Biochimica et Biophysica Acta 1838 (2014) 1501–1508

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Enrichment of phosphatidylinositols with specific acyl chains ☆

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ARTICLE INFO

Article history:
Received 28 July 2013
Received in revised form 25 September 2013
Accepted 3 October 2013
Available online 10 October 2013

Keywords:
Phosphatidylinositol
Phosphatidylinositol cycle
Arachidonoyl
Lipid signal
Acyl chain

ABSTRACT

There are six major species of phospholipids in eukaryotes, each of which plays unique structural and functional roles. One species, phosphatidylinositol (PI) only contributes about 2–10% of the total phospholipid pool. However, they are critical factors in the regulation of several fundamental processes such as in membrane dynamics and signal transduction pathways. Although numerous acyl species exist, PI species are enriched with one specific acyl chain composition at both *sn−1* and *sn−2* positions. Recent work has identified several enzymes that act on lipids to lead to the formation or interconversion of PI species that exhibit acyl chain specificity. These enzymes contribute to this lipid's enrichment with specific acyl chains. The nature of the acyl chains on signaling lipids has been shown to contribute to their specificity. Here we review some of the critical functions of PI and the multiple pathways in which PI can be produced and metabolized. We also discuss a common motif that may confer arachidonoyl specificity to several of the enzymes involved. This article is part of a Special Issue entitled: Membrane Structure and Function: Relevance in the Cell's Physiology, Pathology and Therapy.

Contents

Abbreviations: 2-AG, 2-arachidonoylglycerol; CDS, CDP-diacylglycerol synthase; CDP-DAG, CDP-diacylglycerol; DAG, diacylglycerol; DGK, diacylglycerol kinase; ER, endoplasmic reticulum; IP_3, inositol triphosphate; LPA, lysophosphatidic acid; LPIAT1, lysophosphatidylinositol acyltransferase 1; PA, phosphatidic acid; PI, phosphatidylinositol; $PI(4,5)P_2$, phosphatidylinositol-4,5-bisphosphate; PI4P5K, phosphatidylinositol-4-phosphate 5-kinase; PIP_ns, phosphorylated forms of PI; PIS, PI synthase; PKC, protein kinase C; PLC, phospholipase C; PM, plasma membrane; PUFA, polyunsaturated fatty acids; SAG, 1-stearoyl-2-arachidonoyl glycerol

☆ This article is part of a Special Issue entitled: Membrane Structure and Function: Relevance in the Cell's Physiology, Pathology and Therapy.

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0005-2736/$ – see front matter
http://dx.doi.org/10.1016/j.bbamem.2013.10.003

1. Introduction

The major focus regarding the lipid composition of biological membranes and the roles of lipids in signal transduction has been on the nature of the lipid headgroup. However, it is well established that the acyl chain composition of lipids also has an important biological role. This is at first surprising since acyl chains are only hydrocarbons and do not contain a variety of polar groups that can result in specific interactions. An example of a specific role of an acyl chain is the finding that one specific acyl chain, the arachidonoyl (20:4) chain (Table 1 shows some acyl chain structures) attached to phosphatidylcholine oscillates during the cell cycle and delays cell cycle progression as a consequence of inhibiting the binding of Akt [1]. There are several mechanisms by which acyl chains can modulate function. One mechanism is by changing the physical properties of the membrane, with no specific requirements for a particular chemical structure. One example of this is tafazzin, an acyl transferase that enriches cardiolipin with linoleoyl (18:2) chains, yet exhibits no substrate specificity for particular acyl chains [2]. In contrast to this, there are very specific structural requirements of certain enzymes for particular lipids, such as the specificity of certain lipoxygenases for arachidonic acid [3].

Another indication that acyl chains play a functional role is the observation that they are very unevenly distributed among lipids of different classes, *i.e.* among lipids containing different headgroups. Even within the same organism, the acyl chain composition of specific lipids is different in different organs. Furthermore, changes in the acyl chain composition of a lipid can affect its function and even lead to disease states. The factors determining the specific incorporation of particular acyl chains in certain lipids and the consequences of the loss of this specificity are only recently attracting more attention. One lipid class that is highly enriched in specific acyl chains is phosphatidylinositol (PI).

1.1. Properties of phosphatidylinositols

Phosphatidylinositol (PI) is composed of a glycerol backbone, with an inositol ring and a phosphate at the *sn−3* position and two acyl chains esterified at the *sn−1* and *sn−2* positions [4]. The inositol ring can be phosphorylated at multiple positions, which can yield seven unique species known as phosphoinositides (PIP_ns) (Fig. 1) [5]. PIP_ns are spatially and temporally maintained in distinct sub-cellular compartments through the concerted actions of PI-kinases and phosphatases. For example, phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) is primarily enriched in the plasma membrane (PM), whereas phosphatidylinositol-4-phosphate (PI4P) is high in the Golgi [6,7].

Table 1
Examples of polyunsaturated fatty acids.

