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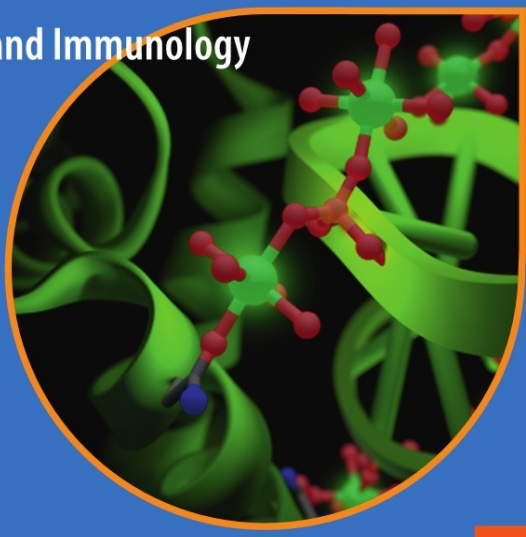

Marco Falasca *Editor*

Phosphoinositides and Disease

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Volume 362

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Contents

Chapter 1
An Introduction to Phosphoinositides

Tania Maffucci

Abstract Phosphoinositides (PIs) are minor components of cellular membranes that play critical regulatory roles in several intracellular functions. This chapter describes the main enzymes regulating the turnover of each of the seven PIs in mammalian cells and introduces to some of their intracellular functions and to some evidences of their involvement in human diseases. Due to the complex interrelation between the distinct PIs and the plethora of functions that they can regulate inside a cell, this chapter is not meant to be a comprehensive coverage of all aspects of PI signalling but rather an introduction to this complex signalling field. For more details of their regulation/functions and extensive description of their intracellular roles, more detailed reviews are suggested on each single topic.

Abbreviations

ArPIKfyve	Associated regulator of PIKfyve
CML	Chronic myelogenous leukemia
CMT	Charcot-Marie-Tooth
DAG	Diacylglycerol
ER	Endoplasmic reticulum
FERM	Four-point one, Ezrin, Radixin, Moesin
FYVE	Fab1/YOTB/Vac1/EEA1
GPCRs	G-protein coupled receptors
GRAM	Glucosyltransferases, Rab-like GTPase activators and Myotubularins
GSK3	Glycogen synthase kinase 3
HIF	Hypoxia inducible factor
LPA	Lysophosphatidic acid

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LPI	Lysophosphatidylinositol
Ins(1,4,5)P_3	Inositol 1,4,5-trisphosphate
MIPS	Myo-inositol-3-phosphate synthase
MTM	Myotubularin
MTMR	Myotubularin-related
mTOR	Mechanistic target of rapamycin
PDK1	3-phosphoinositide-dependent protein kinase 1
PH	Pleckstrin homology
PHD	Plant HomeoDomain
PIKfyve	PhosphoInositide Kinase for five position containing a Fyve finger
PIPP	Proline-rich inositol polyphosphate 5-phosphatase
PIs	phosphoinositides
PI3K	phosphoinositide 3-kinase
PLA	phospholipase A
PLC	Phospholipase C
PLD	Phospholipase D
PtdIns3*P*	Phosphatidylinositol 3-phosphate
PtdIns4*P*	Phosphatidylinositol 4-phosphate
PtdIns5*P*	Phosphatidylinositol 5-phosphate
PtdIns(3,4)P_2	Phosphatidylinositol 3,4-bisphosphate
PtdIns(4,5)P_2	Phosphatidylinositol 4,5-bisphosphate
PtdIns(3,5)P_2	Phosphatidylinositol 3,5-bisphosphate
PtdIns(3,4,5)P_3	Phosphatidylinositol 3,4,5-trisphosphate
PIP4 Ks	PtdIns5P 4-kinases
PIP5Ks	PtdIns4P 5-kinases
PTEN	Phosphatase and tensin homolog
PX	Phox homology
RTK	Receptor tyrosine kinase
SHIP	Src homology 2-domain-containing inositol phosphatase
SKIP	Skeletal muscle and kidney enriched 5-phosphatase
TAPP	Tandem PH domain-containing Protein
TGN	Trans-Golgi network
Vps34	Vacuolar protein sorting 34

The Phosphoinositide Family

Phosphoinositides (PIs) are phospholipids comprising two fatty acid chains linked by a glycerol moiety to a water-soluble inositol (Ins) head group. Ins is a cyclohexanehexol that can exist in nine isomeric forms, of which the *myo*-inositol form is the most used in biology (Michell 2008).

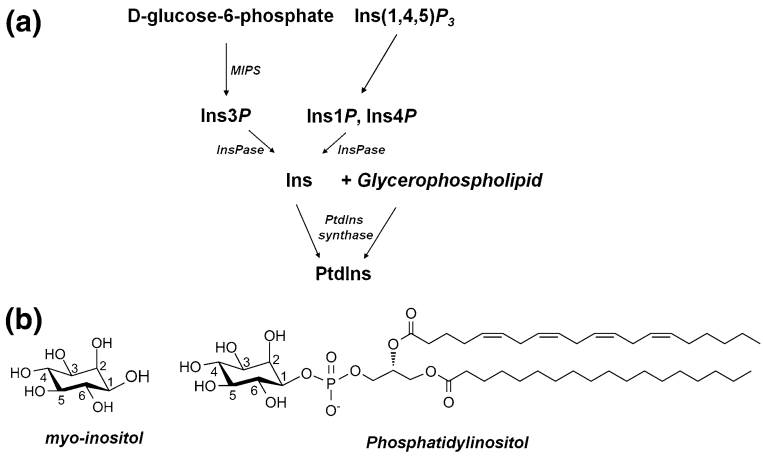

Fig. 1.1 **a** Schematic representation of PtdIns synthesis. **b** Structures of *myo*-inositol and PtdIns

myo-inositol (Fig. 1.1) is synthesised by a NAD^+-dependent *myo*-inositol-3-phosphate synthase (MIPS) that catalyses the cyclisation of D-glucose-6-phosphate to D-*myo*-inositol-3-phosphate (Ins3*P*). Alternatively. *myo*-inositol can derive from Ins1*P* or Ins4*P*, products of dephosphorylation of the Ins(1,4,5)P_3 Ins3*P*, Ins1P_1 or Ins4*P* are dephosphorylated by the inositol monophosphatase (InsPase) to generate Ins (Michell 2008). The synthesis of phosphatidylinositol (PtdIns) is catalysed by the enzyme PtdIns synthase that links the 1-position of the *myo*-inositol to the diester phosphate of a glycerophospholipid (Fig. 1.1). PtdIns is primarily synthesised in the endoplasmic reticulum (ER) and then delivered to other membranes by vesicular transport or via cytosolic PtdIns transfer protein (Di Paolo and De Camilli 2006). Differential phosphorylation of the hydroxyls at the 3-, 4- , and 5-position within the *myo*-inositol headgroup of PtdIns generates seven distinct derivatives, named PIs (Fig. 1.2). The majority of PIs possesses the same fatty acids [a saturated C_{18} residue (stearoyl) in the 1-position and a tetra-unsaturated C_{20} residue (arachidonoyl) in the 2-position]. All seven PIs are naturally occurring in the cell membranes of all higher eukaryotes and they can be inter-converted into each other by the action of specific kinases or phosphatases. Intracellular localisation of PIs-regulating enzymes and/or their relocation upon cellular stimulation is critical for localised modulation of PIs levels.

