Minireview

Phosphoinositide signaling disorders in human diseases

Caroline Pendaries, Hélène Tronchère, Monique Plantavid, Bernard Payrastre*

Inserm U563, Centre de Physiopathologie de Toulouse Purpan, Département d'oncogenèse et signalisation dans les cellules hématopoïétiques, Hôpital Purpan, 31059 Toulouse Cedex, France

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Abstract Phosphoinositides (PIs) play an essential role in diverse cellular functions. Their intracellular level is strictly regulated by specific PI kinases, phosphatases and phospholipases. Recent discoveries indicate that dysfunctions in the control of their level often lead to pathologies. This review will focus on some human diseases whose etiologies involve PI-metabolizing enzymes. The role of PTEN (phosphatase and tensin homolog deleted on chromosome ten) in cancer, the impact of the Src homology 2-containing inositol-5-phosphatase phosphatases in acute myeloid leukemia or diabetes, the involvement of myotubularin family members in genetic diseases and the implication of OCRL1 in Lowe syndrome will be emphasized. We will also review how some bacterial pathogens have evolved strategies to specifically manipulate the host cell PI metabolism to efficiently infect them.

Key words: Phosphoinositides; Cancer; Myotubular myopathy; Charcot–Marie–Tooth neuropathy; Lowe syndrome; Bacterial infection

1. Introduction

Phosphoinositides (PIs) are quantitatively minor phospholipids of cell membranes but their metabolism is highly active and accurately controlled. They exert their role either as precursors of second messengers such as inositol 1,4,5-*tris*phosphate ($Ins(1,4,5)P_3$) and diacylglycerol (DAG) or directly by interacting with proteins and orchestrating the spatio-temporal organization of key intracellular signal transduction pathways [1,2]. Recent discoveries implicate PIs in a number of physiological processes including cell proliferation, death, motility, cytoskeletal regulation, intracellular vesicle trafficking and cell metabolism [2]. Accordingly, the list of PI-metabolizing enzymes linked to the development of human diseases is growing and becomes of great interest in biomedical research (Table 1).

The *myo*-inositol head group of PIs contains five free hydroxyl groups but only three of them have been found to be phosphorylated in vivo. Phosphatidylinositol (PtdIns) is the most abundant PI in mammalian cells (approximately 10% of total cell glycerophospholipids) and can undergo sequential and reversible phosphorylations at position D-3, D-4 and D-5 by specific kinases to generate seven different polyPIs. PtdIns(4)P, $PtdIns(4,5)P_2$ (each representing about 10% of total PIs) and PtdIns are kept in a steady state in cell membranes because of continuous phosphorylation/dephosphorylation reactions by specific 4 and 5 -kinases and -phosphatases which are not yet fully identified. This 'canonical pathway' produces a pool of $PtdIns(4,5)P_2$ that can serve as a substrate for phospholipases C (PLCs) which, upon agonist-dependent activation, make the second messengers $Ins(1,4,5)P_3$ and DAG [1–3]. PtdIns, PtdIns(4)P and $PtdIns(4,5)P_2$ can also be phosphorylated by a family of PI 3-kinases displaying different substrate specificity [4]. As clearly shown these last years, PI 3-kinases play a critical role in agonist-stimulated signaling pathways [2,5]. The best-known PI 3-kinase products, $PtdIns(3,4)P_2$ and $PtdIns(3,4,5)P_3$, are rapidly and transiently produced in response to agonist-mediated cell activation (classically, their level never exceed 10% of $PtdIns(4,5)P_2$). PtdIns(3)P is constitutively present in small quantities and its level is generally rather stable in mammalian cells, although minor changes are noted in some conditions. PtdIns(5)P and $PtdIns(3,5)P_2$ are also quantitatively minor but their level can change upon cell activation. The D-3-phosphorylated PIs, and also $PtdIns(4,5)P_2$, can specifically interact with protein-targeting modules. Indeed, multiple proteins have evolved lipid-binding domains exhibiting some specificity for the different classes of PIs [6]. Pleckstrin homology (PH) domain, phox homology (PX) domain, epsin N-terminal homology (ENTH) domain, band 4.1/ezrin/radixin/moesin (FERM) domain, and Fab1p/YOTB/Vac1p/EEA1 (FYVE) domain are among the best PI-binding domains characterized so far. Through these high-affinity lipid–protein interactions, newly synthesized PIs can locally recruit specific signaling proteins in response to extracellular stimuli. Thus, PI-metabolizing enzymes, which allow rapid and reversible formation

*Corresponding author. Fax: (33)-5-61 77 94 01.
E-mail address: payrastr@toulouse.inserm.fr (B. Payrastre).