Common name	Lipid name	Chemical name
Omega-3 fatty acids		
Alpha-linolenic acid (ALA)	18:3 (n−3)	*All-cis*-9,12,15-octadecatrienoic acid
Eicosatrienoic acid (ETE)	20:3 (n−3)	*All-cis*-11,14,17-eicosatrienoic acid
Eicosatetraenoic acid (ETA)	20:4 (n−3)	*All-cis*-8,11,14,17-eicosatetraenoic acid
Eicosapentaenoic acid (EPA, timnodonic acid)	20:5 (n−3)	*All-cis*-5,8,11,14,17-eicosapentaenoic acid
Docosapentaenoic acid (DPA, clupanodonic acid)	22:5 (n−3)	*All-cis*-7,10,13,16,19-docosapentaenoic acid
Docosahexaenoic acid (DHA, cervonic acid)	22:6 (n−3)	*All-cis*-4,7,10,13,16,19-docosahexaenoic acid
Omega-6 fatty acids		
Linoleic acid	18:2 (n−6)	*All-cis*-9,12-octadecadienoic acid
Gamma-linolenic acid (GLA)	18:3 (n−6)	*All-cis*-6,9,12-octadecatrienoic acid
Dihomo-gamma-linolenic acid (DGLA)	20:3 (n−6)	*All-cis*-8,11,14-eicosatrienoic acid
Arachidonic acid (AA)	20:4 (n−6)	*All-cis*-5,8,11,14-eicosatetraenoic acid
Docosapentaenoic acid (osbond acid)	22:5 (n−6)	*All-cis*-4,7,10,13,16-docosapentaenoic acid

PIP_n species control several different cellular processes such as the regulation of ion channels, actin-cytoskeleton dynamics, vesicular transport, endocytosis, exocytosis and signal transduction pathways [8–10]. The interactions between PIP_ns and their downstream targets are numerous and complex, so only an overview of their interactions will be discussed. Simply, downstream targets of PIP_ns are recruited and/or activated at specific sub-cellular compartments through phosphoinositide binding motifs. These interactions are primarily mediated through a combination of electrostatic and hydrophobic interactions [11]. The role of the headgroup structure in these interactions is currently better understood, however evidence is accumulating to indicate that the acyl chain composition also has an important role. Currently, ten phosphoinositide binding motifs have been characterized, each showing specificity for different PI species [12]. For example, although the adaptor proteins AP1 and AP2 bind similar cargo proteins, AP1 binds PI4P and localizes in the Golgi. AP2 on the other hand, binds $PI(4,5)P_2$ and is enriched in the PM [13,14].

1.2. Phosphatidylinositol biosynthesis

The *de-novo* biosynthesis of PI occurs exclusively in the endoplasmic reticulum (ER) and begins with the precursors, glycerol-3-phosphate or dihydroxyacetonephosphate (Fig. 2) [15]. These molecules undergo two sets of acylations through the actions of acyltransferases; the first acylation forms lysophosphatidic acid (LPA), whereas the second acylation step produces phosphatidic acid (PA) [16]. PA can also be formed through the actions of diacylglycerol kinase (DGK) on diacylglycerol

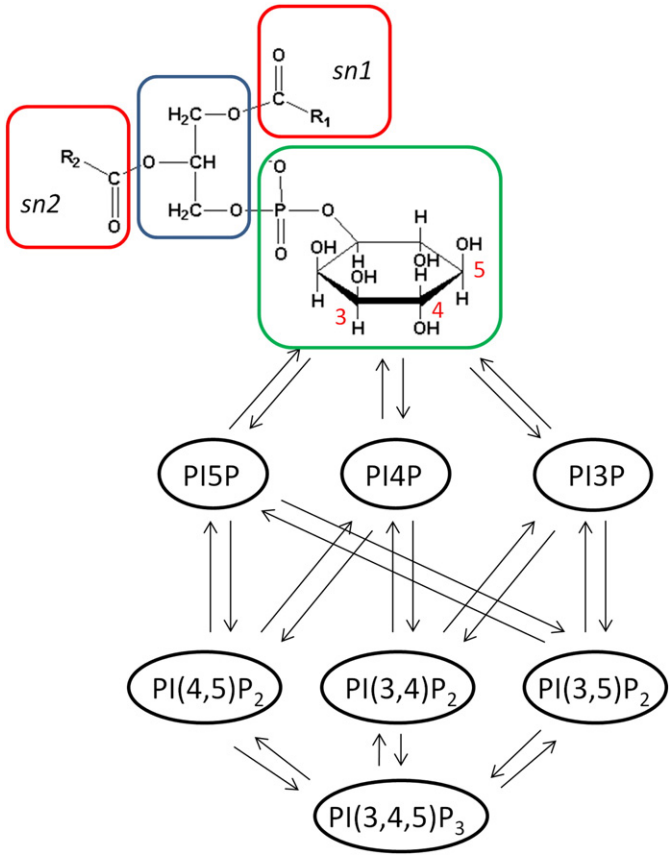

Fig. 1. The structure and production of phosphatidylinositol. Phosphatidylinositol is composed of a glycerol backbone (blue), two acyl chains at the *sn−1* and *sn−2* positions (red) and an inositol headgroup (green). The hydroxyl groups can be phosphorylated at positions 3, 4 and 5, which can yield up to seven unique phosphoinositide species. The production of these species is tightly regulated through the actions of PI kinases and phosphatases. PIP_n species can also be interconverted, as indicated by the arrows.

(DAG) and by the action of phospholipase D on certain phospholipids, such as phosphatidylcholine. PA is then converted to CDP-diacylglycerol (CDP-DAG), through CDP-DAG synthases (CDS), from PA and CTP. In the final step, PI synthase (PIS) catalyzes the coupling of CDP-DAG to *myo*-inositol to form PI [15]. Once formed in the ER, the PI is distributed to other locations in the cell, including the plasma membrane (PM) resulting in the recycling of lipid between the PM and the ER forming the PI cycle as described below.