Mechanisms of PIs-dependent Cellular Signalling

Because of their lipid tail, PIs are obligatory membrane-bound; therefore, they can mark specific membrane compartments, or subdomains within a membrane. Through this property, PIs can act as components of cellular membranes and in some cases they can define the membrane or subdomain of the membranes. PIs can

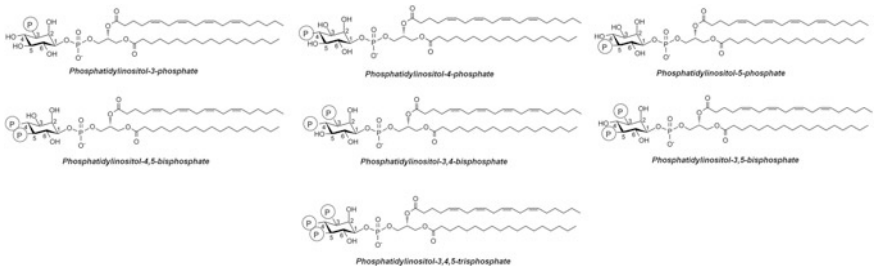

Fig. 1.2 Structures of the seven PIs

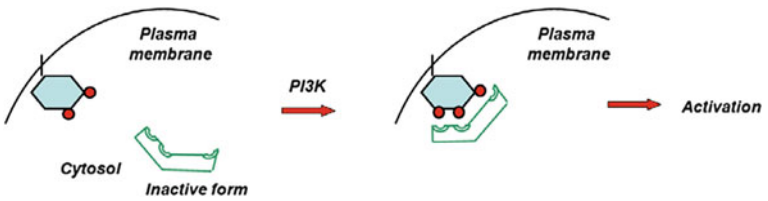

Fig. 1.3 PI-dependent activation of a target protein through recruitment to the plasma membrane. Depicted example refers specifically to the PtdIns(3,4,5)P_3-mediated mechanism of activation

also act as signalling molecules. Their cytosolic soluble headgroup allow PIs to bind to cytosolic proteins or to cytosolic domains of membrane proteins. Binding is mediated by specific PI binding domain(s) within the target proteins and, as a consequence of this interaction, proteins can be activated through conformational changes or through association to a specific cellular membrane (spatial regulation). Moreover, because some PIs are specifically synthesised upon cellular stimulation in normal cells, their regulated synthesis can mediate activation of the target protein not only spatially but also temporally. The rapid synthesis/turnover of some of them is critical to activate signals within the cells only when they are requested to act. Indeed, PIs can rapidly be converted into each other and in particular phosphatases can dephosphorylate specific PIs, often switching off intracellular signals. One of the most studied examples is activation of protein kinase B/Akt that requires translocation of the enzyme to the plasma membrane, a process that is mediated by the interaction of Akt pleckstrin homology (PH) domain and phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3], (Fig. 1.3).

PIs can also act as precursors of other "non PIs" signalling molecules. The paradigmatic example of this is the hydrolysis of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] by phospholipase C (PLC) isoforms. These enzymes convert PtdIns(4,5)P_2 to inositol 1,4,5-trisphosphate and diacylglycerol (Fig. 1.4a), that in turn can mediate release of intracellular calcium and activation of protein kinases Cs, respectively. Similarly, PIs can be hydrolysed by members of the family of PLA (Fig. 1.4b). In particular, the action of PLA_2 on PtdIns releases arachidonic acid, whose role as second messenger has been well studied.

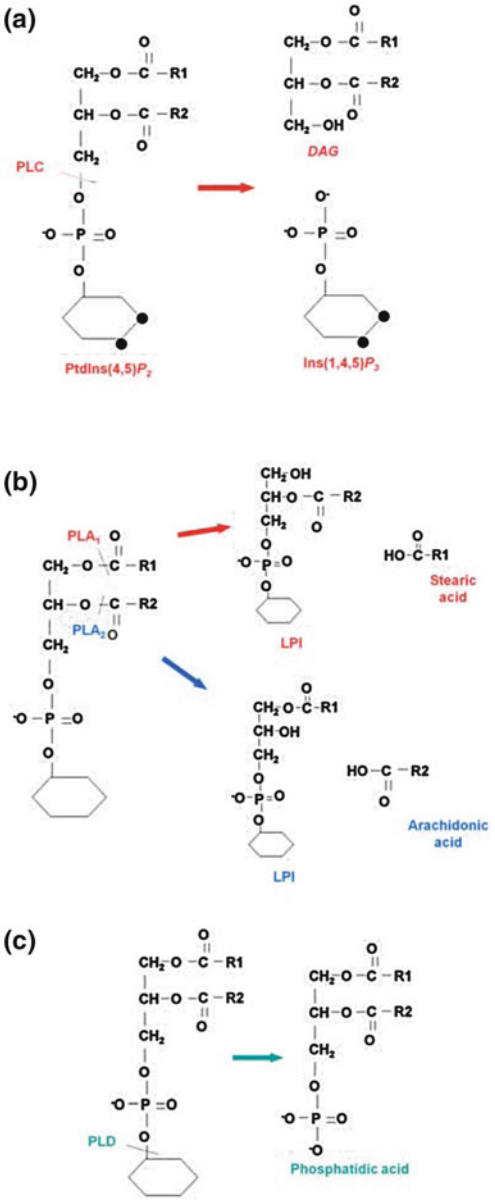

Fig. 1.4 Synthesis of second messengers from PIs: **a** hydrolysis of PtdIns(4,5)P_2 into Ins(1,4,5)P_3 and diacylglycerol (*DAG*) by phospholipase C (*PLC*), **b** hydrolysis of PIs into lysophosphatidylinositol (*LPI*) and stearic or arachidonic acid by phospholipases A, **c** hydrolysis of PIs into phosphatidic acid by phospholipase D (*PLD*)

More recently, an important role in signalling has also been established for the other product of PLA action, lysophosphatidylinositol (LPI) (Piñeiro et al. 2011). LPI can be generated by PLA_1 or PLA_2 (Fig. 1.4b) and it seems that the 2-arachidonoyl species is the most important in signalling.

Finally, PLD can release phosphatidic acid (Fig. 1.4c) which can have a role in signalling or it can be further converted into the second messenger lysophosphatidic acid (LPA).

PIs-Binding Domains

Proteins can bind to PIs through specific protein domains, each showing a distinct affinity and selectivity. The interaction PI–protein domain alone is usually not sufficient to determine the intracellular localisation or re-localisation of the effector protein. Binding of other domains within the protein to other components of the membrane, simultaneous binding of the same domain to a PI and protein(s) (Maffucci and Falasca 2001) or other cooperative mechanisms (Lemmon 2008) guarantee a highly regulated recruitment and activation of signalling molecules. Some of the domains that have been shown to bind PIs are listed below.

PH domains are modules of about 100 amino acids, first identified in pleckstrin, the major protein kinase C substrate in platelets (Lemmon 2008). Although very different in their primary structure, all PH domains possess a similar tertiary structure, consisting of a 7-stranded β-sandwich structure formed by two near-orthogonal β sheets (Lemmon 2008). It has been estimated that only $\sim$10 % of all PH domains bind strongly and specifically to PIs (Lemmon 2008). The majority of PH domains show either low specificity or low affinity (or both) for PIs, and require additional mechanisms to guarantee the specific targeting of the host protein (Maffucci and Falasca 2001).

F*ab*1/***Y****OTB/****V****ac*1/***E****EA*1 (*FYVE*) domains are zinc finger modules of about 60–70 amino acids which specifically bind the monophosphate phosphatidylinositol 3-phosphate (PtdIns3*P*) (Stenmark and Aasland 1999). They can be found as a single finger or as a tandem repeat in proteins and consist of two double stranded antiparallel β sheets and a small C-terminal α-helix. The structure is held together by two tetrahedrally coordinated Zn^{2+} ions (Kutateladze 2006; Lemmon 2008). In contrast to PH, FYVE domains bind more strongly to membrane-embedded PtdIns3*P* than the isolated headgroup Ins(1,3)P_2 (Kutateladze 2006; Lemmon 2008). Endosomal targeting of most FYVE domains requires dimerisation of the domains although this may be crucial for a subset not all FYVE domains (Kutateladze 2006).

Phox homology (*PX*) domains are regions of 130 amino acids named after the two phagocyte NADPH oxidase subunits $p40^{phox}$ and $p47^{phox}$ (Kutateladze 2007). Despite little sequence similarity, PX domains show a highly conserved 3D structure consisting of three stranded β-sheet and a subdomain of three to four α-helices (Kutateladze 2007). All PX domains found in *S. cerevisiae* bind PtdIns3*P* whereas PX domains able to bind phosphatidylinositol 3,5-bisphosphate [PtdIns(3,5)P_2] and phosphatidylinositol 3,4-bisphosphate [PtdIns(3,4)P_2] have also been found in mammals (Lemmon 2008). PX domain binding to the membrane requires a combined action of headgroup binding, electrostatic attraction and membrane insertion, as FYVE and in contrast to PH domain. There are only few

examples of PX domains able to bind PtdIns3*P* with high affinity and whose interaction with this PI is sufficient to target them to membranes. The majority of them bind PtdIns3*P* weakly and are recruited to the membrane only as part of a multicomplex.