Abbreviations: PIs, phosphoinositides; DAG, diacylglycerol; PtdIns, phosphatidylinositol; PLC, phospholipase C; PH, pleckstrin homology; PX, phox homology; ENTH, epsin N-terminal homology; FERM, band 4.1/ezrin/radixin/moesin; FYVE, Fab1p/YOTB/Vac1p/EEA1; SNX, sorting nexin; PDK, 3-phosphoinositide-dependent kinase; PKC, protein kinase C; PTEN, phosphatase and tensin homolog deleted on chromosome ten; SHIP, Src homology 2-containing inositol-5-phosphatase; XLMTM, X-linked myotubular myopathy; MTM1, myotubularin; MTMR, myotubular myopathy-related protein; CMT4B, Charcot–Marie–Tooth disease type 4B; EEA1, early endosome antigen 1; SBF2, SET binding factor 2; SET, Survar 3-9, Enhancer-of-zeste, Trithorax; OCRL, oculocerebrorenal syndrome of Lowe phosphatase; Inl, internalin; NFκB, nuclear factor-κB; IpgD, entry-mediating invasin phosphatase

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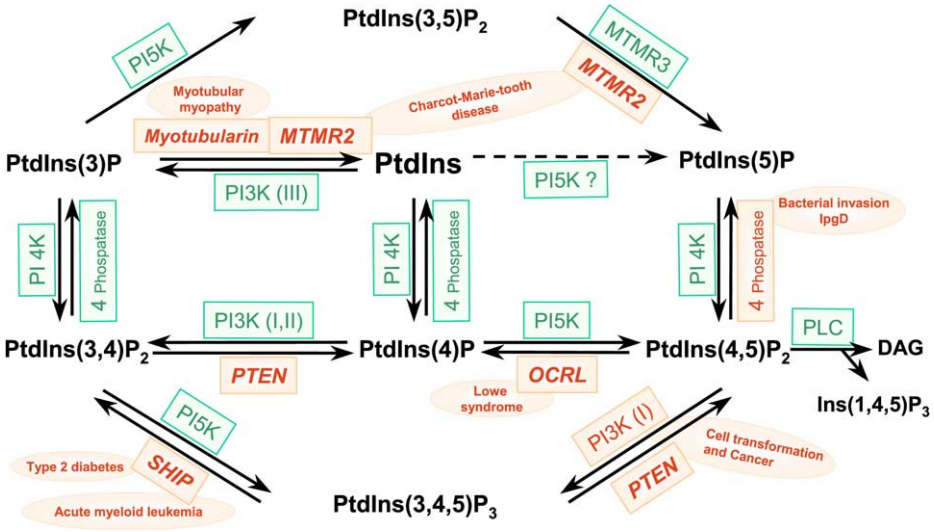

Fig. 1. The PI metabolism and its implications in human diseases. The main pathways of PI synthesis and interconversions in mammalian cells are shown. The enzymes in orange square boxes have been clearly associated with diseases and the corresponding diseases are indicated in orange oblongs. *PTEN*, phosphatase and tensin homologue deleted on chromosome ten; *MTMR*, myotubular myopathy-related proteins; *SHIP*, Src homology 2-containing inositol-5-phosphatase; *OCRL*, phosphatase implicated in the oculocerebrorenal syndrome of Lowe; *IpgD*, entry-mediating invasin of *S. flexneri*.

of microdomains enriched in specific PI species, provide a powerful system to spatially restrict membrane signals. The capacity of some of these enzymes to control the complex interconversions between the different PIs, as illustrated Fig. 1, strongly contributes to the accurate regulation and integration of dynamic intracellular mechanisms.

In this review, we will pay attention to some human pathologies occurring from disruption of PI signaling. Indeed, several PI phosphatases have recently been implicated in human diseases like cancer, myotubular myopathy, neurodegenerative disorder and Lowe syndrome. Moreover, we will summarize how some bacterial pathogens manipulate specific steps of the host cell PI metabolism to develop their virulence.

2. Overview of PI functions

PtdIns is classically considered as precursor of other PIs (polyPIs) through sequential phosphorylations by specific kinases. PtdIns(4)P is the major precursor of PtdIns(4,5)P_2 through phosphorylation by PtdIns(4)P 5-kinases and can also be phosphorylated by type II PI 3-kinases to produce PtdIns(3,4)P_2. PtdIns(4)P has been shown to bind to the cytoskeletal protein talin [1], suggesting that it may have a function in the cell on its own. PtdIns(5)P has long been ignored because it is generally present in small amounts in the cell [7] and is difficult to separate from PtdIns(4)P. Its metabolic pathway in vivo and its role are still unknown but its mass level has been shown to increase during agonist-mediated mammalian cell activation [8]. As described below, the bacterial pathogen *Shigella flexneri* can induce a massive transformation of the host cell PtdIns(4,5)P_2 into PtdIns(5)P during infection [9]. Together, these recent results suggest that PtdIns(5)P may function as an intracellular lipid messenger. However, it is still unknown whether it can activate and/or recruit downstream protein effectors or if it is only a substrate for a local production of quantitatively minor pools of PtdIns(4,5)P_2 or PtdIns(3,5)P_2.