1.2.1. Acyl-chain enrichment

PI is highly enriched at both *sn*−*1* and *sn*−*2* positions with specific acyl chains; the major species being 1-stearoyl-2-arachidonoyl PI (18:0 *sn*−*1*/20:4 *sn*−*2* PI) [17–19]. Depending on the particular tissue, up to 70% of all PI species have this acyl chain composition [18]. Interestingly, studies have shown that the acyl chain composition of PI synthesized through the *de novo* pathway, in contrast to the lipids in the PI cycle, contains mainly saturated and monounsaturated acyl chains [20–23]. So, how do PI species become highly enriched with a different acyl chain composition? There are two potential enzymatic processes that can result in this selective acyl chain incorporation. One involves the cyclical pathway known as the PI cycle [24]. The substrate specificity of the enzymes involved in this cycle can lead to acyl chain enrichment. Since the processes are cyclical and all the lipid intermediates of the cycle are regenerated, any partial enrichment occurring in one cycle will be multiplied by the number of times the cycle repeats. The second pathway, known as the Land's cycle, involves acyl chain remodeling of PI species through acylation and deacylation reactions [25].

1.2.2. The PI cycle

Upon stimulation by growth factors, PI-specific isoforms of phospholipase C (PI-PLC) cleave $PI(4,5)P_2$ in the PM into inositol triphosphate (IP_3) and DAG (Fig. 2) [24]. IP_3 is a water soluble signaling molecule which can activate Ca^{2+} channels and release Ca^{2+} from the ER. DAG itself is a potent lipid secondary messenger which can activate several different DAG-binding proteins such as PKC, PKD, Munc13 and RasGRP [26]. The DAG that is a component of the PI cycle is phosphorylated to PA by DGK in either the ER or the PM [27]. This PA can then be fed into the PI cycle [24]. PA is also produced by the action of phospholipase D, but this PA is not enriched in stearoyl–arachidonoyl acyl chains and therefore must be segregated from the PI cycle. The PI produced in the ER is then transported to the PM, undergoing two phosphorylations to generate $PI(4,5)P_2$ [27]. The cyclical nature of the PI cycle suggests that acyl chain enrichment of PI species could occur if one or more enzymes show acyl chain specificity.

1.2.3. The Land's cycle

All phospholipids undergo acyl chain remodeling through the actions of acyltransferases and phospholipases. This process is collectively known as the Land's cycle (Fig. 3) [25,28–30]. Remodeling allows certain lipids to maintain specific acyl chain compositions, which is important for signaling functions. Several classes of enzymes that are needed for remodeling have been characterized. Lysophosphatidylinositol acyltransferases are enzymes that transfer an acyl group from acyl-CoA to the *sn*−*2* position of a lysophospholipid [31]. Other enzymes required for remodeling include the phospholipase A1 and A2 families, which cleave acyl chains off phospholipids [32,33]. The Land's cycle could result in acyl chain enrichment through the selective incorporation and/or removal at acyl chains of PI.

2. Evidence for the importance of enrichment of DAG with specific acyl chains

The DAG species produced from PC has distinct acyl chain compositions compared with the DAG produced from $PI(4,5)P_2$ [34,35]. DAG

