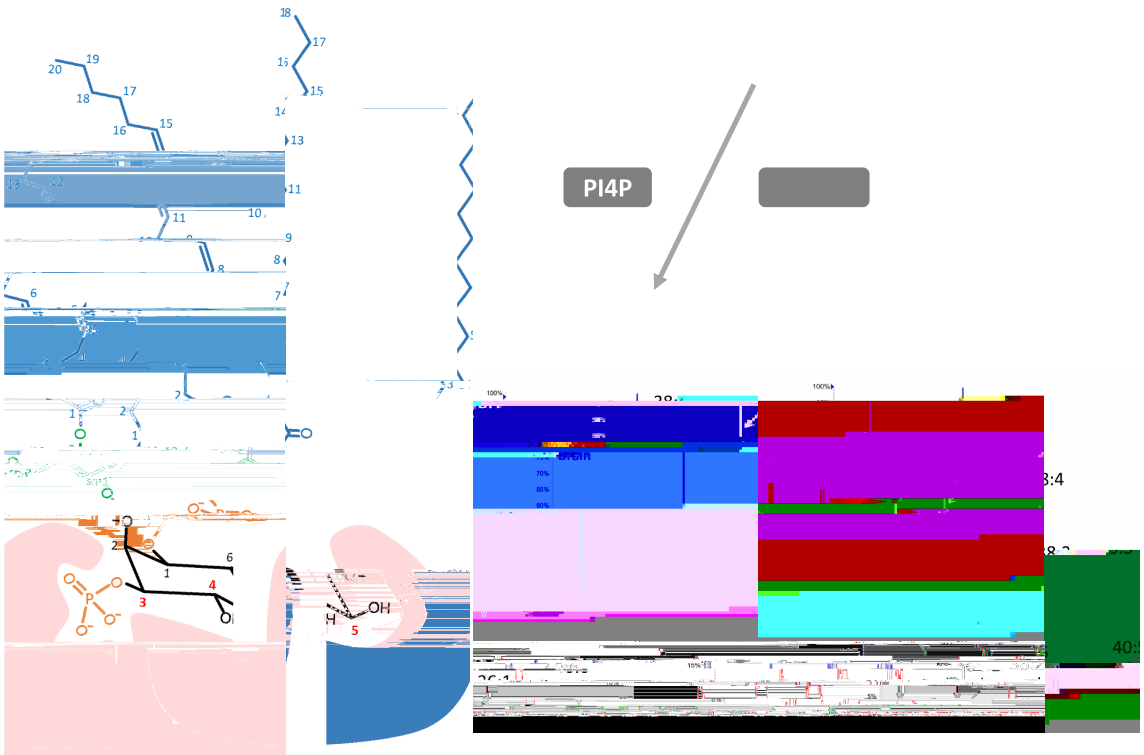

[5–7]. As both chains are simultaneously measured in most routine, mass spectrometry (MS)-based analyses, this molecular species is usually referred to as ‘C38:4’ PI. Early analyses focussed only on PI, but recent MS studies have shown that the acyl compositions of PI, PIP, PIP_2 and PIP_3 are very similar (current MS methods do not discriminate between regio-isomers), consistent with rapid interconversion between the various phosphorylated forms of these lipids and parental PI (Figure 1) [8,9]. These observations suggest evolution has favoured the selective enrichment of this species of PIPn in mammals, but we know very little about the advantages provided by this acyl chain species and the molecular mechanisms responsible for creating it.

How is the acyl composition of mammalian PIPn determined?

In principle, the C38:4 species of PI could be selectively synthesized and/or enriched by subsequent acyl chain ‘remodelling’. The pathways for synthesis of PI and acyl chain remodelling by the ‘Lands

Cycle’ (Figure 2

CDS2 has a significant preference for C20:4 in the sn-2 position of PA, but CDS1 is less selective [16], although it prefers unsaturated species of PA compared with fully saturated species [17]. CDS2 is ubiquitously expressed, while CDS1 is restricted to a subset of tissues [16,18] and might be induced when a high rate of PI synthesis is required [19]. Furthermore, overexpressed CDS1 and CDS2 show slightly different patterns of localization in the ER [17]. These observations suggest that the formation of CDP-DG from PA is likely to be a regulated step and CDS1 and 2 have evolved to play differing roles, although definitive evidence for their

pro-inflammatory phenotype may be related to defective processing of C20:4 by MBOAT7 rather than small changes in PI species.