PtdIns(3)P is constitutively present in cells at low level and is implicated in membrane trafficking through the recruitment of FYVE domain-containing proteins such as EEA1 (early endosome antigen 1) [10] or PX domain-containing proteins like the sorting nexin SNX3 [11]. In agreement, using GFP-FYVE probes, this PI has been shown to be mainly present in early endosomes [12]. PtdIns(3)P is phosphorylated by PIKfyve to produce PtdIns(3,5)P_2 [2]. In yeast, PtdIns(3,5)P_2 is predicted to maintain vacuole membrane integrity, recycling and turnover [1]. The PtdIns(3,5)P_2 level is dramatically increased upon hyperosmotic shock in yeast cells, whereas, in mammalian cells, it seems to be engaged in other responses to stress such as UV radiation [2]. Although PH or PX domains are good candidates to interact with this PI, little is known on the putative targets of PtdIns(3,5)P_2.

PtdIns(4,5)P_2 plays an essential role as substrate for the different PI-specific PLCs and is therefore the precursor of Ins(1,4,5)P_3 and DAG. It is also the substrate of the type I PI 3-kinases producing PtdIns(3,4,5)P_3. Although its global level does not rise dramatically in stimulated cells, local increases in PtdIns(4,5)P_2 concentrations are likely to occur and evidence is accumulating that this PI also acts as a signaling molecule on its own. It can influence actin cytoskeleton organization through interactions with actin-binding proteins [13]. For instance, PtdIns(4,5)P_2 can dissociate profilin–actin complexes, thereby regulating the free actin monomer concentration in the cell. Moreover, through its interaction with CapZ-related proteins or gelsolin, PtdIns(4,5)P_2 can uncap actin filaments, allowing elongation of pre-existing filaments. Interestingly, Raucher et al. [14] found that PtdIns(4,5)P_2 can control local adhesion energy between the plasma membrane and the underlying cytoskeleton. Basically, when the PtdIns(4,5)P_2 concentration decreases, the adhesion energy becomes smaller, leading to the formation of membrane blebs. The interaction of this PI with several cytoskeletal anchoring proteins such as vinculin, talin or ERM family proteins (Ezrin, Radixin and Moesin) could support this observation, which has broad implications for the dynamics of the plasma membrane, as we will see below.

PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 are two quantitatively minor PIs rapidly and transiently produced in response to various agonist-mediated cell stimulations. PtdIns(3,4,5)P_3 is a critical signaling molecule able to interact with high affinity with several PH domain-containing proteins. The serine/threonine kinases PDK (3-phosphoinositide-dependent kinase) and Akt, the tyrosine kinase Btk, several exchange factors for small G proteins, PLCγ and the adapter molecule Grb2-associated protein 1 (Gab1) are among the best-characterized targets of PtdIns(3,4,5)P_3. The local production of this PI, at the site of PI 3-kinase stimulation, results in the recruitment of interacting proteins, whose activation often requires a synergistic effect of phosphorylations or a binding with other partners [2,4,5]. One of the best-characterized PI 3-kinase-dependent pathways is the activation of Akt/PKB, the cellular homolog of the retroviral oncogene v-Akt [15]. Following PI 3-kinase activation, PtdIns(3,4,5)P_3 recruits Akt at the plasma membrane by direct interaction with its PH domain. PDK-1, which also contains a PH domain with affinity for PtdIns(3,4,5)P_3, is targeted to the same area and phosphorylates the Thr308 of Akt. Further phosphorylation of the Ser473 of Akt by a putative PDK-2 allows full activation of this enzyme, which then plays an important role in the regulation of many biological processes, including proliferation, apoptosis and growth. PtdIns(3,4)P_2 can be produced by hydrolysis of PtdIns(3,4,5)P_3 by 5-phosphatases, phosphorylation of PtdIns(4)P by a type II PI 3-kinase or phosphorylation of PtdIns(3)P by a putative 4-kinase. It is also able to bind with high affinity to the PH domain of Akt [2,15]. Moreover, this PI can directly activate in vitro novel or atypical protein kinases C (PKCs) such as PKCδ, -ε and -ζ [2].

3. From PI signaling to human cancer

PI 3-kinase was discovered because of its association with the viral oncoproteins v-Src and polyomavirus middle T antigen [16]. Since this initial finding, many other observations have provided compelling evidence that the type IA PI 3-kinase-mediated signaling pathway is involved in mitogenesis and transformation [2,15]. Numerous oncogenes activate type IA PI 3-kinase and several components of the PI 3-kinase/Akt pathway are dysregulated in a wide range of human cancers, including breast, colon, ovarian, pancreatic, lymphoid and prostate cancers [15]. Immunohistochemical studies, using phospho-specific antibodies that only recognize Akt in an active state, have shown that Akt activity is elevated in the various cancers listed above as well as in multiple myeloma, head and neck cancers [15]. A recent analysis of the PI 3-kinase/Akt pathway in 450 tumor samples from eight different tumor types has shown that Akt was activated in 55% of these samples [17].