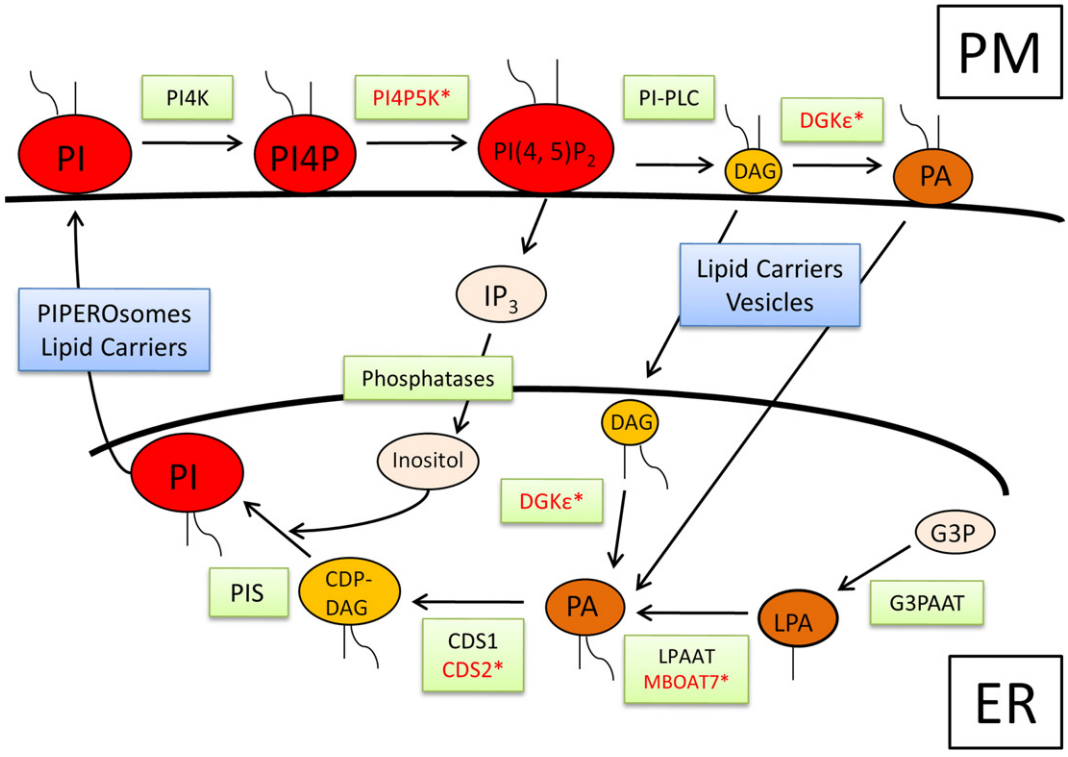

Fig. 2. The multiple pathways of PI synthesis. The *de novo* synthesis of PI begins with G3P, which is converted to PA. CDS1/2 converts PA to CDP-DAG and PI is formed through PIS enzymes. The *de novo* pathway takes place in the ER. The PI cycle also contributes to PI synthesis, feeding PA into the *de novo* pathway. PA is produced through the phosphorylation of DAG by DGKε, which is formed by $PI(4,5)P_2$ cleavage. $PI(4,5)P_2$ is re-synthesized in the PM. The PI cycle requires both the ER and PM. Enzymes that have arachidonoyl specificity are highlighted in red and marked with an *.

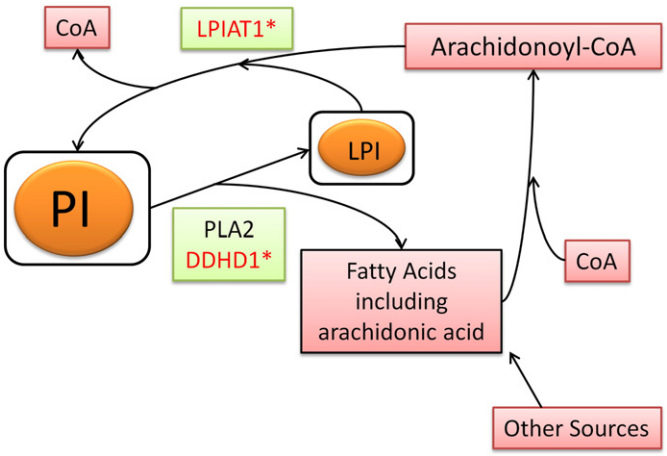

Fig. 3. The Land's cycle of acyl chain remodeling for PA and PI species. The acyl chains of PI can be remodeled through phospholipases and acyltransferases. DDHD1 is a phospholipase A1 that has dual specificity for PA and PI. Other phospholipases, both A1 and A2, cleave acyl chains of phospholipids with varying specificity for the headgroup. Conversely, acyltransferases can transfer an acyl chain to lysophospholipids from other phospholipids or from acyl-CoA, creating new phospholipids. Both phospholipases and acyltransferases are critical as they not only maintain lipid asymmetry, but also regulate the levels of phospholipids, lysophospholipids and fatty acids. Enzymes that may contribute to arachidonoyl enrichment in PI are highlighted in red and marked with an *.

produced by cleavage of PC is either saturated or monounsaturated at its *sn−2* position. The principle form of DAG produced from PI(4,5)P_2, as a step in the PI cycle, is 1-stearoyl-2-arachidonoyl glycerol (SAG) formed by the action of PI-PLC on PI(4,5)P_2. The nature of the acyl chains on DAG determines its potency in stimulating protein kinase C (PKC) [36]. Sustained PKC activation has been shown to be oncogenic and contributes to malignant phenotypes seen in cancers [37,38]. Only polyunsaturated DAG species (derived from PI(4,5)P_2) were shown to be able to bind and activate PKC (α, ε, δ) isoforms both *in vitro* and *in vivo* [34,35,39]. In particular, SAG is more effective in activating PKCδ compared with other DAG species. The relative activating potency of different DAG species is different for different PKC isoforms [35], showing that the acyl chain composition of DAG affects its signaling properties. Other evidence of acyl chain specificity of DAG comes from recent studies of the activation of caged DAGs with different acyl chains [40]. It was demonstrated that the photoactivation to liberate SAG, and not other DAGs, resulted in a massive increase of intracellular Ca^{2+} levels. This can be understood in terms of the PI cycle. SAG is an intermediate in the PI cycle and its liberation will activate the cycle. This is because in biochemical cycles all of the intermediates in the cycle act as catalysts for the cycle because they are regenerated by the cycle itself. While all of the lipid intermediates are regenerated by the cycle, the overall process utilizes nucleotide triphosphates and inositol to generate IP_3, a ligand for opening calcium channels in the ER and raising the intracellular levels of Ca^{2+}. Thus, these experiments tie in specific acyl chains with signaling resulting from the PI cycle.

3. Phospholipids as precursors of bioactive lipids

Phospholipids themselves can act as signaling molecules, as well as being precursors for other bioactive lipids through the action of phospholipases. For example, arachidonic acid, liberated by hydrolysis of phospholipids, is a critical precursor for a large family of inflammatory compounds known as eicosanoids [41,42]. Free fatty acids and lysophospholipids have roles as lipid signaling agents or as precursors to such lipids. However, fatty acids and lysolipids also have detergent-like properties and are toxic to cells at high concentration. Hence they are maintained at low concentrations in the cell. They are produced as signaling agents from phospholipid precursors and the quantity of fatty acids and lysolipids that can be generated is highly dependent on the presence of certain acyl chains in the phospholipid. Hence the amount of phospholipids in a cell with certain acyl chains, in particular arachidonoyl and docosahexanoyl chains, will determine the ability to form downstream lipid signaling molecules.