The first committed step in PI remodelling is its hydrolysis into LPI by PLAs, and thus coupling this process to PI synthesis might be envisaged to facilitate remodelling. Indeed, it has been suggested that the faster remodelling of atypical PE and PS species produced involves their selective degradation by PLAs [41]. Similarly, PIs generated from exogenous C32:0 CDP-DG in liver microsomes were rapidly hydrolyzed into LPI, while little LPI was formed from pre-existing PIs, suggesting a selective de-acylation of newly synthesized PIs [42]. As yet, no PI-PLA activity selective for these intermediate species has been identified and the roles of the PLA superfamily of enzymes in phospholipid remodelling are not clearly defined [43]. The inhibition of Ca^{2+}-independent phospholipase A2b (iPLA2b) reduces the incorporation of C20:4 into phospholipids [44,45] while its overexpression increases the levels of C38:4 PI [46]. The analysis of brains from iPLA2b−/− mice, however, suggest that it is not required for C38:4 enrichment [47]. DDHD1 seems to be the PLA1 involved in the generation of C20:4 LPI [48], an endogenous activating ligand of G protein-coupled receptor GPR55 [49,50]. Although loss of the homologue of DDHD1 in *C. a* , IPLA-1, results in a dramatic change in their predominant PI species, from C18:0/20:5 to 18:1/20:5 [29], in DDHD1−/− mice there were no changes in C38:4 PIPn whilst there was a relative increase in 18:1/C20:4 PIPn at the expense of shorter and more saturated species [51]. Regarding the products of PLA activity on PIs, C18:0 LPI shows the highest levels in mouse tissues, with C20:4 LPI also being abundant in the nervous system [52]. However, this provides little information about the kinetics of remodelling in sn-1 and sn-2 as these species can be formed as remodelling intermediates but also from C38:4 PI for signalling purposes. Thus, if it is not reacylated, LPI can be metabolized by lysophospholipases A, C or D to generate glycerophosphoinositol [53], monoacylglycerol [54] or LPA [55], which will all have different signalling roles [56].

(Figure 3; [63]). The activation of a PI(4,5)P_2-selective PLC by receptors is now accepted to be a central signal transduction mechanism in multicellular organisms and liberates the 'second messengers' IP_3 and DG, which stimulate intracellular Ca^{2+}-release and the activation of PKC, respectively [64,65]. Whilst PIPn can be interconverted by kinases and phosphomonoesterases, the action of a PLC produces DG, which is a common biosynthetic intermediate in lipid metabolism. This creates a potential problem for cells in segregating PLC-derived-DG from other sources of DG, enabling it to both act as a selective signal for the activation of PKC and also as a source of PA for the PI-cycle. Furthermore, PLC activation is often observed to occur in parallel with activation of a PLD which usually appears to be directed predominantly against PC, adding a further source of complexity in segregating pools of PA (and DG via PA phosphatases) destined for different purposes [66].

C32:0 species were used to derive the SF-1 crystals (Figure 4E). It has also been described that nuclear p53 can bind PI(4,5)P_2, which results in its stabilization [94]. This interaction was resistant to denaturation, and was observed with natural PI(4,5)P_2 but not with dioctanoyl-PI(4,5)P_2.

The synthesis of PI from CDP-DG and free inositol via PIS is conserved in all eukaryotes (reviewed in [95]), which also present most of their phosphorylated forms [96]. While these PIPn participate in homologous functions in different organisms, the PI enrichment in C38:4 backbones has only been observed thus far in vertebrates. Therefore, tracing the emergence of C38:4 enrichment during the evolution of PIPn signalling could provide some insights into the evolutionary pressures favouring a distinct and homogeneous acyl chain composition in this key family of signalling lipids.