***P**lant **H**omeo**D**omain* (*PHD*) consists of two atypical zinc fingers, characterised by a Cys4-His-Cys3 structure that coordinates two Zn^{2+} ions. Several chromatin-regulating proteins contain PHD fingers, including members of the ING family which can modulate the activity of histone acetyl transferase and histone deacetylase (Gozani et al. 2003). ING2 has been reported to possess a PHD finger which specifically binds phosphatidylinositol 5-phosphate [PtdIns5*P*] and PtdIns3*P* in vitro and possibly PtdIns5*P* in vivo (Gozani et al. 2003).

***F**our-point one, **E**zrin, **R**adixin, **M**oesin* (*FERM*) domains are modules of approximately 300 amino acids found in a family of peripheral membrane proteins that are able to link membrane to cytoskeleton (Chishti et al. 1998). The FERM domain is composed of three subdomains which can interact with numerous protein-binding partners and can bind to PtdIns(4,5)P_2 (Tepass 2009).

***G**lucosyltransferases, **Ra**b-like GTPase activators and **M**yotubularins* (*GRAM*) domains consist generally of 70 amino acids (Doerks et al. 2000). Data have suggested that at least GRAM domains from myotubularins can bind or contribute to binding to PtdIns(3,5)P_2 (Berger et al. 2003; Tsujita et al. 2004). Binding to phosphatidylinositol 4-phosphate [PtdIns4*P*] has also been reported for one GRAM domain (Yamashita et al. 2006).

ENTH and ANTH domains form a superhelical solenoid of α-helices which can bind PIs with relatively little stereospecificity (Lemmon 2008). Oligomerisation of multiple low-affinity PtdIns(4,5)P_2-binding sites seems to be critical for membrane association of the ANTH domain while a well defined pocket within the ENTH domain forms the PtdIns(4,5)P_2-binding site (Lemmon 2008).

Monophosphates: Phosphatidylinositol 3-phosphate

Kinases

PtdIns3*P* (Fig. 1.2) is one of the lipid products of the family of enzymes phosphoinositide 3-kinase (PI3K) which catalyse phosphorylation at position 3 of the inositol ring of some PIs (Falasca and Maffucci 2006; Falasca and Maffucci 2009). Of the three classes of PI3Ks (Vanhaesebroeck et al. 2010), PtdIns3*P* can derive from direct phosphorylation of PtdIns by class III PI3 K (vacuolar protein sorting 34, Vps34) or members of class II PI3Ks (Fig. 1.5). These isoforms will be discussed in this paragraph. Class I PI3Ks will be discussed within the paragraph on PtdIns(3,4,5)P_3.

Class III PI3K (*hVps*34) is ubiquitously expressed in mammalian cells and only catalyses the synthesis of PtdIns3*P* (Backer 2008). It is generally considered responsible for the synthesis of a constitutive, mostly endosomal-associated, pool

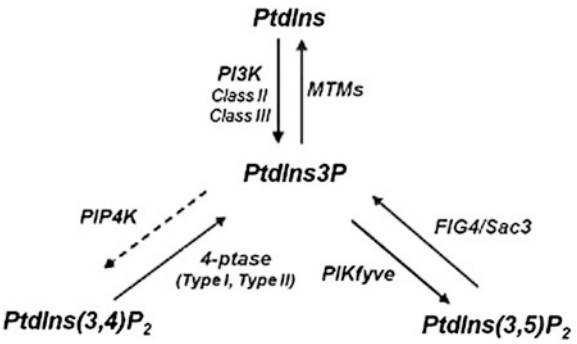

Fig. 1.5 Schematic representation of PtdIns3*P* turnover

of PtdIns3*P*. Vps34 is closely associated with the protein kinase Vps15 which has been described as a Vps34 regulatory protein although the precise role of Vps15 in Vps34 regulation is still unclear (Backer 2008). Questions still remain of whether the activity of Vps34 is modulated by cellular stimulation (Vanhaesebroeck et al. 2010).

Class II PI3Ks are monomers of high molecular weight and exist in three isoforms, PI3K-C2α, PI3K-C2β and PI3K-C2γ (Falasca and Maffucci 2007, 2012). Class II PI3Ks can phosphorylate both PtdIns and PtdIns4*P* but not PtdIns(4,5)P_2 in vitro, with a preferential activity for PtdIns (Arcaro et al. 1998). Work from our laboratory has demonstrated that PI3K-C2α and PI3K-C2β specifically generate PtdIns3*P* at the plasma membrane of muscle and cancer cells upon insulin and LPA stimulation, respectively, (Maffucci et al. 2003; 2005a; Falasca et al. 2007) indicating that class II PI3Ks are mostly responsible for the synthesis of the stimulated pool of this PI. A PI3K-C2α-dependent synthesis of PtdIns3*P* has also been detected in neurosecretory granules (Meunier et al. 2005). No evidence of a role for PI3K-C2γ in modulating PtdIns3*P* levels in vivo has been provided so far. Evidence is also emerging indicating that there is a pool of PtdIns3*P*, likely synthesised by class II PI3Ks, and directly regulated by the phosphatases myotubularins (MTMs). For instance, down-regulation of MTMs in *C. elegans* is able to rescue the phenotype of a Vps34 null mutant (Xue et al. 2003). Similarly, down-regulation of PI3K-C2β specifically rescues the inhibition of Akt phosphorylation induced by down-regulation of MTM1 (Razidlo et al. 2011). The mechanism of activation of class II PI3Ks is still not clear although data suggest that it may involve translocation of the enzymes to the plasma membrane (Maffucci et al. 2005; Falasca et al. 2007; Falasca and Maffucci 2012).

Phosphatases

PtdIns3*P* can be dephosphorylated by several phosphatases, mostly MTMs which are members of the protein tyrosine phosphatases superfamily (Robinson and Dixon 2006) (Fig. 1.5). Originally thought to be protein phosphatases, it was soon observed that recombinant myotubularin MTM1 and myotubularin-related (MTMR) 1, 2, 3, 4, 6 and 7 were able to selectively dephosphorylate PtdIns3*P*. Data later revealed that

some MTM/MTMR (such as MTM1, MTMR 2, 3, 6) were also able to dephosphorylate PtdIns(3,5)P_2 (see below). MTMRs are conserved among eukaryotes and 14 family members have been detected in humans. Interestingly, nearly half of them are predicted to be catalytically inactive. Although overexpressed MTMs are usually found diffusely distributed into the cytosol, localisation in other intracellular compartments has also been reported. For instance, endogenous MTMR7 has been described as partially localised to Golgi-like structures in a neuroblastoma cell line; MTM1 can partially localise to the plasma membrane (Robinson and Dixon 2006) and it has also been observed in Rab5-positive early endosomes (Cao et al. 2007) where it critically regulates PtdIns3*P* levels (Cao et al. 2008). MTMR2 specifically localizes to Rab7-positive late endosomes (Cao et al. 2007) and can regulate late endosomal PtdIns3*P* levels (Cao et al. 2008). Overexpressed MTMR3 is largely cytosolic but also present in punctuate elements and in the ER.

The mechanism of regulation of MTMs is not completely understood. It has been suggested that intracellular relocation may represent one way of regulating their activity or that the several non-catalytic domains may have a role in regulation of their enzymatic activity (Choudhury et al. 2006). For instance, binding of PtdIns5*P* seems to activate MTM1, MTMR3 and MTMR6 at least in vitro (Clague and Lorenzo 2005). Moreover evidence seems to suggest that binding of inactive MTMs to active family members can represent some sort of mechanism of regulation (Zou et al. 2009). A recent hypothesis has suggested that MTM specificity can be regulated by their recruitment to different protein complexes (Lecompte et al. 2008).

Other Routes of PtdIns3P Turnover

PtdIns3*P* levels are also regulated by kinases/phosphatases that can convert it into other PIs (Fig. 1.5) and these routes will be discussed in the paragraphs related to the respective PI. In particular, interconversion of PtdIns3*P* and PtdIns(3,5)P_2 is regulated by the enzyme PIKfyve and the 5-phosphatase FIG4/Sac3. Direct synthesis of PtdIns3*P* and PtdIns(3,4)P_2 can be potentially mediated by PIP4 Ks, detected in vitro and possibly occurring in vivo (Divecha and Halstead 2004). Finally, dephosphorylation of PtdIns(3,4)P_2 to PtdIns3*P* has also been described.