3.1. Arachidonic acid as a precursor of bioactive lipids

Polyunsaturated acyl chains also serve as precursors to several classes of lipid modulations. These lipid modulators play critical roles in processes such as inflammation, blood pressure, immune function and neuroprotection [43,44]. Arachidonic acid, derived from the *sn−2* acyl chain of PI and phosphatidylethanolamine (PE), is a precursor to a family of oxygenated derivatives known as eicosanoids. Eicosanoids encompass a large family of lipid molecules, most of which are pro-inflammation [41,42]. Chronic inflammation often results in fever and is implicated in several diseases, such as arthritis, atherosclerosis and cancer [41,44,45].

3.2. Polyunsaturated acids (PUFA) as precursors of anti-inflammatory lipids

Many of the eicosanoid lipids are pro-inflammatory. Polyunsaturated fatty acids are also precursors for anti-inflammatory lipids. Arachidonic acid is an n−3 PUFA. Another common n−3 PUFA are eicosapentaenoic acid (20:5) that is a precursor for E-resolvins, and docosahexaenoic acid (22:6) that is a precursor for D-resolvins and for protectin D1 [46]. All of these products are anti-inflammatory and reverse the inflammation caused by eicosanoids [47].

3.3. 2-Arachidonoylglycerol (2-AG) as a signaling lipid

2-Arachidonoylglycerol (2-AG) is derived from the cleavage of SAG by DAG lipases [48]. 2-AG is a known ligand for the CB_1 and CB_2 cannabinoid receptors, which are implicated in the regulation of food intake, neurotransmitter release and pain [49–51]. Aberrant 2-AG signaling has also been clinically linked to Alzheimer's disease in patients [52].

4. Enzymes contributing to the enrichment of lipids with arachidonoyl groups

While several different PUFA have specific roles in lipid signaling, in this review we wish to focus on the role of arachidonoyl enrichment which is important for the lipid intermediates of the PI cycle, as well as for the generation of eicosanoids. Several enzymes show arachidonoyl specificity and could play significant roles in PI's acyl chain enrichment. In the following sections, we will examine several of these enzymes, with specific emphasis on the physiological significance of DGKε, phosphatidylinositol-4-phosphate 5-kinase (PI4P5K) and lysophosphatidylinositol acyltransferase 1 (LPIAT1).

4.1. Diacylglycerol kinase epsilon (DGKε)

DGKε catalyzes the phosphorylation of DAG to PA using ATP as a phosphate donor [53]. Out of the ten mammalian isoforms of DGK, DGKε is the only isoform that shows specificity for the acyl chains of the substrate [54]. *In vitro* and *in vivo* experiments have shown that DGKε prefers DAG substrates with an *sn−1* stearoyl and an *sn−2* arachidonoyl group, the species being SAG (18:0 *sn−1*/20:4 *sn−2* DAG) [54–56].

Since the acyl chain composition of SAG is also predominant in PI species, it is believed that DGKε is committed to PI re-synthesis. We should point out that although DGKε can contribute significantly to acyl chain enrichment in PI, it is not the only factor. There are tissues in which DGKε is not highly expressed, yet they still can synthesize PI. However, in tissues such as the brain in which DGKε is highly expressed, acyl

chain enrichment, with *sn−1* stearoyl and *sn−2* arachidonoyl, is particularly large. Studies in DGKε$^{-/-}$ MEFs have indicated that there is a roughly 30% decrease in the content of PA and PI in the plasma membrane of these cells as compared to wild type MEFs [24]. Similar lipidomic experiments in DGKε$^{-/-}$ MEFs have also shown a reduction in the levels of both stearoyl and arachidonoyl contents in PI lipids [17,54]. The decrease in arachidonoyl content also extends to other lipid classes, including PC [17]. This change in arachidonoyl-PC would be expected to increase cell cycling and promote Akt activation [1]. Over-expression of DGKε in porcine aortic endothelial cells, that would phosphorylate SAG to make the corresponding PA, results in significant decreases in polyunsaturated DAGs [34]. In DGKε$^{-/-}$ mouse models, electrical stimulation of these mice resulted in decreases in the levels of arachidonoyl-PI(4,5)P_2 and free arachidonic acid [57]. Thus it appears that DGKε not only contributes to the re-synthesis of PI, but also increases its arachidonoyl content.

The functional importance of DGKε in PI-synthesis and acyl chain enrichment has been demonstrated through several studies, most notably in neural function. DGKε appears to regulate seizure susceptibility and long term potentiation through PI signaling [43,57,58]. Using electroconvulsive shocks, DGKε$^{-/-}$ mice were shown to have reduced levels of free arachidonic acid, 20:4-DAG and 20:4-PI(4,5)P_2. These lipids were also shown to take longer to return to resting levels. Physiologically, DGKε$^{-/-}$ mice were shown to be resistant to electroconvulsive shock and kindling, conditions tied to deficiencies in long-term neural potentiation. DGKε$^{-/-}$ mice also showed a lack of morphological changes in hippocampal glial cells, including hypertrophied cell bodies and elongated processes [57]. The involvement of the PI-cycle and DGKε in kindling and long-term potentiation makes it an attractive target for epilepsy.