PTEN/MMAC1/TEP1 (for 'phosphatase and tensin homolog deleted on chromosome ten', 'mutated in multiple advanced cancers 1', 'transforming growth factor β-regulated and epithelial cell-enriched phosphatase 1') [18] is a dual-specificity phosphatase that recognizes both protein substrates and

Table 1
PI phosphatases implicated in human syndromes

Gene	Activity	Substrate specificity	Syndromes in human	Phenotype in mice	References
PTEN	3-Phosphatase	PtdIns(3,4)P_2, PtdIns(3,4,5)P_3	Cowden disease. Increased susceptibility to develop various cancers. Bannayan–Zonana syndrome	Embryo lethality of knockout mice. Tumorigenesis in heterozygous mice	[18–25,58]
SHIP1	5-Phosphatase	PtdIns(3,4,5)P_3	Inhibitory mutation associated with a case of acute myeloid leukemia and chemotherapy resistance. Potential involvement in Paget's disease?	Myeloproliferative phenotype with progressive splenomegaly, massive myeloid infiltration of the lungs, shortened lifespan of knockout mice. Severe osteoporotic phenotype. No tumor susceptibility described so far	[26–28,56,59]
SHIP2	5-Phosphatase	PtdIns(3,4,5)P_3	Type 2 diabetes	Increased sensitivity to insulin, severe neonatal hypoglycemia, perinatal death of knockout mice. Increased glucose tolerance and insulin sensitivity in heterozygous adult mice	[29–31]
Myotubularin (MTM1)	3-Phosphatase	PtdIns(3)P	X-linked myotubular myopathy	Generalized and progressive myopathy starting at around 4 weeks of age, with amyotrophy and accumulation of central nuclei in skeletal muscle fibers, leading to death at 6–14 weeks of knockout mice	[32–36]
MTMR2	3-Phosphatase	PtdIns(3)P, PtdIns(3,5)P_2	Charcot–Marie–Tooth disease 4B1	not described	[18,37–39]
SBF2	Inactive		Charcot–Marie–Tooth disease 4B2	not described	[41]
OCRL1	5-Phosphatase	PtdIns(4,5)P_2	Lowe syndrome	Asymptomatic. Show none of the signs of Lowe syndrome (possible compensation by the 5-phosphatase Inpp5b in mice)	[42–46,60]

PtdIns(3,4,5)P_3. It was first identified as a tumor-suppressor gene located on chromosome 10q23.3, a region associated with high-grade glioblastomas, prostate and breast cancers [19,20]. PTEN tumor-suppressor function is linked to its ability to dephosphorylate the 3′-phosphate of PtdIns(3,4,5)P_3 [18,21]. Somatic mutations of PTEN are frequently found in a variety of human cancers and germ-line mutations in *PTEN* are also associated with two inherited hamartoma tumor syndromes, the Cowden disease and the Bannayan–Zonana syndrome [18,21,22,58]. It has also been reported that Cowden patients suffer a high risk of breast cancer.

PTEN plays a pivotal role in downregulating PDK-1 and Akt-dependent pathways [18,21,22]. Mutations that abolish its catalytic activity lead to the accumulation of PtdIns(3,4,5)P_3 promoting unrestrained signaling through Akt and increased cell survival, growth and proliferation. PTEN can also affect cell cycle progression and induce a G1 arrest. Indeed, PTEN-mediated downregulation of Akt stimulates transcription of the cyclin-dependent kinase inhibitors (p27Kip1, p21Waf1 and p57Kip2) by Forkhead family transcription factors [23]. Interestingly, recent studies in transgenic mice have confirmed the role of PTEN in the negative regulation of PI 3-kinase/Akt-mediated tumorigenesis [24].

The negative regulation of Akt activity can also be exerted by other inositol phosphatases either independently or in cooperation with PTEN [25]. For instance, the Src homology 2-containing inositol-5-phosphatases, SHIP1 and SHIP2, are important regulators of PtdIns(3,4,5)P_3 level by removing its 5′-phosphate [26,27]. SHIP1 is a hematopoietic-restricted enzyme that plays a crucial role in the negative regulation of immunocompetent cells [27]. Recently, a somatic mutation within the signature motif of the phosphatase domain of the human SHIP1 gene was reported in primary myeloid leukemia cells [28]. This mutation, in the phosphatase active site (V684E), generates a mutant catalytically defective in 5-phosphatase activity. The consequences are an enhanced Akt phosphorylation in response to IL-3, promotion of cell survival under conditions of serum deprivation and resistance to VP16-induced apoptosis. These results suggest a role of the mutated SHIP1 gene in the development of acute leukemia and chemotherapy resistance through deregulation of the PtdIns(3,4,5)P_3/Akt signaling pathway.

