrophils recruitment [29]. Recently, other pathogen signatures like lipopolysaccharides (LPS) and viral dsRNA have been found to positively affect PI5P amounts in host cells, which have been described as being involved in toll-like receptor-related pathways [30]. All these reports showed that fluctuations of PI5P in cells can be linked to different external stimuli and signaling pathways.

2.3. A role for PI5P in nuclear outcomes

The role of PI5P as a second messenger has been widely investigated. In particular, many processes regulated by changes in PI5P levels have been described in relation to the nuclei. This began with evidence collected during the study of the cell cycle of murine erythroleukemia (MEL) cells, which showed a strong increase of the nuclear PI5P pool during G1/S transition [14][31]. This led to the first ideas about possible roles of this phosphoinositide in the regulation of nuclear processes [32]. In fact, throughout the years, it has turned out that PI5P is involved in many nuclear outputs such as chromatin remodeling, gene expression, or responses to stressors like UV irradiation or genotoxic factors. For example, Gozani et al. described a role for nuclear PI5P in the regulation of ING2 mediated p53 acetylation and apoptosis during stress response [33][34]. This was possible through direct interaction between ING2 and PI5P, and controlled by the activity of two different classes of enzymes named PI5P4K/PIP4K and Type I PI(4,5)P2 4-phosphatases (see later) [35][36]. Another report, indicated PI5P as able to impact on oxidative stress cellular response and reactive oxygen species (ROS) production through Pin1 [37]. Nuclear PIs are often involved in chromatin remodeling and gene expression. In particular, PI5P has been reported to negatively control Arabidopsis Thritorax (ATX) which encodes plant methylatransferase proteins, able to methylate lysine residues on the tail of H3 histones [38][39]. On the other hand, the Ubiquitin-like, containing PHD and RING Finger domains 1 (Uhrf1), an E3 ubiquitin ligase, which plays a pivotal role in DNA methyltransferase 1 (DNMT1) DNA docking, has been shown to interact with PI5P. This interaction modulates the DNA accessibility of DNMT1 and, in turn, DNA methylation [40]. Recently, we demonstrated that silencing PIP4K2B and PIP4K2C in human T regulatory cells (Treg) impact on Uhrf1 levels and Treg function and proliferation [41]. Finally, nuclear PI5P has been connected to the regulation of myogenic differentiation affecting the DNA binding to specific DNA regions of the TATA-Box Binding Protein Associated Factor 3 (TAF3), and impacting on the expression of several genes involved in myogenesis [42].

3. Enzymes Involved in the Turnover of PI5P

Although PI5P levels can change upon different stimuli, how this occurs is not always understood. Several pathways underlying PI5P synthesis have been described, including direct processes through phosphorylation of PI on position 5, or indirect-like de-phosphorylation of $PI(3,5)P_2$ (Figure 1). Moreover, the balance between $PI(4,5)P_2$ and PI5P mediated by PIP4K has also been found to be important for cellular control of the PI5P pool.