DGKε also appears to be an attractive target for the attenuation of Huntington's disease. Blocking DAG-activated transient receptor potential has been shown to block Huntington's neurotoxicity [59]. Mutant Huntington (Htt) protein also appears to bind subsets of PIP_ns more strongly that the wild type protein. Inhibition of DGKε using siRNAs reduced caspase activity and striatal toxicity of a Hdh$^{111Q/111Q}$ cell line. Similarly, DGKε protein levels were also increased in the striatum of HD transgenic mice, which suggests a potential role for this protein in the observed toxicity of mutant Htt.

Recent work has also identified roles for DGKε in renal function; mutations in the *DGKE* gene were identified in membranoproliferative-like glomerular microangiopathy (MPGN) and atypical hemolytic–uremic syndrome (HUS) [60,61]. Both MPGN and HUS present with several renal phenotypes, such as thrombosis and frequently leading to renal failure. DAG signaling is believed to be a key for the development of these diseases through the production of pro/anti-thrombic factors and the regulation of slit diaphragm function in endothelial cells and podocytes, respectively. These mutant DGKε variants were primarily localized to its catalytic domain and were shown to result in increasing DAG levels.

DGKε restores cardiac dysfunction and improves survival under chronic pressure overload by controlling cellular DAG levels and TRPC-6 expression. It is suggested that DGKε may be a novel therapeutic target to prevent cardiac hypertrophy and progression to heart failure [62].

4.2. CDP-diacylglycerol synthase 2 (CDS2)

CDS2 is involved in the next step of PI re-synthesis after DGKε, catalyzing the conversion of PA to CDP-diacylglycerol [63]. There are two CDS isoforms, both of which are found in the ER [64,65]. CDS2 appears to show substrate specificity for PA species, preferentially acting on 1-stearoyl-2-arachidonoyl PA *in vitro* [66]. It is not currently known whether CDS1 also shows the same specificity. There is evidence that antidepressant drugs affect CDS activity [67].

4.3. Phosphatidylinositol-4-phosphate 5-kinase (PI4P5K)

PI4P5K phosphorylates PI4P to PI(4,5)P_2 using ATP as a phosphate donor. These enzymes are mainly responsible for the production of PI(4,5)P_2 and are the rate limiting step in the PI cycle [68]. PI(4,5)P_2 regulates several critical processes such as actin polymerization and reorganization, vesicular trafficking, neurotransmitter release and signal transduction pathways [69,70]. Deregulation of PI(4,5)P_2 metabolism has been demonstrated in a wide range of neuronal disorders, including Down syndrome, psychiatric disorders, Alzheimer's and Huntington's disease [71]. Additionally, PI(4,5)P_2 serves as a precursor to PIP_3, a potent signaling lipid implicated in cell survival, growth and migration. PIP_3 is up-regulated in several cancers and PTEN, a PIP_3 phosphatase is frequently mutated in cancers [72].

There are three PI4P5K isoforms (α, β, γ), all of which have been shown to prefer SAPI4P (18:0–*sn−1*/20:4–*sn−2* PI4P) and SOPI4P (18:0–*sn−1*/18:1–*sn−2* PI4P) species when compared to DPPI4P (16:0–*sn−1*/16:0–*sn−2* PI4P) [55,73]. Kinetic analysis also shows that these isoforms have the highest activity for SAPI4P, with the difference in selectivity being largest for the γ isoform [73]. PI4P5Ks also show acyl chain specificity for its lipid activator, the major activator being unsaturated PA species, such as DAPA (20:4–*sn−1*/20:4–*sn−2* PA) [73]. Thus there appears to be more arachidonoyl specificity for the PA activation of PI4P5K than there is for the PI4P substrate of this enzyme [24,39,54].

Through its production of PI(4,5)P_2, PI4P5Ks play critical roles in many physiological processes; like DGKε and LPIAT1, these enzymes play especially critical roles in neural function and development. For example, PI4P5Kα is involved in ganglioside-stimulated astrocytes, helping to contribute and modulate the cell's inflammatory response [74]. Additionally, the α isoform was also shown to mediate TLR-dependant inflammation in microglial cells [75]. Prolonged inflammation by astrocytes is also recognized to play a role in the progression of neurodegenerative diseases [76]. It is currently not known whether regulation of these inflammatory processes is due to PI(4,5)P_2's acyl chain composition or through interaction with PI(4,5)P_2 binding interaction.

PI4P5Kγ was shown to be important for both cardiovascular development and neuronal development, being essential for cell junction formation in myocardiocytes and neural tube closure in the cranial region of mice [77]. Mutations in PI4P5Kγ were found to be present in Lethal Congenital Contracture Syndrome, a disease characterized by muscle contractures, wasting and atrophy [78]. A cluster of PI-metabolizing genes has also been mapped to chromosomal regions linked to psychiatric diseases [78]. It has been suggested that these enzymes may play a potential role in the progression of these diseases; however, no evidence for this correlation exists.