Intracellular Roles

Membrane trafficking. PtdIns3*P* intracellular localisation has been studied by using specific PtdIns3*P*-binding domains fused to the GFP, mostly tandem FYVE domains. Despite several limitations and drawbacks of these tools, these experiments clearly revealed a strong accumulation of this PI in the endosomal system in resting mammalian cells (Gillooly et al. 2000) where it regulates membrane transport and membrane dynamics by recruiting proteins containing FYVE, PX

and PH domains (Lindmo and Stenmark 2006). It is generally accepted that hVPs34 is responsible for the synthesis of the endosomal PtdIns3*P* and indeed inhibition of hVps34 blocks homotypic early endosomes fusion, the formation of internal vesicles in multivesicular bodies and it delays trafficking of receptors (Backer 2008). Vps34 also controls protein sorting to the lysosome and membrane homeostasis. Emerging data indicate that the endosomal pool of PtdIns3*P* can also contribute to growth factor signalling by critically regulating maturation of an early endocytic intermediate, known as APPL endosomes (Zoncu et al. 2009). Evidence suggests that other PI3Ks can also control the endosomal pool of PtdIns3*P* (Falasca and Maffucci 2009).

Autophagy. hVps34 forms a multiprotein complex with the proautophagic Beclin1/Atg6, Bif-1 and UVRAG that initiates autophagosome formation (Backer 2008; Vanhaesebroeck et al. 2010) and it is critical for autophagosome–lysosome fusion during late steps of autophagy. These data support the hypothesis of a key role for PtdIns3*P* in this process, as also suggested by data indicating that knockdown of MTMR14 and, possibly MTMR6, increases autophagy and the total numbers of autophagic organelles (Vergne et al. 2009); that overexpression of a dominant negative mutant of MTMR3 as well as down-regulation of MTMR3 induce autophagosome formation (Taguchi-Atarashi et al. 2010) and that the local pools of PtdIns3*P* are critical for both autophagy initiation and regulation of autophagosome membrane structure (Taguchi-Atarashi et al. 2010). A direct involvement of PtdIns3*P* in autophagosome formation has been proposed (Axe et al. 2008) as well as a role in the recruitment of Atg18, a protein involved in autophagosome formation (Proikas-Cezanne et al. 2007).

Exocytosis. Evidence indicates that PtdIns3*P* has a role in exocytosis, possibly in different cellular contexts. A PI3K-C2α-dependent pool of PtdIns3*P* plays a critical role in exocytosis of neurosecretory granules by regulating the ATP-dependent priming of the vesicles (Meunier et al. 2005; Wen et al. 2008). Similarly, PI3K-C2α is involved in glucose-induced insulin secretion (Leibiger et al. 2010) and insulin granules' exocytosis (Dominguez et al. 2011). Although a role for PtdIns(3,4)P_2 in this process has been suggested (Liebiger et al. 2010) it has been reported that PtdIns3*P* can directly support fusion in particular experimental conditions using reconstituted proteoliposomes (Mima and Wickner 2009a) and this phosphoinositide is part of a minimal set of lipids required for fusion (Mima and Wickner 2009b). The possibility, therefore, exists that PI3K-C2α may regulate insulin granule fusion directly by maintaining a pool of PtdIns3*P* necessary for this event. A role for an insulin-dependent PtdIns3*P* in regulation of glucose disposal into fat and muscle cells through modulation of the translocation of the glucose transporter protein GLUT4 to the plasma membrane has also been demonstrated (Maffucci et al. 2003a). Synthesis of this stimulated pool of PtdIns3*P* occurs at the plasma membrane of muscle cells and adipocytes (Maffucci et al. 2003a) and it is mediated by PI3K-C2α (Falasca et al. 2007). Indeed, down-regulation of PI3K-C2α inhibits glucose transport (Falasca et al. 2007). Consistent with a role for PtdIns3*P*, overexpression of MTM impairs insulin-induced GLUT4 translocation (Chaussade et al. 2003). More recently, it has been reported that a Rab5-dependent

PtdIns3*P* synthesis is important for GLUT4 translocation (Lodhi et al. 2008). The precise role of PtdIns3*P* in GLUT4 translocation remains to be addressed (Ishiki et al. 2005; Kanda et al. 2005; Kong et al. 2006). Whether PtdIns3*P* itself is directly involved in this process or whether the de novo synthesis of this PI through PI3K-C2α activation is required for its further conversion into PtdIns(3,5)P_2, whose role in GLUT4 translocation has also been reported (Shisheva 2008a), is still a matter of investigation.

Cell migration. LPA activates PI3K-C2β which in turn catalyses the synthesis of PtdIns3*P* at the plasma membrane of ovarian and cervical cancer cells (Maffucci et al. 2005a). Translocation to the plasma membrane of exogenous PI3K-C2β upon LPA stimulation has been observed (Maffucci et al. 2005a), suggesting that a stimulated targeting of the class II enzymes can be responsible for temporally and spatially regulated synthesis of PtdIns3*P*. Data indicate that the LPA-dependent pool of PtdIns3*P* regulates cell migration (Maffucci et al. 2005a), later confirmed in other cellular systems (Domin et al. 2005).

Regulation of ion channels. PtdIns3*P* indirectly activates the Ca^{2+}-activated K^+ channel, KCa3.1 (Srivastava et al. 2006a). A role for MTMR6-dependent modulation of PtdIns3*P* levels has been suggested in this context (Srivastava et al. 2005, 2006b). Involvement of the class II PI3K isoform PI3K-C2β in this process has also been reported (Srivastava et al. 2009).

Studies using specific PtdIns3*P* binding PH domains tagged to GFP have revealed the presence of a nuclear pool of this PI (Safi et al. 2004; Maffucci et al. 2003b), possibly specifically restricted to nuclear speckles (Maffucci et al. 2003b). In this respect, it is noteworthy that PI3K-C2α has also been found confined in this specific intranuclear structures (Didichenko and Thelen 2001). Nuclear localisation was also reported for PI3K-C2β (Sindić et al. 2001; Visnjić et al. 2002). It remains to be established what the role of nuclear PtdIns3*P* is.

Human Disease

Since PtdIns3*P* plays a key role in autophagy, it would be interesting to determine whether it might be involved in human pathologies characterised by deregulation of this process. Autophagy is altered in several diseases, including cancer, some myopathies (where autophagic vacuoles accumulate) and several neurodegenerative disorders (where the accumulation of misfolded proteins can derive from defective autophagy). No mutation in PIK3C3 has been found in this kind of disease (Nicot and Laporte 2008) although mutation or deletion of components of the VPS34 complexes, such as beclin 1 and ultraviolet radiation resistance-associated gene protein have been found in breast, ovarian and colon cancer (Levine and Kroemer 2008). Data are also now revealing a key role for autophagy in pancreatic islets of Langerhans (Ebato et al. 2008) and β cells homeostasis (Jung et al. 2008) and altered autophagy has been detected in β cells from Type 2 diabetic individuals (Masini et al. 2009; Marchetti and Masini 2009).

A rare variant in the promoter of PIK3C3 gene has been reported to be associated with bipolar disorder and schizophrenia in a candidate gene study, (Nicot and Laporte 2008). A potential role for PtdIns3*P* in cancer is suggested by data indicating a key role for PI3K-C2β in migration of some cancer cells (Maffucci et al. 2005a; Katso et al. 2006) and data suggesting a role for PI3K-C2β in regulation of apoptosis in some cell lines (Elis et al. 2008). PI3K-C2α and PI3K-C2β expression is elevated in a large number of human small cell lung cancer cell lines compared with normal lung epithelial cells (Arcaro et al. 2002). The potential contribution of PtdIns3*P* to cancer still needs to be properly investigated.

Mutations in the MTMs have been associated with distinct forms of Charcot-Marie-Tooth (CMT) diseases, a heterogeneous group of genetic peripheral neuropathies affecting motor and sensory nerves and characterised by progressive distal muscle atrophy and weakness (McCrea and De Camilli 2009; Nicot and Laporte 2008). Mutations all over the gene encoding for MTMR2 were detected in the autosomal recessive demyelinating neuropathy CMT type 4B (CMT4B1) and five distinct mutations in the *MTMR*13 gene have been reported to cause CMT4B2. About 200 mutations in the gene encoding MTM1, resulting in reduced protein levels or loss of protein, have been found in patients affected by X-linked centronuclear myopathy (Nicot and Laporte 2008), a very severe congenital myopathy.

Monophosphates: Phosphatidylinositol 4-phosphate

Kinases

PtdIns4*P* (Fig. 1.2) is generated from the precursor PtdIns through the action of PtdIns 4-kinases (Balla and Balla 2006; D'Angelo et al. 2008). Four isoforms of PtdIns 4-kinases have been identified in mammals, originally classified as Types II and III PI4Ks (Fig. 1.6).