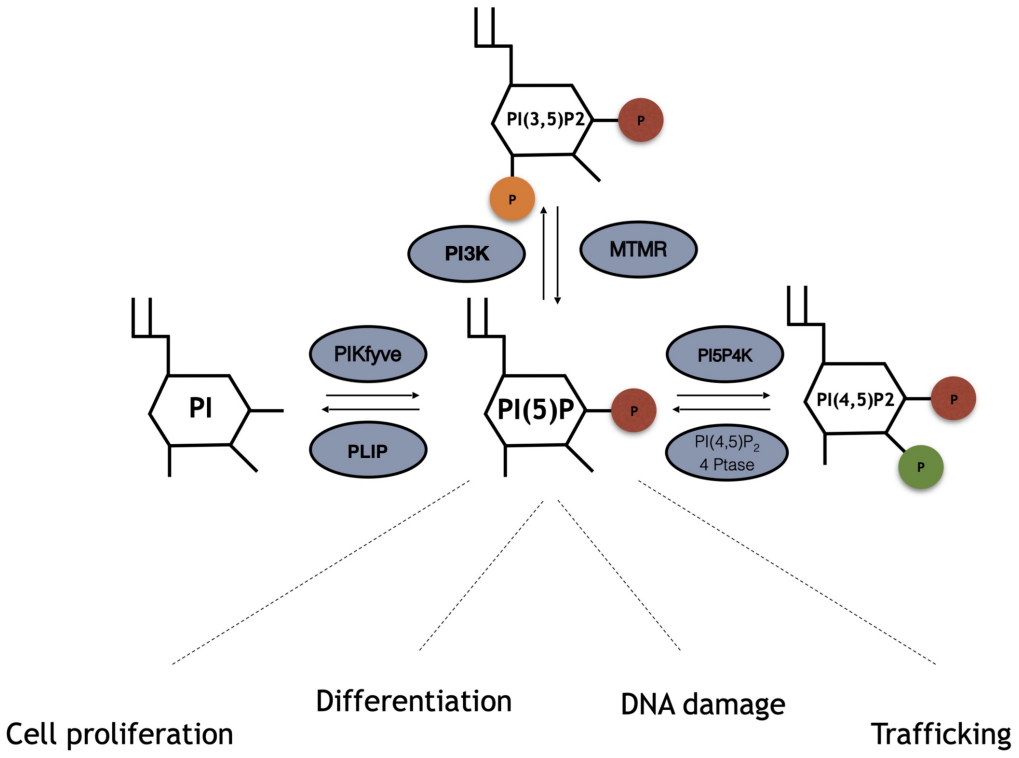

Figure 1. Enzymes involved in phosphatidylinositol 5 phosphate (PI5P) turnover and array of kinases and phosphatases involved in PI5P turnover. PI5P can be directly synthesized by PIKFyve phosphotransferases through direct phosphorylation of phosphatidylinositol (PI) on position 5 of the inositol ring. Moreover, MTMR phosphatases can remove a phosphate group on position 3 from $PI(3,5)P_2$, leading to increased amount of PI5P levels. Finally, PI5P4K/PIP4Ks directly phosphorylate PI5P on position 4 leading to $PI(4,5)P_2$ synthesis, an event that can be counterbalanced by type I/II 4-phosphatases which remove the phosphate group on position 4.

3.1. PIKfyve/Phosphatidylinositol-3-Phosphate 5-Kinase

PIKfyve, also known as phosphatidylinositol-3-phosphate 5-kinase type III or PIPKIII, is an established evolutionarily conserved PIK present in animals, plants and fungi. It possesses a FYVE zinc finger domain, named after the proteins in which it was first identified: Fab1p (the yeast orthologue of PIKfyve), YOTB, Vac1 (vesicle transport protein), and EEA1 (Early Endosome Antigen 1) [43][44][45][46]. This domain has a small cysteine-rich Zn_{2+} binding domain, characterized by a basic motif in the first β-strand (R/K) (R/K) HHCR which primarily allows phosphatidylinositol 3 phosphate (PI3P) binding. PIKfyve is a large protein involved in endosome processing, HIV and Salmonella replication, and type 2 diabetes, while mutations in its coding gene are connected to corneal fleck dystrophy (CFD) [46][47]. Interestingly, together with its capacity to phosphorylate PIs, it possesses protein kinase activity towards non lipid substrates [48]. Nevertheless, in vitro and in vivo evidence has described PIKfyve as able to bind and phosphorylate the lipids PI3P and PI, leading to the synthesis of $PI(3,5)P_2$ and PI5P respectively [43][44][45][46] (Figure 1). Overexpression or silencing/inhibition of PIKfyve leads to changes in the levels of these two PIs. Most PI5P production in cells is thought to be due to PIKfyve activity via both direct and indirect pathways. Indeed, as stated, PIKfyve is able to directly phosphorylate PI rings in position 5, leading to synthesis of PI5P (the direct

pathway) [43][44][45][46][49]. Another proposed model of PI5P synthesis is related to dephosphorylation of PIKfyve-derived PI(3,5)P_2 by 3-phosphatases named myotubularins (the indirect pathway, see next) [50][51].