4. The PtdIns(3,4,5)P_3 5-phosphatase SHIP2 in type 2 diabetes

Although SHIP2, whose expression is ubiquitous, can inhibit the proliferation of erythroleukemia cells in vitro [29], this protein has rather been implicated so far in the genetic susceptibility of type 2 diabetes. Transgenic studies in mice support a role for SHIP2 as a critical negative regulator of insulin signaling and sensitivity [30]. Recently, Marion et al. [31] have reported the presence of SHIP2 gene mutations associated with type 2 diabetes in humans. Comparison of SHIP2 cDNA from eight subjects with type 2 diabetes and the cDNA from four control subjects shows no difference in the coding sequence but one of the diabetic subjects exhibited a 16-bp deleted sequence in the proximal part of the SHIP2 3′ untranslated region. This mutation leads to an increased SHIP2 expression and a decreased insulin sensitivity. These results are in agreement with the fact that PI 3-kinase is an important mediator of insulin actions and glucose homeostasis [2,4].

5. Phosphatases of the myotubularin family in myotubular myopathy and Charcot–Marie–Tooth disease

X-linked myotubular myopathy (XLMTM) is a severe congenital disorder characterized by generalized muscle weakness and hypotonia that affects one out of 50 000 newborn males. MTM1, the gene mutated in XLMTM, was located on chromosome Xq28 and cloned in 1996 [32]. More than 133 mutations affecting the MTM1 gene have been characterized in 328 unrelated families. MTM1 codes for a 603 amino acid ubiquitously expressed protein, myotubularin, presenting the conserved sequence of the phosphotyrosine phosphatases active site. However, studies on its substrate specificity have shown that myotubularin's preferred substrate is PtdIns(3)P [33,34], a PI implicated in intracellular vesicular trafficking [2]. This discovery has raised considerable interest, as myotubularin is the first PtdIns(3)P-specific phosphatase. Thus, myotubularin may regulate the level of specific PtdIns(3)P pools and relocate PtdIns(3)P-binding proteins in the cell. This is supported by the fact that overexpression of myotubularin delocalizes EEA1, a PtdIns(3)P-binding protein, from the early endosomes [35]. Mice deleted for MTM1 reproduce the muscle phenotype observed in humans [36] and provide an interesting model to better understand the links between the PI metabolism and the etiology of the disease.

Myotubularin is the founding member of a highly conserved family of genes that comprises 13 members in eukaryotes grouped under the name of myotubular myopathy-related proteins (MTMR) [37]. Mutations in the MTMR2 gene (65% homologous to myotubularin) are responsible for the development of an autosomal recessive motor and sensory demyelinating neuropathy, the Charcot–Marie–Tooth disease type 4B1 (CMT4B1) [38]. This disease, affecting the peripheral nervous system, results from focally unfolded myelin sheaths and Schwann cell proliferation in peripheral nerves [18]. Like myotubularin, MTMR2 is a potent PtdIns(3)P phosphatase but is also able to dephosphorylate PtdIns(3,5)P_2 in vitro and in yeast leading to the generation of PtdIns(5)P [39] (recent results suggest that myotubularin may actually also hydrolyze PtdIns(3,5)P_2; Tronchère et al., unpublished observations). Thus, MTMR2 and myotubularin could be a source of the newly described PtdIns(5)P, whose exact function in the cell is still unknown. A differential regulation of expression of these two proteins and the use of different pools of PtdIns(3)P [35] could explain why these two structurally and functionally redundant ubiquitous and cytosolic phosphatases [40] are involved in different pathologies.

Recently, a new member of the myotubularin family, SBF2 (SET binding factor 2), was located to 11p15 by linkage analysis and found to be mutated in the autosomal recessive Charcot–Marie–Tooth disease type 4B2 (CMT4B2) with focally folded myelin. SBF2, a member of the MTMR non-active phosphatase/antiphosphatase group, presents homology with MTMR2. Interestingly, the non-active SBF2 and the phosphatase-active MTMR2 are implicated in two subforms of CMT4B, suggesting that both proteins act on the same signaling pathway [41]. One hypothesis is that the inactive SBF2 plays the role of an adapter or regulator of the active MTMR2 form.

Characterization of the regulatory mechanisms of myotubularin family members and identification of the pools of hydrolyzed substrates should help to define their role in these pathologies more precisely.