Type II PI4Ks, PI4KIIα and PI4KIIβ, are characterised by their insensitivity to the inhibitor wortmannin (Balla and Balla 2006). Because of their palmitoylation, Type II PI4Ks are tightly membrane-bound proteins, although a significant larger fraction of PI4KIIβ than PI4KIIα is cytosolic. PI4KIIα was cloned from membranes of large dense core vesicles in neurons and although it has been suggested that both isoforms may be associated with synaptic vesicles, it seems that the main isoform in these vesicles is PI4KIIα (Hammond and Schiavo 2007). Both PI4KIIα and PI4KIIβ localised to intracellular membranes, mostly TGN and endosomes (Balla and Balla 2006) but they are also present in compartments that contain the AP-3 adaptor complex and in GLUT4-containing vesicles (D'Angelo et al. 2008). A plasma membrane localisation has also been reported for both Type II PI4Ks either in basal conditions (PI4KIIα) or upon stimulation of cells with platelet derived growth factor (PI4KIIβ) in a Rac-dependent manner (D'Angelo et al.

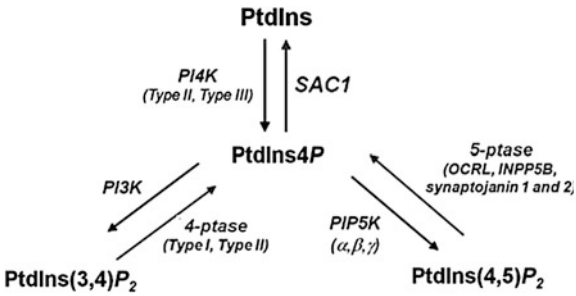

Fig. 1.6 Schematic representation of PtdIns4*P* turnover

2008). Regulation of Type II PI4Ks is not completely clear but it has been reported that calcium inhibits both isoforms whereas membrane association increases PI4KIIβ activity (Balla and Balla 2006).

Type III PI4Ks, PI4KIIIα and PI4KIIIβ, are sensitive to wortmannin (Balla and Balla 2006). In mammalian cells, PI4KIIIα is mainly localised in the ER, in a perinuclear compartment and in the nucleolus (Balla and Balla 2006). PI4KIIIβ is mainly present in the Golgi complex (D'Angelo et al. 2008) although it has also been reported in the nucleus (Balla and Balla 2006). Both Arf1 and neuronal calcium sensor 1 regulate PI4KIIIβ activity which can be further activated through phosphorylation by protein kinases D1 and D2 (D'Angelo et al. 2008).

Phosphatases

PtdIns4*P* can be dephosphorylated by the phosphatase Sac1 and this process is responsible for its turnover at the ER and the Golgi (Fig. 1.6). Original data on mammalian Sac1 revealed that this enzyme was able to dephosphorylate PtdIns4*P* and PtdIns3*P* in vitro (Rohde et al. 2003); however, its specific localisation within the ER and the Golgi complex supports a main role for this enzyme in modulation of PtdIns4*P* levels in vivo. It has been suggested that redistribution of Sac1 between the Golgi complex and the ER upon growth factor stimulation can induce an accumulation of PtdIns4*P* within the Golgi, which in turn can enhance stimulated secretion in proliferating cells (Blagoveshchenskaya et al. 2008).

Other Routes of PtdIns4P Turnover

PtdIns4*P* can be converted into the bisphosphates PtdIns(4,5)P_2 and PtdIns(3,4)P_2 by the action of PtdIns4*P* 5-kinases (PIP5Ks, Type I PIPK) and PI3K respectively (Fig. 1.6). Similarly, dephosphorylation of both PIs by specific 5- and 4-phosphatases has been reported to generate PtdIns4*P* (Fig. 1.6).

Intracellular Roles

Consistent with its subcellular localisation, PI4KIIα has a role in trans-Golgi network (TGN)-to-endosome and TGN-to-plasma membrane transport, in the association of AP-3 with endosomal compartments and in degradation of the epidermal growth factor receptor (D'Angelo et al. 2008). Similarly, PI4KIIIβ can regulate the structural architecture of the Golgi complex, TGN-to-plasma membrane transport and the sphingolipid synthetic pathway (D'Angelo et al. 2008).

The intracellular roles of PtdIns4*P* have been mostly ascribed to its subsequent conversion to PtdIns(4,5)P_2 and possibly PtdIns(3,4,5)P_3. In this respect, it must be noted that the pool of PtdIns4*P* to be converted into PtdIns(4,5)P_2 can be either delivered to the plasma membrane by membrane carriers derived from the Golgi complex and from recycling organelles or it can be produced locally at the plasma membrane by PI4Ks (Di Paolo and De Camilli 2006). For instance, it seems that de novo synthesis of PtdIns4*P*, followed by action of PIP5K, is required for generation of synaptic PtdIns(4,5)P_2 rather than stored PtdIns4*P* (Hammond and Schiavo 2007). Synthesis of this PI, therefore, represents a key step in modulation of the levels of PtdIns(4,5)P_2.

Beside its role as precursor of PtdIns(4,5)P_2, the identification of selective PtdIns4*P* effectors within the cell has now suggested that PtdIns4*P* itself may regulate some intracellular functions. In particular, it has been shown that PtdIns4*P* can participate in recruitment of adaptor and coat proteins to specific domains of the TGN. Examples include AP-1, which promotes clathrin-dependent TGN-to-endosome trafficking and epsinR which can induce membrane curvature. PtdIns4*P* seems also to regulate assembly of COPII at the ER exit sites. Some lipid transport proteins can also bind PtdIns4*P*, including oxysterol-binding proteins, which binds cholesterol and oxysterols, and ceramide transport protein, which transfers ceramide between the ER and the TGN (Balla and Balla 2006). Recently, it has been reported that inhibition of PtdIns4*P* production results in impairment of glycosphingolipid synthesis through its role in regulation of the glucosylceramide-transfer protein FAPP2 (D'Angelo et al. 2007). Some evidence of a role for PI4KIIIβ in insulin secretion has also been provided (Balla and Balla 2006).

Although the roles of PI4KIIIα in the ER are not completely known it is likely that this enzyme is responsible for the synthesis of a pool of PtdIns4*P* at the level of the ER-plasma membrane sites. Indeed, it has been reported that the production of a plasma membrane pool of PtdIns4*P* is mediated by PI4KIIIα (Balla and Balla 2006). The importance of this is highlighted by mass measurements of PtdIns(4,5)P_2 revealing that almost 50 % of the total cellular pool of this PI is synthesised via a wortmannin-sensitive PI4K (Balla and Balla 2006).

Human Disease

Accumulation of PI4KIIα has been observed in some cancer types and specific down-regulation of this enzyme reduces growth of different xenografts in mice in a mechanism involving regulation of angiogenesis through control of hypoxia inducible factor (HIF)-1α expression (Li et al. 2010). Interestingly, down-regulation of HIF-1α expression induced by knockdown of PI4KIIα is rescued by exogenous addition of PtdIns4*P*, confirming that the lipid kinase activity of the enzyme is crucial for its role (Li et al. 2010). Although expression of PI4KII*β* appears to be up regulated in some but not all analysed xenografts, overexpression of this specific isoform does not increase HIF-1α expression, as observed in the case of PI4KIIα overexpression (Li et al. 2010).

Monophosphates: Phosphatidylinositol 5-phosphate

Kinases

PIKfyve/MTMs. The main route of PtdIns5*P* synthesis in mammalian cells seems to involve synthesis of PtdIns(3,5)P_2 (which is catalysed by Type III PIPK, PIKfyve, as described below) and subsequent dephosphorylation by MTMs (Fig. 1.7). MTM1, MTMR1, MTMR2 and MTMR3 were all able to dephosphorylate PtdIns(3,5)P_2 and the product of the reaction was identified as PtdIns5*P* (Tronchere et al. 2004). Moreover, overexpression of MTM1 in Jurkat cells was able to increase PtdIns5*P* levels (Tronchere et al. 2004), indicating that this enzyme can induce PtdIns5*P* synthesis in mammalian cells.