3.2. MTM-MTMR/Myotubularins

Myotubularin 3-phosphatases represent a family of proteins conserved in eukaryotes and composed of 15 members named MTM1 and MTMR1–14 [52][53][54][55]. These enzymes share a structural motif which is represented by a PH-GRAM (pleckstrin homology-glucosyltransferases, rab-like GTPase activators, and myotubularin), catalytic protein tyrosine phosphatases (PTP) domains, and a coiled-coil motif. Some of the isoforms also contain FYVE-, PH-, and PDZ-binding sites [52][53][54][55]. The active site of the protein is represented by a Cys-X_5-Arg motif, which allows hydrolyzation of phosphodiester bonds on a cysteine nucleophile and an arginine residue, binding an oxygen atom onto the phosphate groups [52][53][54][55]. These catalytic residues can be altered by missense substitutions in several isoforms. This divides myotubularins into active (MTM1, MTMR1–4, MTMR6, MTMR7–8, and MTMR14) and inactive (MTMR5 and MTMR9–13) phosphatases [56][57]. Throughout the years, MTMs have been described as being able to bind and dephosphorylate PI3P and PI(3,5)P_2 to PI and PI5P, respectively. Reports on the crystal structure of MTMR2 have unraveled the molecular basis of PI3P and PI(3,5)P_2 binding through its PH-GRAM domain [57][58][59] (Figure 1). Finally, this class of PI phosphatases is known to play a role in endocytosis and membrane trafficking, cell proliferation, differentiation, and cell junction dynamics. Mutations on the genes encoding myotubularin enzymes have been found in neuromuscular diseases or have been associated with metabolic syndromes, obesity, and cancer.

3.3. Phosphatidylinositol 5 Phosphate 4 Kinases (PI5P4K/PIP4K)

Phosphatidylinositol 5 phosphate 4 kinases, or type II PIPKs, represent a family of enzymes able to phosphorylate PI5P in order to produce PI(4,5)P_2 [23]. PIP4Ks are conserved in different species spanning from flies and worms to mice and humans. Mammalians are characterized by the presence of three different isoforms, namely, PIP4K2A, PIP4K2B, and PIP4K2C [4][8][60][61][62]. They share dimerization and lipid kinase domains and carry main differences in amino acid sequences found at -N and -C termini, which confer each isoform specific characteristics [4][8][61][62][63]. PIP4K2A is considered the most active isozyme if compared to PIP4K2B, while PIP4K2C possesses a limited capacity to phosphorylate PI5P [4][8][60][61][62]. Interestingly, PIP4K2B preferentially uses GTP instead of ATP for its kinase function [64][65]. PIP4K isozymes also differ from each other for their intracellular localization: 2A is mostly located in the cytoplasm/membrane, 2B can also be found in the nucleus, and 2C is found in not well defined membraneless compartments. As already indicated, this class of enzymes was first discovered in 1997 by members of Cantley's lab, who were able to overcome an issue related to the mix between PI4P and PI5P in bovine brain preparations [23]. This finding rendered the study of PIP4K substrate specificity possible and described those proteins as different from their related family of PIP5K [66]. In any case, the capacity of PIP4K to produce PI(4,5)P_2 is considered minor with respect to PIP5K, so they are suggested as being involved in the regulation of PI5P levels in cells [4][8][61][62] (Figure 1). Indeed, knockdown/inhibition of PIP4Ks in mammalian cells or knockout in drosophila leads to increased levels of PI5P, with almost no effects on the pool of PI(4,5)P_2 [61][67][68]. These proteins have been recently connected with many cellular functions, including DNA damage, cell proliferation, and

chromatin remodeling, and have been proposed as possible targets for treatment of cancer or autoimmune diseases [69][70][71][72][41][73].

3.4. Type I/II PI(4,5)P_2 4-Phosphatases

Type I/II PI(4,5)P_2 4-phosphatases are human phosphatases able to specifically target PI(4,5)P_2 and hydrolyze the phosphodiester bond of D4 phosphate on the inositol ring, leading to synthesis of PI5P in human cells. Type I/II 4-phosphatases share a Cys-X_5-Arg motif with the *Shigella flexenery* PI(4,5)P_2 4-phosphatase IpgD. This, once injected or expressed into host cells, leads to a specific and strong increase in PI5P levels through PI(4,5)P_2 hydrolysis [74][75][76] (Figure 1). Mammalian type I/II PI(4,5)P_2 4-phosphatases were described for the first time by Ungewickell et al. and were reported as being located in endosomal/lysosomal membranes in epithelial cells. In addition, upon cellular stress, type I 4-phosphatase can localize in the nucleus, where it regulates p53 dependent apoptosis [35].

4. Conclusions

Despite the low abundance in the cells, PI5P is a lipid second messenger involved in the regulation of several cellular functions, spanning from cytoskeletal organisation to nuclear dynamics. Specifically, PI5P production and turnover is due to the activity of different enzymes comprising myotubularins, PIKFyve, PIP4Ks, and type I/II 4-phosphatases. However, the study of PI5P as a second messenger is still quite challenging. This is due to a lack of specific antibodies or ways to detect it, i.e., more sensitive cellular probes, as well as methods to specifically and singularly alter its levels. For all these reasons, many new chapters in the story of this rare and little understood phosphoinositide will be written in future.

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