4.4. Lysophosphatidylinositol acyltransferase 1 (LPIAT1)

LPIAT1, also known as membrane bound O-acyltransferase containing domain 7 (also referred to as MBOAT7), catalyzes the transfer of an acyl-CoA to lysoPI [79]. As an acyltransferase, LPIAT1 is involved in the Land's cycle of acyl chain remodeling. Recently, it was shown that LPIAT1 has a high preference for arachidonoyl-CoA [79]. It is not know whether LPIAT1 also shows preference for particular lyso-PI species as this species was not varied. However, LPIAT1's arachidonoyl specificity contributes to both the production and the enrichment of arachidonoyl-PI.

Recently, two papers have characterized LPIAT1$^{-/-}$ KO mice and have demonstrated that this enzyme is critical for neural function. Both studies showed that knocking out LPIAT1 resulted in a significant decrease in arachidonoyl-containing PI, and PI(4,5)P_2 [80,81]. LPIAT1 appears to be the major arachidonoyl-CoA acyltransferase, as loss of this enzyme resulted in an almost complete loss of activity in the brain, liver, kidney and testis of mice. LPIAT1 also appears to be critical for neural development of mice; LPIAT$^{-/-}$ mice were viable up to 30 days after birth, but exhibited a smaller, atrophied cerebral cortex and hippocampus. The laminal structure of the neocortex was also

disordered due to delayed neural migration, which indicated a role for LPIAT1 in cortical lamination [81].

4.5. DDHD domain containing 1 (DDHD1)

DDHD1 belongs to a family of intracellular phospholipase A1s, which act to remodel acyl chains [82,83]. DDHD1 cleaves the *sn−1* acyl chain of both PI and PA, forming two important bioactive lipids, LPI and LPA [84]. LPI is an activator of a proposed cannabinoid receptor, GPR55, which can activate ERK signaling pathways and increase intracellular Ca^{2+} levels [85]. LPA too, can stimulate cell proliferation through binding several GPCRs and activating Rho GTPase [86]. DDHD1 was shown to have higher activity for cleaving PA species over PI. Although DDHD1's substrate specificity has been poorly characterized, initial experiments have shown that it forms arachidonoyl-LPI at a much higher rate than stearoyl-LPI [84]. It is currently unknown whether DDHD1 shows substrate specificity for its substrate's *sn−1* chain or what impact this has on PI enrichment.

5. A common motif for arachidonoyl specificity?

Could there be a common amino acid motif that contributes to arachidonoyl specificity of several diverse enzymes? In the case of the family of enzymes known as lipoxygenases, several crystal structures have been solved that have identified a potential arachidonoyl preferring motif [87]. Lipoxygenases catalyze the dioxygenation of arachidonic acid; these enzymes are required for the synthesis of both inflammatory and anti-inflammatory leukotrienes and lipoxins, respectively [88]. In crystal structures of an 8R-lipoxygenase from *Plexaura homomalla* and human 5-lipoxygenase, the authors noted a U-shaped channel that allowed arachidonic acid access to the catalytic site [3,88]. In lipoxygenases, the segment with the required residues forms an arched helix, with the side chains of each of the required residues projecting into the channel [3,88]. This motif is highly conserved in certain lipoxygenases through evolution [88] (see Fig. 4).

We have referred to the conserved pattern of amino acid residues in the lining of this channel as the LOX-like motif. A similar consensus sequence $I/L\text{-}X_{(3-4)}\text{-}R\text{-}X_{(2)}\text{-}L\text{-}X_{(4)}\text{-}G$, where X can be any amino acid residue, is found not only in lipoxygenases, but also in the amino acid sequence of these enzymes discussed above, including DGKε, CDS2 and LPIAT1. A recent study also concluded that the LOX-like motif is critical for DGKε's arachidonoyl specificity [55]. Similar studies on CDS2 and LPIAT1 also support a role for the LOX-like motif in the function of these enzymes (K.D., unpublished results).

6. Enrichment of PI and the brain

There are multiple factors contributing to the specific acyl chain content of PI. This includes the specificity of DGKε for SAG. This does not mean that DGKε is the only isoform of DGK that participates in the PI cycle. Other DGK isoforms will also phosphorylate SAG, but they are just not specific for SAG. DGKε is preferentially expressed in certain organs of mammals, such as the brain. Brain PI is also more highly enriched with stearoyl–arachidonoyl forms than that of other organs. It is likely that this is a result of the high expression of DGKε in the brain. However, we do not believe that DGKε is essential for the functioning of the PI cycle, since some cells survive with minimal expression of DGKε and DGKε-knockout mice have a rather mild phenotype. In addition to DGKε, we have shown that CDS2 also has arachidonoyl-specificity that can contribute to enrichment of this acyl chain in PI. However, it is not known if CDS has any stearoyl specificity for the *sn−1* position, nor has it yet been determined what the role of CDS1 is with regard to acyl chain selectivity. In the case of PI4P5K there is little acyl chain specificity for the substrate. This is not surprising since the substrate, PI4P, as well as the product, $PI(4,5)P_2$, are both enriched with stearoyl–arachidonoyl acyl chains to comparable extents. However, activation of PI4P5K by PA is acyl chain specific. This would increase the incorporation of stearoyl–arachidonoyl acyl chains into PI because of activation of the PI cycle. In addition, LPIAT1 will further enrich PI with these acyl chains as a result of acyl chain transfer from arachidonoyl-CoA to LPI by LPIAT1. In addition to enzyme specificities, there could also be compartmentalization within the cell, causing segregation of certain lipids. This has not yet been explored.