The direct phosphorylation of PtdIns by PIKfyve has also been suggested (Shisheva 2008b). For instance, an increase in PtdIns5*P* levels has been detected in Ba/F3 and NIH3T3 cells expressing the oncogenic tyrosine kinase nucleophosmin anaplastic lympohoma kinase compared to control cells. Treatment with siRNA specifically targeting PIKfyve reduces PtdIns5*P* levels (Coronas et al. 2008). Consistent with this, overexpression of PIKfyve seems to induce an accumulation of PtdIns5*P* in some cell lines (Sbrissa et al. 2002). However, whether this is due to a direct PIKfyve-dependent phosphorylation of PtdIns or rather to PIKfyve-mediated generation of PtdIns(3,5)P_2 and subsequent dephosphorylation by MTMs is difficult to be assessed.

PIP4Ks. Levels of PtdIns5*P* can also be regulated by PtdIns5*P* 4-kinases (PIP4Ks, Type II PIPKs) which can reduce PtdIns5*P* levels by phosphorylating it at position 4, leading to PtdIns(4,5)P_2 (see below). Three isoforms of PIP4Ks exists, namely PIP4Kα, *β*, and *γ*, with the *β* showing the highest expression of all isoforms and the *γ* being highly expressed in the kidney and in neurons (Clarke et al. 2009). Although studies have reported that overexpression of PIP4Kα (Roberts et al. 2005) and *β* (Roberts et al. 2005; Jones et al. 2006) does not affect

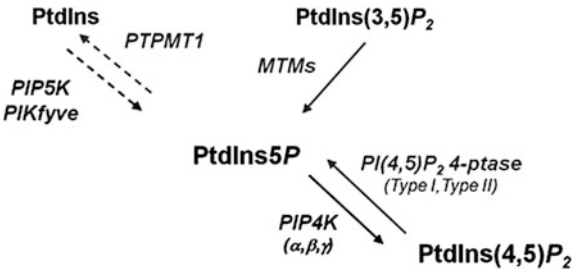

Fig. 1.7 Schematic representation of PtdIns5*P* turnover

the basal levels of PtdIns5*P*, UV irradiation induces phosphorylation of PIP4Kβ in a mechanism involving p38 activation, resulting in inhibition of the enzyme and subsequent accumulation of nuclear PtdIns5*P* (Jones et al. 2006). PIP4Kβ possesses an almost exclusive nuclear localisation (Divecha et al. 1993; Richardson et al. 2007) where it can specifically localise in nuclear speckles (Bunce et al. 2008), therefore it was generally accepted that this isoform was specifically involved in modulation of a nuclear pool of PtdIns5*P*. By contrast, it has been suggested that PIP4Kα can modulate increases in a cytoplasmic pool of PtdIns5*P* in HeLa cells upon inhibition of the phospho-tyrosine phosphatase (Wilcox and Hinchliffe 2008), consistent with a preferred cytosolic intracellular localisation. Nevertheless, it has been recently demonstrated that PIP4Kβ can interact with PIP4Kα in vitro and in vivo and modulate the nuclear localisation of PIP4Kα (Bultsma et al. 2010), consistent with original observations (Divecha et al. 1993). PIP4Kγ appears to be associated with vesicles in kidney and brain. PIP4Kα, first purified and cloned from erythrocytes (Boronenkov and Anderson 1995) and platelets (Divecha et al. 1995), possesses a much higher enzymatic activity than the other two isoforms (Clarke et al. 2009; Bultsma et al. 2010).

PIP5Ks (PtdIns4*P* 5-kinase, Type I PIPK) can also directly phosphorylate PtdIns in vitro to generate PtdIns5*P* (Tolias et al. 1998) but whether this occurs in vivo remain to be addressed, with one study showing that transfection of PIP5Kα does not increase the levels of PtdIns5*P* in COS7 (Roberts et al. 2005). No study so far has described a PI3K-dependent phosphorylation of PtdIns5*P* to generate PtdIns(3,5)P_2.

Phosphatases

A phosphatase with a highly specific 5-phosphatase activity towards PtdIns5*P* in vitro has been described in mammals (Merlot et al. 2003; Pagliarini et al. 2004). This enzyme, originally named phospholipid-inositol phosphatase and later renamed PTPMT1 (Blero et al. 2007), can potentially convert PtdIns5*P* to PtdIns but whether this enzyme has a role in reducing the levels of PtdIns5*P* in vivo is not clear.

By contrast, the role of some phosphatases in promoting an increase in PtdIns5*P* levels has been demonstrated. For instance, MTMs are key regulators of

PtdIns5P levels, as already discussed. Accumulation of PtdIns5*P* can also derive from dephosphorylation of PtdIns(4,5)P_2 by the action of 4-phosphatases. Actually the identification of a 4-phosphatase, invasion plasmid gene D (IpgD), in *Shigella flexneri* able to convert PtdIns(4,5)P_2 into PtdIns5*P* (Niebuhr et al. 2002) represented an important step towards the identification of some of the intracellular functions of PtdIn5*P* (see below). Two mammalian PtdIns(4,5)P_2 4-phosphatases have been identified (Ungewickell et al. 2005) which can generate PtdIns5*P* through dephosphorylation of PtdIns(4,5)P_2 (see below). Some increase in PtdIns5*P* levels have been detected in HEK293 cells overexpressing Type I but not Type II PtdIns(4,5)P_2 4-phosphatase (Zou et al. 2007).

Intracellular Roles

Levels of PtdIns5*P* increase 20-fold during G1 suggesting that this PI may have a role in regulation of cell cycle progression (Clarke et al. 2001). As discussed above, accumulation of a PIP4Kβ-dependent pool of PtdIns5*P* has been detected in the nucleus upon stress signals and it has been shown that nuclear PtdIns5*P* can modulate ING2 binding to chromatin (Jones et al. 2006) and this in turn can regulate the ability of ING2 to activate p53 (Gozani et al. 2003). This role of nuclear PtdIns5*P* seems to be supported by data indicating that overexpression of Type I PtdIns(4,5)P_2 4-phosphatase promotes p53 acetylation and cell death (Zou et al. 2007). Increased cellular levels of PtdIns5*P* stimulate the complex Cul3-SPOP which can regulate ubiquitylation of several substrates, including PIP4KIβ in a mechanism involving p38 MAPK (Bunce et al. 2008).

A role for a cytosolic pool of PtdIns5*P* has been discovered in cells during *S. flexneri* infection. Accumulation of this PI at the entry foci of the bacteria has been reported as a result of release of the 4-phosphatase IpgD (Pendaries et al. 2006). Moreover, it has been reported that expression of IpgD but not its inactive mutant is able to induce Akt phosphorylation in host cells and this is inhibited by expression of PIP4Kβ (Pendaries et al. 2006; Ramel et al. 2009). Moreover, incubation of cells with exogenous PtdIns5*P* is also specifically able to induce Akt phosphorylation and this was suggested to occur through activation of class I PI3Ks (Pendaries et al. 2006). More recently, it has been suggested that PtdIns5*P* has a role in protecting Akt from dephosphorylation (Ramel et al. 2009).

Data have also suggested that PtdIns5*P* may have a regulatory role in the osmotic response pathway, since its levels are decreased upon hypo-osmotic shock in 3T3-L1 fibroblasts and adipocytes (Sbrissa et al. 2002). Using an indirect assay to measure PtdIns5*P* accumulation, it was also reported that insulin increases the levels of this PI in 3T3-L1 adipocytes and CHO-IR cells (Sbrissa et al. 2004) and data also suggested a role for this PI in GLUT4 translocation (Sbrissa et al. 2004). Accumulation of PtdIns5*P* has been detected in platelets upon thrombin stimulation (Morris et al. 2000).

Bisphosphates: Phosphatidylinositol 4,5-bisphosphate

Phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2, Fig. 1.2) was originally mainly studied as a precursor of key second messengers Ins(1,4,5)P_3/diacylglycerol (Fig. 1.3) or PtdIns(3,4,5)P_3. Nevertheless in the last years, it has become increasingly evident that PtdIns(4,5)P_2 can also act as a second messenger itself.

Kinases

PIP4Ks. PtdIns(4,5)P_2 can be synthesised through the action of PtdIns5*P* 4-kinases that phosphorylates PtdIns5*P*, as already discussed (Fig. 1.8). However, this route is mostly studied for its role in modulation of PtdIns5*P* levels rather than for its contribution to PtdIns(4,5)P_2 synthesis.