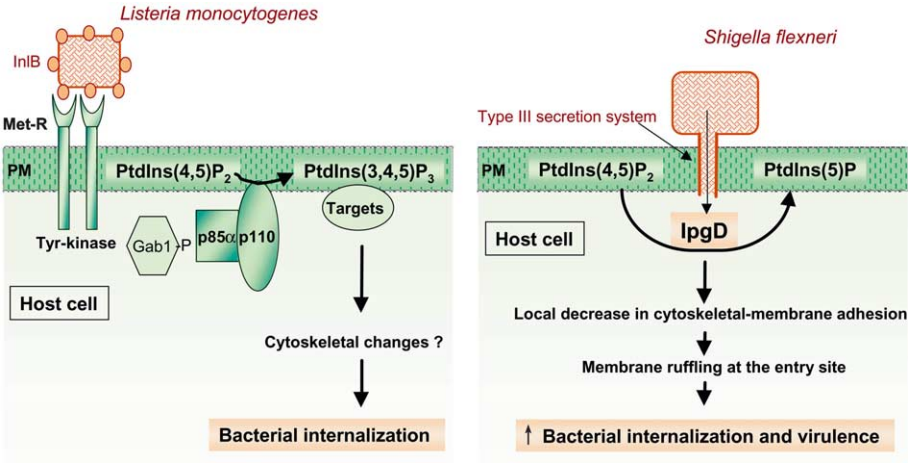

Fig. 2. Schematic representation of how bacterial pathogens disturb and use the PI metabolism to increase their virulence. A pathway implicating InlB/Met receptor/Gab1/PI 3-kinase is critical for InlB-dependent entry of *L. monocytogenes* in several cell lines (left panel). *S. flexneri* can specifically transform PtdIns(4,5)P_2 into PtdIns(5)P by injecting its effector IpgD into the host cell. This modification leads to a local decrease in cytoskeleton–membrane adhesion which, in cooperation with Cdc42 and Rac, allows the formation of membrane ruffles at the entry site of the bacteria (right panel). Host cell is represented in green and bacteria in orange. PM, plasma membrane.

6. The PtdIns(4,5)P_2 5-phosphatase OCRL1 in Lowe syndrome

Lowe syndrome or oculocerebrorenal syndrome of Lowe (OCRL) is an X-linked disorder characterized by bilateral congenital cataracts (with defects in lens epithelial cells), renal Fanconi syndrome (with defects in kidney epithelial cells), and severe mental retardation. It is caused by mutations in the *OCRL1* gene, which encodes a 105 kDa PtdIns(4,5)P_2 5-phosphatase [42,43]. This phosphatase is located in the trans-Golgi network [42], a major sorting site involved in directing proteins to the apical or basolateral domains in epithelial cells. Cell lines from kidney proximal tubules of a patient with Lowe syndrome accumulate PtdIns(4,5)P_2 [44], consistent with an abrogation of the phosphatase activity. It has also been reported that OCRL-deficient cells secrete an abnormally high proportion of lysosomal enzymes [45] and immunofluorescence studies show that OCRL colocalizes with a lysosomal membrane protein in normal proximal tubule cell lines [44]. Thus, OCRL may function in regulating a specific pool of PtdIns(4,5)P_2 associated with lysosomal membranes such that a misregulation due to its loss of function would lead to abnormal delivery of lysosomal enzymes.

Moreover, cellular abnormalities of the actin cytoskeleton have been observed in fibroblasts from patients with Lowe syndrome. These cells presented a decrease in long actin stress fiber, a higher sensitivity to actin depolymerizing agents and an increase in punctuate F-actin staining in their center. These defects correlate with an abnormal distribution of gelsolin and α-actinin, two actin-binding proteins known to be regulated by PtdIns(4,5)P_2 and Ca^{2+} [46]. Actin polymerization plays a key role in the formation, maintenance, and proper function of tight and adherent junctions, which are critical in renal proximal tubule function, and in the differentiation of lens.

Thus the OCRL protein may play a role in the regulation of cell junctions, trafficking, secretion and delivery of lysosomal enzymes. Although the etiology of this pathology remains to be fully understood, these data give some clues to explain how this PI(4,5)P_2 5-phosphatase deficiency may produce the Lowe syndrome phenotype.

7. Roles of PIs in pathogenic bacterial invasion

The ability of an intracellular bacterium to breach the intestinal barrier, penetrate, survive and replicate in mammalian cells is crucial for its pathogenicity and is dependent on multiple virulence factors. The invasion process starts by the attachment to the host cell membrane, followed by the internalization. Some pathogens, like *Listeria monocytogenes*, express surface proteins that bind to host cell receptors, leading to membrane zippering around the bacterium and internalization. Others, such as *Shigella flexneri* or *Salmonella typhimurium*, use a type III secretion system to inject effector proteins into the host cell, leading to membrane ruffling and uptake through a process resembling macropinocytosis [47]. In all cases, the pathogens have evolved several mechanisms to hijack the cellular machinery of the host cell to promote their entry. The three following examples will emphasize how bacterial pathogens can specifically manipulate the PI metabolism of the host cell to develop or increase their virulence.

The Gram-positive food-borne pathogen *L. monocytogenes* can cause meningitis and abortion in pregnant women. Like many microbial pathogens, this intracellular bacterium induces uptake into host cells that are normally non-phagocytic (such as epithelial cells or hepatocytes). Entry is mediated by the *Listeria* surface proteins InlA (internalin) and InlB. Mammalian cell lines have different susceptibilities to InlA- or InlB-mediated uptake. The host receptor for InlA is the calcium-dependent cell-surface adhesion molecule E-cadherin [48]. InlB is a 630 amino acid protein that mediates internalization into Vero, Hep-2, HeLa cells and some hepatocytes. The receptor for InlB has been identified as the tyrosine kinase Met receptor [49]. Entry of *Listeria* is an active process requiring tyrosine phosphorylation and reorganization of the actin cytoskeleton in the host cell. Interestingly, infection of Vero cells with *Listeria* results in activation of the host type IA PI 3-kinase, leading to the production of PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 [50]. *Listeria* entry into these cells is blocked by genetic or chemical inhibition of PI 3-kinase activity. Purified InlB is sufficient to induce tyrosine phosphor-

ylation of the mammalian adapter proteins Gab1 and Cbl, the formation of Gab1/PI 3-kinase complexes and the activation of PI 3-kinase in the host cell [51].