While C38:4 enrichment might be restricted to vertebrates, a common feature of PIPn across many different organisms is the concentration of saturated acyl chains in sn-1 compared with other phospholipids [29,97,98]. In yeast, the enrichment in C16:0 or C18:0 in sn-1 appears to take place by distinct mechanisms; the PI synthase Pis1p seems to select CDP-DG molecules containing C16:0 [99], while C18:0 chains are incorporated via remodelling by the LPI acyltransferase Psi1p, their closest homologue to mammalian AGPAT8 [100]. *P 1Δ* yeast strains present an abnormal localization of PI4P and PI(4,5)P_2 and alterations in vesicle trafficking and cell polarity, which has been attributed to non-redundant functions of the PIPn pool containing C18:0 [101]. The main lysophospholipid sn-2 acyltransferase in yeast microsomes is Ale1p, an MBOAT family enzyme with a strong preference for mono- and poly-unsaturated acyl-CoA chains, though the longer chain C20:4-CoA is a poor substrate [102]. Studies investigating the substrate preferences of the mammalian MBOAT family suggest some overlapping selectivity for the lyso-phospholipid head group and varying selectivity for acyl-CoA chain length and degree of unsaturation [26], but MBOAT7 appears remarkably selective for both LPI and C20:4-CoA [28]. Similar results are observed with homologues in nematode [27] and fly [103], though C20:4 is absent from flies [104]. Therefore, during the evolution of PIPn signalling, two different families of acyltransferases (AGPAT and MBOAT) have evolved to generate a pool of PIPn with a stearoyl chain in sn-1 and a polyunsaturated fatty acid in sn-2, which in vertebrates becomes C20:4. Remarkably, the social amoeba *D c c* , which branched before the divergence of fungi and metazoa, also presents a unique hydrocarbon backbone in PIPn, composed of an ether-linked C16:0 chain in sn-1 and a ester-linked C18:1 chain in sn-2 (C34:1e) [105]. Taken together, a theme emerges that suggests a core functional advantage to the selected PI backbone, such as availability within the bilayer and/or IPn head-group presentation to effectors. This advantage may be fine-tuned in individual organisms to the precise temperature and lipid composition of the membranes in which they must act. Moreover, in some organisms, such as vertebrates and *D c* , there appears to have been further enrichment to more precise molecular species, which may imply additional advantages, such as metabolic identity and optimal PIPn–protein interactions.

While the hydrolysis of PI(4,5)P_2 by PLC seems to be ubiquitous in eukaryotes, the emergence of PLC signalling in metazoans might have increased the pressure for PIPn homeostatic mechanisms. In fly photoreceptor cells, which present heterogeneous PIPn species and require the rapid replenishment of PI(4,5)P_2 to sustain their light-activated PLC signalling, an efficient PI cycle is organised by a specialized ER-PM contact site, the Submicrovillar Cisternae, where PI is resynthesized using PA molecules transferred from the PM by RdgB proteins (a homologue of mammalian Nir2) [68]. In mammals, the efficient operation of a PI cycle in different cell types might be facilitated by an additional layer of selectivity provided by the C38:4 backbone (see discussion above). This may be reflected in the divergence of C38:4-selective CDS2 and non-selective CDS1 in vertebrates [106] and it would be informative to investigate whether other proteins involved in the mammalian PI cycle, such as Nir2/3, have evolved selectivity for C38:4 substrates.

The presence of alternative PIPn species

Whilst the forgoing discussion has focussed on the enrichment of the C38:4 species of PIPn in primary mammalian tissue, several examples have been reported where this enrichment does not occur, or at least not to the same extent [107thesubstr rinsn-168] and

same manner as C38:4 PIPn. In addition, the stimulation of the PI3K and PLC pathways in platelets indicated that some minor PIPn species present different dynamics to the C38:4 PIPn pool [108].

Alternative PIPn species might also be enriched in certain cell compartments. The exosomes released by prostate cancer cell line PC-3, showed a higher proportion of C34:1 and C36:1 species in PI compared with the parental cells [110]. As PI levels were very low in exosomes, their unusual composition could indicate an exclusion of polyunsaturated PI species during exosome formation. This lack of C38:4 enrichment in PIs has also been observed in extracellular vesicles derived from other prostate cell lines [111] or differentiated 3T3-L1 adipocytes [112], although their levels were not compared with those of the parental cells. Similarly, PI in lung surfactant showed a predominance of saturated/monounsaturated species, which could be related to its biophysical functions [25,113].