PIP5Ks. PtdIns(4,5)P_2 is mainly synthesised through the phosphorylation of PtdIns4*P* at the 5-position by PIP5Ks (van den Bout and Divecha 2009), (Fig. 1.8). Ectopic expression of PIP5Ks but not PIP4Ks increases the synthesis of PtdIns(4,5)P_2 in cells (Halstead et al. 2005). Three isoforms of PIP5Ks exist (PIP5Kα, PIP5Kβ and PIP5Kγ) and data indicate that the three isoforms are not redundant. PIP5Kα localises at the plasma membrane, the Golgi complex, in nuclear speckles and in membrane ruffles. PIP5Kβ localises at the plasma membrane and it has also been detected in perinuclear region. Mouse PIP5Kγ661 can localise to focal adhesions and at adherens junctions in epithelial cells (van den Bout and Divecha 2009). Several different mechanisms of regulation of PIP5Ks have been reported, including regulation through the small GTP-binding proteins Rac and Rho, the ADP-ribosylation factors, the protein talin or through phospholipase D and phosphatidic acid (van den Bout and Divecha 2009).

Phosphatases

5-*phosphatases*. PtdIns(4,5)P_2 can be dephosphorylated by 5-phosphatases to generate PtdIns4*P* (Fig. 1.8). The first identified enzyme of this family, Type I, does not appear to dephosphorylate PIs (Blero et al. 2007). All the other 5-phosphatases are Type II enzymes and nine different forms have been described in humans. Some of them are listed in this paragraph. *OCRL* exists in two isoforms, termed a and b (Lowe 2005) and it preferentially dephosphorylates PtdIns(4,5)P_2 although it can also dephosphorylate PtdIns(3,5)P_2 and PtdIns(3,4,5)P_3 (Ooms et al. 2009). OCRL1 localises to the TGN, lysosome and endosomes in particular in clathrin-coated buds at the TGN and clathrin-coated vesicles between the TGN and endosomes (Lowe 2005). OCRL can also translocate to membrane ruffles upon cellular stimulation, in a mechanism dependent on Rac1 (Ooms et al. 2009). *INPP5B* possesses a domain organisation very similar to OCRL and it preferentially dephosphorylates

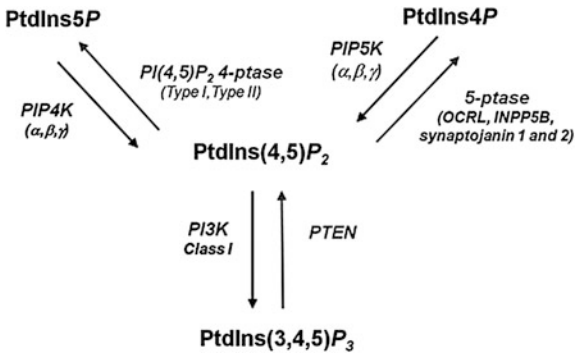

Fig. 1.8 Schematic representation of PtdIns(4,5)P_2 turnover

PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 and localises to the Golgi and endocytic pathway. It is also able to translocate to lamellipodia upon cellular stimulation, where it can co-localise with Rab5 and actin. *Synaptojanin* 1 is a nerve terminal protein of 145 kDa crucial for synaptic vesicle trafficking and recycling (Blero et al. 2007). Mice deficient for this phosphatase die shortly after birth (Cremona et al. 1999). *Synaptojanin* 2 was first identified by polymerase chain reaction and it is necessary for the formation of clathrin-coated pits and for lamellipodia formation during cell migration. The proline rich domains of the two synaptojanins can associate with different proteins, locating the enzymes in different cellular compartments (Blero et al. 2007). *SKIP* is a 5-phosphatase which can dephosphorylate PtdIns(3,4,5)P_3 (see below). Nevertheless, in vitro kinetic analysis has revealed a preference for PtdIns(4,5)P_2 compared to PtdIns(3,4,5)P_3 but whether this can occur in vivo is still not clear. Similarly, SHIP2, INPP5E and PIPP have been shown to be able to dephosphorylate PtdIns(4,5)P_2 in vitro (see below).

4-*phosphatases*. Dephosphorylation of PtdIns(4,5)P_2 by a 4-phosphatase was first observed in studies on IpgD. Two mammalian PtdIns(4,5)P_2 4-phosphatases have been identified [named PtdIns(4,5)P_2 4-phosphatase type I and type II] which lead to accumulation of PtdIns5*P*. Both exogenous and endogenous proteins appear to localise in the late endosomes/lysosomal membranes with data suggesting that they may be lysosomal transmembrane proteins (Ungewickell et al. 2005).

Intracellular Roles

Channel activity. Activity of several ion channels has been reported to depend on PIs (a list can be found in Logothetis et al. 2010). In particular, PtdIns(4,5)P_2 is involved in regulation of inward rectifier and voltage-gated potassium channels, calcium channels and pumps, transient receptor potential channels, epithelial sodium channels, ion exchangers (Di Paolo and De Camilli 2006; Logothetis et al. 2010). PtdIns(4,5)P_2 can regulate channel function through direct binding (Halstead et al. 2005). On the other hand, several channels appear to be dependent on PtdIns(4,5)P_2 even if they lack specific PtdIns(4,5)P_2 binding sites (van Rossum et al. 2005).

Actin modulation. One of the most studied roles of PtdIns(4,5)P_2 is regulation of the actin cytoskeleton, often in concert with PtdIns(3,4,5)P_3 or small GTPases. PtdIns(4,5)P_2 can regulate actin filament assembly through different mechanisms including: (1) inhibition of actin monomer sequestering; (2) filament depolymerisation; (3) filament barbed ends capping; (4) activation of the Arp2/3 complex (Saarikangas et al. 2010). On the other hand, PtdIns(4,5)P_2 can positively regulate actin-membrane linkage by releasing proteins containing FERM domain from their autoinhibitory state (Saarikangas et al. 2010). Through these actions, PtdIns(4,5)P_2 participates in regulation of cell shape, motility, cytokinesis and several other functions (Di Paolo and De Camilli 2006). PtdIns(4,5)P_2 is also critical for regulating local polymerisation of actin during phagocytosis.

Trafficking. PtdIns(4,5)P_2 is directly involved in exocytosis, acting both on plasma membrane proteins and on vesicle proteins and it can probably cooperate with SNARE interactions (Di Paolo and De Camilli 2006). PtdIns(4,5)P_2 appears to be required for all forms of endocytosis by recruiting and regulating critical endocytic proteins at the plasma membrane (Ooms et al. 2009). A role for OCRL in regulation of transport from early endosomes to TGN and for INPP5B in the early secretory pathways has also been suggested (Ooms et al. 2009). Whether PtdIns(4,5)P_2 is directly involved in these processes is not clear.

Apoptosis. As precursor of PtdIns(3,4,5)P_3, PtdIns(4,5)P_2 has been indirectly implicated in regulation of apoptosis. Evidence that PtdIns(4,5)P_2 itself can have a role in regulation of apoptosis has been provided by studies indicating that this PI can directly inhibit purified caspase 3 and 8 activity in vitro and that overexpression of PIP5Kα can suppress apoptosis in a mechanism that does not involves PtdIns(3,4,5)P_3 synthesis or Akt activation (Mejillano et al. 2001). PtdIns(4,5)P_2 levels are reduced during apoptosis induced by oxidative stress and UV irradiation in a mechanism independent of caspase activation and involving down-regulation of PIP5Kα (Halstead et al. 2006).

Human Disease

Lowe Syndrome. Mutations in OCRL causes Lowe Syndrome (also known as **O**culo**C**erebro**R**enal syndrome of **L**owe), an X-linked disorder which induces bilateral congenital cataracts, mental retardation, neonatal hypotonia and renal Fanconi syndrome (McCrea and De Camilli 2009). The mechanisms by which OCRL loss determine the clinical onset of Lowe syndrome is not clear. Magnetic resonance imaging brain scans of Lowe Syndrome's patients show cystic abnormalities in the white matter. High levels of lysosomal enzymes are also found in the plasma, suggesting a defect in the endosomal trafficking (Ooms et al. 2009). Fibroblasts from Lowe syndome's exhibit reduced actin stress fibres, increased F-actin puncta, enhanced sensitivity to actin-depolymerising agents and an altered localisation of actin-binding proteins such as gelsolin and α-actinin (Ooms et al. 2009).

Dent disease. Mutations of OCRL have been observed also in a subset of cases of Dent disease, an X-linked disease with renal disorder similar but not identical to the defects detected in Lowe Syndromes (McCrea and De Camilli 2009).