Thus, the InlB/Met receptor/Gab1/PI 3-kinase pathway is critical for InlB-dependent entry of *Listeria* into host cell (Fig. 2). It is still unknown how PI 3-kinase regulates *Listeria* uptake but a role of its lipid products in the reorganization of the actin cytoskeleton and/or the first phases of the phagocytosis is envisaged. In J774 macrophage-like cells, InlB can activate the small G protein Ras upstream of PI 3-kinase and Akt in a pathway leading to nuclear factor-κB (NFκB) activation [52]. This pathway could be involved in the host defense or the inhibition of apoptosis during infection by *Listeria*.

The human intestinal pathogen *S. flexneri* is a Gram-negative bacillus that causes bacillary dysentery in humans. Among the proteins injected by the type III secretion system of *Shigella* during invasion, four Ipas (invasion plasmid antigens A–D), IpgD and its chaperone IpgE are implicated in entry focus formation. IpgD, which has two motifs related to the active site of mammalian inositol polyphosphate 4-phosphatase [53], is injected into the host cell during the first steps of infection of epithelial cells by *Shigella*. It acts as an efficient PtdIns(4,5)P_2 4-phosphatase and the product of host-cell PtdIns(4,5)P_2 hydrolysis, which accumulates, is PtdIns(5)P. The transformation of PtdIns(4,5)P_2 into PtdIns(5)P by IpgD promotes a local decrease in cytoskeletal–membrane adhesion, allowing the formation of membrane ruffling at the entry site [9,54]. In agreement, expression of IpgD in fibroblasts facilitates the development of Cdc42 and Rac-mediated cytoskeleton rearrangement, such as filopodia and lamellipodia, upon agonist stimulation. Thus, transformation of PtdIns(4,5)P_2 into PtdIns(5)P by IpgD, in cooperation with Cdc42 and Rac, two GTPases activated and involved in the entry process of *Shigella*, allows the formation of membrane ruffles at the entry site to improve the bacterial virulence (Fig. 2). The role of the newly formed PtdIns(5)P in this process is still unknown.

Like *Shigella*, *S. typhimurium* actively invades intestinal epithelial cells by inducing membrane ruffling and macropinocytose. Cytoskeletal and membrane remodeling required for bacterial entry depend on the secretion into the host cell of virulence proteins exported by the type III secretory system. Interestingly, SigD (also known as SopB), the homolog of IpgD in *Salmonella*, is also an effective inositol phosphatase [55]. SigD was recently shown to translocate into host cells via the specialized type III secretion system and to mediate a removal of PtdIns(4,5)P_2 from the base of the ruffle [55]. A reduced rigidity of the membrane was observed after SigD action. As in the case of IpgD, this was likely due to a release of membrane–cytoskeleton interactions, thereby facilitating plasmalemmal deformation at the entry foci. This focal disappearance of PtdIns(4,5)P_2 facilitates sealing of the invaginations by contributing to the removal of F-actin and its associated proteins [55]. This event appears to be required for an efficient formation of *Salmonella*-containing vacuoles. Accordingly, the invasion of SigD-deficient *Salmonella* is significantly delayed.

The results obtained with the two bacterial effectors, IpgD and SigD, demonstrate that manipulation of a very specific step of the PI metabolism (i.e. local removal of PtdIns(4,5)P_2) leads to membrane/cytoskeleton remodeling, a critical feature of the endocytic process.

8. Concluding remarks

As reviewed above, recent studies have placed several key enzymes of the PI metabolism at the forefront of the current biomedical research. This is particularly true for phosphatases acting on D-3 PIs (PTEN, SHIP and MTM family members) in which mutations or deficiency lead to human diseases. The critical role of PI signaling is highlighted by bacterial pathogen invasion models, providing an exciting source of information about the functions of these lipids.

Mouse knockout models have confirmed or pointed out the implication of several genes encoding for enzymes of the PI metabolism in the development of various pathologies. In some cases, these transgenic models with dysregulated PI signaling have highlighted unexpected potential implications of PI-metabolizing enzymes in diseases. For instance, a severe osteoporotic phenotype has recently been observed in SHIP1 knockout mice due to increased numbers of hyper-resorptive osteoclasts that mimics the human Paget's disease [56]. This observation may represent an important contribution to the understanding of this disease. Another example is provided by Weeble mutant mice showing a mutation in the gene encoding a type I inositol 4-phosphatase (which hydrolyzes mainly PtdIns(3,4)P_2) and suggesting a potential role of this enzyme in cell proliferation and maturation of inhibitory synapses in neurons [57].