A higher saturation degree in alternative PIPn species could render functional advantages, such as facilitating their packing [3]. PIPn can aggregate in microdomains, as seen with clusters of synthasin-1A/PI(4,5)P_2 control-

- Ne d ar e fr m e d f e me ab c d am c f PIP m ec ar ec e a d e c f e ac c a ar ab e f a a d r a a .

AGPAT, ac cer -3- a e ac ra fera e; CDP-DG, CDP-d ac cer ; CDS, CDP-d ac cer

19 Blunsom, N.J., Gomez-Espinosa, E., Ashlin, T.G. and Cockcroft, S. (2019) Sustained phospholipase C stimulation of H9c2 cardiomyoblasts by vasopressin induces an increase in CDP-diacylglycerol synthase 1 (CDS1) through protein kinase C and cFos.

47 Cheon, Y., Kim, H.-W., Igarashi, M., Modi, H.R., Chang, L., Ma, K. et al. (2012) Disturbed brain phospholipid and docosahexaenoic acid metabolism in calcium-independent phospholipase A2-VIA (iPLA2β)-knockout mice. Biochim. Biophys. Acta Mol. Cell Biol. Lipids **1$_8$21**, 1278–1286 https://doi.org/10.1016/j.bbalip.2012.02.003

48 Yamashita, A., Kumazawa, T., Koga, H., Suzuki, N., Oka, S. and Sugiura, T. (2010) Generation of lysophosphatidylinositol by DDHD domain containing 1 (DDHD1): possible involvement of phospholipase D/phosphatidic acid in the activation of DDHD1. Biochim. Biophys. Acta Mol. Cell Biol. Lipids **1$_8$01**, 711–720 https://doi.org/10.1016/j.bbalip.2010.03.012

49 Oka, S., Nakajima, K., Yamashita, A., Kishimoto, S. and Sugiura, T. (2007) Identification of GPR55 as a lysophosphatidylinositol receptor. Biochem. Biophys. Res. Commun. **362**, 928–934 https://doi.org/10.1016/j.bbrc.2007.08.078

50 Alhouayek, M., Masquelier, J. and Muccioli, G.G. (2018) Lysophosphatidylinositols, from cell membrane constituents to GPR55 ligands. Trends Pharmacol. Sci. **39**, 586–604 https://doi.org/10.1016/j.tips.2018.02.011

51 Inloes, J.M., Jing, H. and Cravatt, B.F. (2018) The spastic paraplegia-associated phospholipase DDHD1 is a primary brain phosphatidylinositol lipase. Biochemistry **57**, 5759–5767 https://doi.org/10.1021/acs.biochem.8b00810

52 Masquelier, J. and Muccioli, G.G. (2016) Development and validation of a specific and sensitive HPLC-ESI-MS method for quantification of lysophosphatidylinositols and evaluation of their levels in mice tissues. J. Pharm. Biomed. Anal. **126**, 132–140 https://doi.org/10.1016/j.jpba.2016.04.011

53 Mariggiò, S., Sebastià, J., Filippi, B.M., Iurisci, C., Volonté, C., Amadio, S. et al. (2006) A novel pathway of cell growth regulation mediated by a PLA2α-derived phosphoinositide metabolite. FASEB J. **20**, 2567–2569 https://doi.org/10.1096/fj.05-5397fje

54 Murase, S. and Okuyama, H. (1985) A membrane-bound phospholipase C with an apparent specificity for lysophosphatidylinositol in porcine platelets. J. Biol. Chem. **260**, 262–265 PMID:3917432

55 Aoki, J., Taira, A., Takanezawa, Y., Kishi, Y., Hama, K., Kishimoto, T. et al. (2002) Serum lysophosphatidic acid is produced through diverse phospholipase pathways. J. Biol. Chem. **277**, 48737–48744 https://doi.org/10.1074/jbc.M206812200

56 Yamashita, A., Oka, S., Tanikawa, T., Hayashi, Y., Nemoto-Sasaki, Y. and Sugiura, T. (2013) The actions and metabolism of lysophosphatidylinositol, an endogenous agonist for GPR55. Prostaglandins Other Lipid Mediat. **107**, 103–116 https://doi.org/10.1016/j.prostaglandins.2013.05.004

57 Yamashita, A., Hayashi, Y., Matsumoto, N., Nemoto-Sasaki, Y., Koizumi, T., Inagaki, Y. et al. (2017) Coenzyme-A-independent transacylation system; possible involvement of phospholipase A2 in transacylation. Biology **6**, 23 https://doi.org/10.3390/biology6020023