Channelopathies. Mutations that appear to impair the interactions between channels and PtdIns(4,5)P_2, as established by in vitro studies, and resulting in decreased open probability have been detected in Andersen–Tawil syndrome and in hyperprostaglandin E syndrome (HPS), the antenatal form of Bartter syndrome (Halstead et al. 2005; Logothetis et al. 2010). Similarly, disruption of the interaction between the channel and PtdIns(4,5)P_2 seems to result from few mutations linked to LQT syndrome (Logothetis et al. 2010). Few of the mutations in Kir6.2 that have been linked to congenital hyperinsulinism can be mapped to the putative PtdIns(4,5)P_2 binding site (Logothetis et al. 2010). Finally, few mutations identified in patients with thyrotoxic hypokalemic periodic paralysis were also shown to alter the interaction between PtdIns(4,5)P_2 and Kir2.6 (Logothetis et al. 2010).

Lethal contractural syndrome type 3. An inactivating mutation in the gene encoding for PIP5Kγ has been found to be responsible for this autosomal recessive lethal congenital contractural syndrome (Narkis et al. 2007).

Bisphosphates: Phosphatidylinositol 3,4-bisphosphate

Kinases

PI3K. The direct route of phosphatidylinositol 3,4-bisphosphate [PtdIns(3,4)P_2] synthesis occurs through phosphorylation of PtdIns4*P* by a member of the PI3K family (Fig. 1.9). While in vitro studies have revealed that both class I and class II PI3K isoforms can generate PtdIns(3,4)P_2, the relative contribution of each isoform to in vivo synthesis of PtdIns(3,4)P_2 is still not clear. In fact, demonstration that accumulation of PtdIns(3,4)P_2 can occur in vivo through direct action of a PI3K-dependent on PtdIns4*P*, as opposed to PtdIns(3,4,5)P_3 synthesis followed by the action of a 5-phosphatase has proven to be very difficult because several stimuli are able to simultaneously generate PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 making difficult to discriminate which effect are due to each PI and to identify intracellular functions specifically dependent on PtdIns(3,4)P_2. The relative levels of PtdIns(3,4)P_2 compared to PtdIns(3,4,5)P_3 seem to depend on the cell types, receptor type activated, relative levels of phosphatase and tensin homolog (PTEN), tissue- and cell-specific expression of 5- and 4-phosphatases (Ooms et al. 2009).

PIP4K. PIP4Ks have also been shown to be able to phosphorylate PtdIns3*P* to generate PtdIns(3,4)P_2 in vitro (Rameh et al. 1997) and this observation, together with data showing that PtdIns(3,4)P_2 can be converted to PtdIns(3,4,5)P_3 by PIP5Ks during oxidative stress (Halstead et al. 2001) has led to the hypothesis of the existence of this alternative pathway to generate PtdIns(3,4,5)P_3 which for instance may have been developed before evolution of class I PI3Ks (Divecha and Halstead 2004).

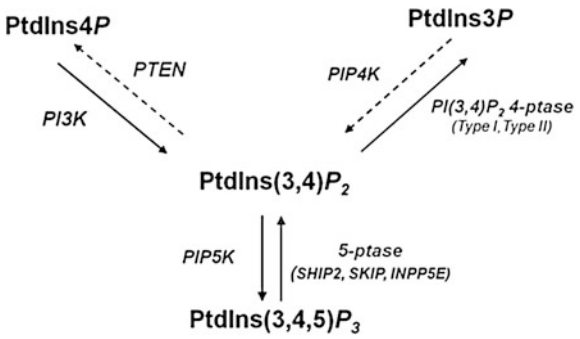

Fig. 1.9 Schematic representation of PtdIns(3,4)P_2 turnover

Phosphatases

4-*phosphatases*. Dephosphorylation of PtdIns(3,4)P_2 by inositol polyphosphate 4-phosphatases (4-ptases) leads to PtdIns3*P* (Fig. 1.9). Two PtdIns(3,4)P_2 4-ptases have been identified, Type I and II, that exist as two alternative splice mutants (α and β). Recombinant 4-ptase I is able to hydrolyse PtdIns(3,4)P_2 in vitro and its overexpression has been shown to decrease total levels of PtdIns(3,4)P_2 but not PtdIns(3,4,5)P_3 (Kisseleva et al. 2002). Moreover, Weeble cells (which lacks 4-ptase mRNA and protein) show an increase in PtdIns(3,4)P_2 but not PtdIns(3,4,5)P_3 (Shin et al. 2005). The inositol polyphosphate 4-phosphatase Type II (INPP4B) preferentially dephosphorylates PtdIns(3,4)P_2 both in vitro and in vivo with no effect on PtdIns(3,4,5)P_3 (Gewinner et al. 2009).

PTEN. In vitro studies have suggested that PTEN can dephosphorylate PtdIns(3,4)P_2 but it is not clear whether this occurs in vivo.

Other Routes of PtdIns(3,4)P_2 Turnover

PtdIns(3,4)P_2 can accumulate upon dephosphorylation of PtdIns(3,4,5)P_3 by 5-phosphatases (Fig. 1.9) and this route is still believed to be the main pathway for PtdIns(3,4)P_2 accumulation upon cellular stimulation.

Intracellular Roles

The identification of intracellular functions specifically regulated by PtdIns(3,4)P_2 has proven to be extremely challenging because of the simultaneous synthesis of PtdIns(3,4,5)P_3 that almost always occurs upon cellular stimulation. Furthermore, some PH domains able to bind PtdIns(3,4)P_2 can also bind PtdIns(3,4,5)P_3 (including the highly characterised Akt PH domain), making difficult to assess the contribution of each PI in activation of the target protein. A specific role for PtdIns(3,4)P_2 in Akt activation has been suggested by data reporting that mouse embryonic fibroblasts lacking 4-ptase I display an increased Akt activation in

serum-starved conditions and enhanced activation upon growth factor stimulation which results in increased cell proliferation and colonies in soft agar, reduced apoptosis, and show an enhanced ability to form tumours in vivo (Ivetac et al. 2009).

The identification of PH domains specifically able to bind PtdIns(3,4)P_2 such as the C-terminal domains of TAPP1 and TAPP2 (**Ta**ndem **P**H domain-containing **P**rotein) (Dowler et al. 2000) has been useful to investigate the intracellular localisation of PtdIns(3,4)P_2 and to analyse stimulus-dependent increase. These data suggest a specific role for PtdIns(3,4)P_2 during oxidative stress. Different pools of PtdIns(3,4)P_2 exist within the cells and can be modulated upon cellular stimulation. In particular, an increase in PtdIns(3,4)P_2 was detected at the plasma membrane upon certain cellular stimulation but also detected in ER, MVBs, nuclear envelope and mitochondria and data suggested that the pool in the ER may be regulated by a different mechanism compared to the plasma membrane and endosome pool (Watt et al. 2004). Type Iα 4-ptase has been involved in regulation of megakaryocyte and fibroblast differentiation (Blero et al. 2007).

Human Disease

INPP4B acts as a tumour suppressor in epithelial cancers and a positive correlation between loss of INPP4B protein expression and reduced overall survival has been reported for breast cancer and ovarian cancer patients (Gewinner et al. 2009). INPP4B down-regulation increases anchorage-independent growth, cell proliferation and motility whereas INPP4B overexpression reduces tumour growth. Since INPP4B does not appear to dephosphorylate PtdIns(3,4,5)P_3, a key role for PtdIns(3,4)P_2 in these processes is suggested. Further evidence of a role for INP44B in cancer comes from the observation that INPP4B is frequently deleted in breast cancers and it is silenced in malignant proerythroblasts with high levels of phosphorylated Akt (Bunney and Katan 2010). More recently, down-regulation of INPP4B has been detected in prostate cancer and it has been shown that the expression levels of the phosphatase are regulated by activation of the androgen receptor (Hodgson et al. 2011).

A model of spontaneous mutant mouse (weeble mutant mice) has revealed that mutation in the type I 4-ptase gene is associated with neuronal loss in the cerebellum, ataxia and neonatal death (Nystuen et al. 2001).

Bisphosphates: Phosphatidylinositol 3,5-bisphosphate

Kinases

PtdIns(3,5)P_2 is synthesised by a member of the family of Type III PIPKs through PtdIns3*P* phosphorylation (Dove et al. 2009) (Fig. 1.10). The first PtdIns(3,5)P_2-synthesising enzyme to be identified in higher eukaryotes was the mouse