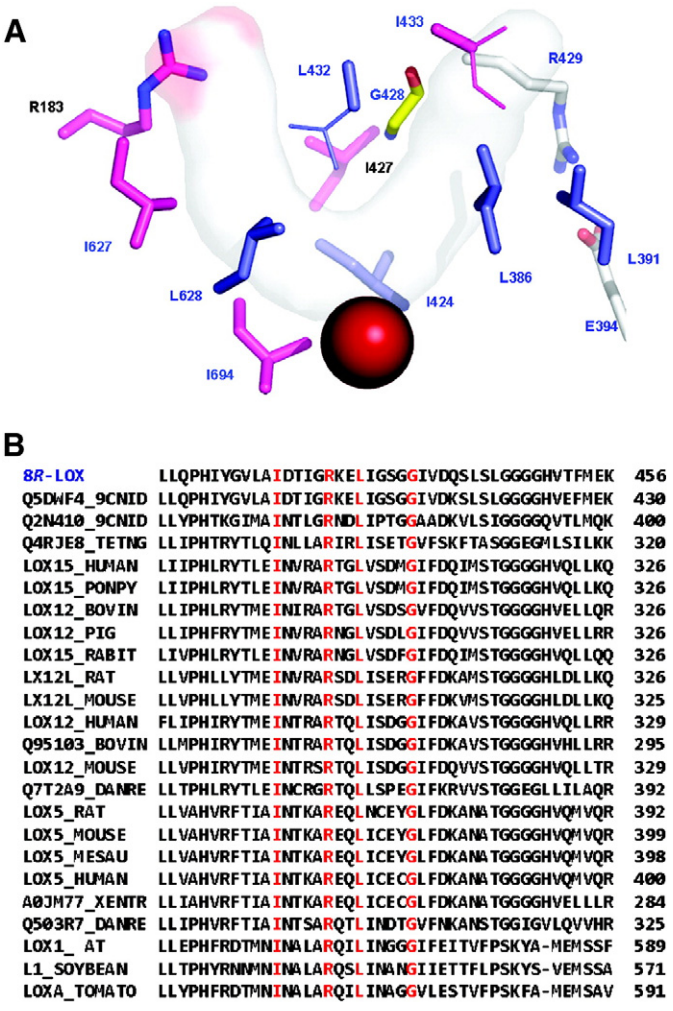

Fig. 4. Channel in lipoxygenase to which arachidonic acid binds. Below the figure are the sequences of lipoxygenase from different species, with the residues that are invariant in evolution shown in red.
Taken from [88] with permission.

While several defects have been associated with enzymes contributing to acyl chain enrichment in PI, these enzymes are frequently associated with defects in brain function. Thus, DGKε has a role in epilepsy [57,58] and in Huntington's disease [59]. DGKε has also recently been shown to play a role in light-dependent mechanisms in mammalian photoreceptor cells [89]. CDS is activated by anti-depressants [67], PI4P5K is associated with schizophrenia [90] and loss of LPIAT1 results in severe developmental brain defects [80]. It is thus suggestive that modulation of arachidonoyl-enrichment of PI can be developed as a method for treating psychiatric disorders.

PI in the brain can also be a source of free arachidonic acid. It is found that arachidonic acid enhances brain healing following injury [91] and can reverse age-related brain decline [92].

7. Future directions

Acyl chain enrichment is a critical determinant in many of PI's cellular processes, including activation of downstream proteins and

generation of precursor molecules. However, several questions remain about acyl chain enrichment. Several of these enzymes, such as DGKε, also show stearoyl preference at the *sn−1* position [55]. However, there have been no motifs identified that can confer this specificity. Changes in the acyl chain at the *sn−1* position are more tolerable than at the *sn−2* position [93]. What role does the *sn−1* acyl chain play in substrate recognition? Could it just serve to determine the depth of burial of these substrates in membranes or help position the substrate for catalysis?

Similarly, does the LOX-like motif form a similar channel in all enzymes that show arachidonoyl specificity? There are key differences between the substrates of lipoxygenases and enzymes involved in PI synthesis. For one, arachidonoyl containing substrates are only preferred substrates of enzymes involved in PI synthesis, whereas lipoxygenases generally only show activity for arachidonic acid. The arachidonoyl group in these substrates is also esterified to a glycerol backbone, unlike arachidonic acid. How then does the LOX-like motif guide these bulkier substrates to the catalytic site? The LOX-like motif could allow transfer of the *sn−2* arachidonoyl chain from the bilayer to the catalytic site, thus extracting this group from the membrane; however other explanations are possible.

Finally, are there similar motifs that also confer acyl chain specificity for other enzymes involved in phospholipid synthesis? For example, PE and PS species show high enrichment in both arachidonoyl and docosahexanoyl acyl chains at its *sn−2* position [94]. How does this enrichment occur? Are there enzymes in PE and PS synthesis that show dual acyl chain preference or multiple enzymes that show either arachidonoyl or docosahexanoyl selectivity? Why don't other phospholipids, like PC, show similar acyl chain enrichment?

The answers to these questions can provide insight into the incredibly complex process of phospholipid biosynthesis and acyl chain enrichment. Continued research into PI's stearoyl and arachidonoyl enrichment can also prove invaluable, as these lipids are involved in critical biochemical processes and their alteration results in disease states.

Acknowledgements

This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada, Grant 9848.

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