58 Schlame, M. (2013) Cardiolipin remodeling and the function of tafazzin. Biochim. Biophys. Acta Mol. Cell Biol. Lipids **1$_8$31**, 582–588 https://doi.org/10.1016/j.bbalip.2012.11.007

59 Kim, Y.J., Guzman-Hernandez, M.L. and Balla, T. (2011) A highly dynamic ER-derived phosphatidylinositol-synthesizing organelle supplies phosphoinositides to cellular membranes. Dev. Cell **21**, 813–824 https://doi.org/10.1016/j.devcel.2011.09.005

60 English, A.R. and Voeltz, G.K. (2013) Rab10 GTPase regulates ER dynamics and morphology. Nat. Cell Biol. **15**, 169 https://doi.org/10.1038/ncb2647

61 Tábara, L.C., Vicente, J.-J., Biazik, J., Eskelinen, E.-L., Vincent, O. and Escalante, R. (2018) Vacuole membrane protein 1 marks endoplasmic reticulum subdomains enriched in phospholipid synthesizing enzymes and is required for phosphoinositide distribution. Traffic **19**, 624–638 https://doi.org/10.1111/tra.12581

62 Nishimura, T., Tamura, N., Kono, N., Shimanaka, Y., Arai, H., Yamamoto, H. et al. (2017) Autophagosome formation is initiated at phosphatidylinositol synthase-enriched ER subdomains. EMBO J. **36**, 1719 https://doi.org/10.15252/embj.201695189

63 Michell, R.H. (1975) Inositol phospholipids and cell surface receptor function. Biochim. Biophys. Acta Rev. Biomembr. **415**, 81–147 https://doi.org/10.1016/0304-4157(75)90017-9

64 Thillaiappan, N.B., Chakraborty, P., Hasan, G. and Taylor, C.W. (2018) IP3 receptors and Ca^{2+} entry. Biochim. Biophys. Acta Mol. Cell Res. **1$_8$66**, 1092–1100 https://doi.org/10.1016/j.bbamcr.2018.11.007

65 Gallegos, L.L. and Newton, A.C. (2008) Spatiotemporal dynamics of lipid signaling: protein kinase C as a paradigm. IUBMB Life **60**, 782–789 https://doi.org/10.1002/iub.122

66 Pettitt, T.R. and Wakelam, M.J. (1999) Diacylglycerol kinase ε, but not ζ, selectively removes polyunsaturated diacylglycerol, inducing altered protein kinase C distribution in vivo. J. Biol. Chem. **274**, 36181–36186 https://doi.org/10.1074/jbc.274.51.36181

67 Chen, Y.-J., Quintanilla, C.G. and Liou, J. (2019) Recent insights into mammalian ER–PM junctions. Curr. Opin. Cell Biol. **57**, 99–105 https://doi.org/10.1016/j.ceb.2018.12.011

68 Cockcroft, S. and Raghu, P. (2018) Phospholipid transport protein function at organelle contact sites. Curr. Opin. Cell Biol. **53**, 52–60 https://doi.org/10.1016/j.ceb.2018.04.011

69 Saheki, Y. and Camilli, P.D. (2017) Endoplasmic reticulum–plasma membrane contact sites. Annu. Rev. Biochem. **86**, 659–684 https://doi.org/10.1146/annurev-biochem-061516-044518

78 Takemasu, S., Ito, M., Morioka, S., Nigorikawa, K., Kofuji, S., Takasuga, S. et al. (2019) Lysophosphatidylinositol-acyltransferase-1 is involved in cytosolic Ca^{2+} oscillations in macrophages. Genes Cells **24**, 366–376 https://doi.org/10.1111/gtc.12681
79 Stephens, L., Anderson, K., Stokoe, D., Erdjument-Bromage, H., Painter, G.F., Holmes, A.B. et al. (1998) Protein kinase B kinases that mediate phosphatidylinositol 3,4,5–trisphosphate-dependent activation of protein kinase B. Science **279**, 710–714 https://doi.org/10.1126/science.279.5351.710
80 Rohács, T., Chen, J., Prestwich, G.D. and Logothetis, D.E. (1999) Distinct specificities of inwardly rectifying K^+ channels for phosphoinositides. J. Biol. Chem. **274**, 36065–36072 https://doi.org/10.1074/jbc.274.51.36065
81 Wong, L.H., Gatta, A.T. and Levine, T.P. (2019) Lipid transfer proteins: the lipid commute via shuttles, bridges and tubes. Nat. Rev. Mol. Cell Biol. **20**, 85–101 https://doi.org/10.1038/s41580-018-0071-5
82 Wang, Y., Mousley, C.J., Lete, M.G. and Bankaitis, V.A. (2019) An equal opportunity collaboration between lipid metabolism and proteins in the control of membrane trafficking in the trans-Golgi and endosomal systems. Curr. Opin. Cell Biol. **59**, 58–72 https://doi.org/10.1016/j.ceb.2019.03.012
83 Antonny, B., Bigay, J. and Mesmin, B. (2018) The oxysterol-binding protein cycle: burning off PI(4)P to transport cholesterol. Annu. Rev. Biochem. **87**, 809–837 https://doi.org/10.1146/annurev-biochem-061516-044924
84 Schauder, C.M., Wu, X., Saheki, Y., Narayanaswamy, P., Torta, F., Wenk, M.R. et al. (2014) Structure of a lipid-bound extended synaptotagmin indicates a role in lipid transfer. Nature **510**, 552 https://doi.org/10.1038/nature13269
85 Chung, J., Torta, F., Masai, K., Lucast, L., Czapla, H., Tanner, L.B. et al. (2015) PI4P/phosphatidylserine countertransport at ORP5-and ORP8-mediated ER–plasma membrane contacts. Science **349**, 428–432 https://doi.org/10.1126/science.aab1370
86 Ghai, R., Du, X., Wang, H., Dong, J., Ferguson, C., Brown, A.J. et al. (2017) ORP5 and ORP8 bind phosphatidylinositol-4, 5–biphosphate (Ptdlns (4, 5) P 2) and regulate its level at the plasma membrane. Nat. Commun. **8**, 757 https://doi.org/10.1038/s41467-017-00861-5
87 Nishimura, T., Gecht, M., Covino, R., Hummer, G., Surma, M.A., Klose, C. et al. (2019) Osh proteins control nanoscale lipid organization necessary for PI(4,5)P2 synthesis. Mol. Cell **75**, 1043–1057.e8 https://doi.org/10.1016/j.molcel.2019.06.037
88 Garner, K., Hunt, A.N., Koster, G., Somerharju, P., Groves, E., Li, M. et al. (2012) Phosphatidylinositol transfer protein, cytoplasmic 1 (PITPNC1) binds and transfers phosphatidic acid. J. Biol. Chem. **287**, 32263–32276 https://doi.org/10.1074/jbc.M112.375840
89 Jacobsen, R.G., Mazloumi Gavgani, F., Edson, A.J., Goris, M., Altankhuyag, A. and Lewis, A.E. (2019) Polyphosphoinositides in the nucleus: roadmap of their effectors and mechanisms of interaction. Adv. Biol. Regul. **72**, 7–21 https://doi.org/10.1016/j.jbior.2019.04.001
90 Fiume, R., Faenza, I., Sheth, B., 1338-1.2-29780JismMzetti,ng C. et al. (2019)nucare:
J. Mol. Sem.

109 Ren, M., Xu, Y., Erdjument-Bromage, H., Donelian, A., Phoon, C.K.L., Terada, N. et al. (2019) Extramitochondrial cardiolipin suggests a novel function of mitochondria in spermatogenesis. J. Cell Biol. **21$_8$**, 1491–1502 https://doi.org/10.1083/jcb.201808131
110 Llorente, A., Skotland, T., Sylvänne, T., Kauhanen, D., Róg, T., Orłowski, A. et al. (2013) Molecular lipidomics of exosomes released by PC-3 prostate cancer cells. Biochim. Biophys. Acta Mol. Cell Biol. Lipids **1$_8$31**, 1302–1309 https://doi.org/10.1016/j.bbalip.2013.04.011
111 Brzozowski, J.S., Jankowski, H., Bond, D.R., McCague, S.B., Munro, B.R., Predebon, M.J. et al. (2018) Lipidomic profiling of extracellular vesicles