An important challenge is now to better understand the functional specificity within the large and complex family of PI-metabolizing enzymes. Future work should bring information about the expression, the localization and the regulation of these enzymes. The development of new technologies and tools for membrane and lipid signaling studies is mandatory to improve our understanding of the very dynamic integration and spatio-temporal regulation of different pools of individual PIs within the cell. Elucidation of the signaling mechanisms regulated by PI synthesis, degradation or interconversion should unravel new important targets for future drug design.

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References

[1] Payrastre, B., Missy, K., Giuriato, S., Bodin, S., Plantavid, M. and Gratacap, M. (2001) Cell. Signal. 13, 377–387.
[2] Toker, A. (2002) Cell. Mol. Life Sci. 59, 761–779.
[3] Berridge, M.J. (1987) Annu. Rev. Biochem. 56, 159–193.
[4] Katso, R., Okkenhaug, K., Ahmadi, K., White, S., Timms, J. and Waterfield, M.D. (2001) Annu. Rev. Cell Dev. Biol. 17, 615–675.
[5] Rameh, L.E. and Cantley, L.C. (1999) J. Biol. Chem. 274, 8347–8350.
[6] Itoh, T. and Takenawa, T. (2002) Cell. Signal. 14, 733–743.
[7] Rameh, L.E., Tolias, K.F., Duckworth, B.C. and Cantley, L.C. (1997) Nature 390, 192–196.
[8] Morris, J.B., Hinchliffe, K.A., Ciruela, A., Letcher, A.J. and Irvine, R.F. (2000) FEBS Lett. 475, 57–60.
[9] Niebuhr, K., Giuriato, S., Pedron, T., Philpott, D.J., Gaits, F., Sable, J., Sheetz, M.P., Parsot, C., Sansonetti, P.J. and Payrastre, B. (2002) EMBO J. 21, 5069–5078.
[10] Odorizzi, G., Babst, M. and Emr, S.D. (2000) Trends Biochem. Sci. 25, 229–235.
[11] Xu, Y., Hortsman, H., Seet, L., Wong, S.H. and Hong, W. (2001) Nat. Cell. Biol. 3, 658–666.

[12] Gaullier, J.M., Ronning, E., Gillooly, D.J. and Stenmark, H. (2000) J. Biol. Chem. 275, 24595–24600.
[13] Yin, H.L. and Janmey, P.A. (2003) Annu. Rev. Physiol. 65, 761–789.
[14] Raucher, D., Stauffer, T., Chen, W., Shen, K., Guo, S., York, J.D., Sheetz, M.P. and Meyer, T. (2000) Cell 100, 221–228.
[15] Vivanco, I. and Sawyers, C.L. (2002) Natl. Rev. Cancer 2, 489–501.
[16] Cantley, L.C., Auger, K.R., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R. and Soltoff, S. (1991) Cell 64, 281–302.
[17] West, K.A., Sianna Castillo, S. and Dennis, P.A. (2002) Drug Resist. Updates 5, 234–248.
[18] Wishart, M.J. and Dixon, J.E. (2002) Trends Cell Biol. 12, 579–585.
[19] Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S.I., Puc, J., Miliaresis, C., Rodgers, L., McCombie, R., Bigner, S.H., Giovanella, B.C., Ittmann, M., Tycko, B., Hibshoosh, H., Wigler, M.H. and Parsons, R. (1997) Science 275, 1943–1947.
[20] Steck, P.A., Pershouse, M.A., Jasser, S.A., Yung, W.K., Lin, H., Ligon, A.H., Langford, L.A., Baumgard, M.L., Hattier, T., Davis, T., Frye, C., Hu, R., Swedlund, B., Teng, D.H. and Tavtigian, S.V. (1997) Nat. Genet. 15, 356–362.
[21] Maehama, T. and Dixon, J.E. (1999) Trends Cell Biol. 9, 125–128.
[22] Cantley, L.C. and Neel, B.G. (1999) Proc. Natl. Acad. Sci. USA 96, 4240–4245.
[23] Nakamura, N., Ramaswamy, S., Vazquez, F., Signoretti, S., Loda, M. and Sellers, W.R. (2000) Mol. Cell. Biol. 20, 8969–8982.
[24] You, M.J., Castrillon, D.H., Bastian, B.C., O'Hagan, R.C., Bosenberg, M.W., Parsons, R., Chin, L. and DePinho, R.A. (2002) Proc. Natl. Acad. Sci. USA 99, 1455–1460.
[25] Moody, J.L., Pereira, C.G., Magil, A., Fritzler, M.J. and Jirik, F.R. (2003) Genes Immun. 4, 60–66.
[26] Erneux, C., Govaerts, C., Communi, D. and Pesesse, X. (1998) Biochim. Biophys. Acta 1436, 185–199.
[27] Rohrschneider, L.R., Fuller, J.F., Wolf, I., Liu, Y. and Lucas, D.M. (2000) Genes Dev. 14, 505–520.
[28] Luo, J.M., Yoshida, H., Komura, S., Ohishi, N., Pan, L., Shigeno, K., Hanamura, I., Miura, K., Iida, S., Ueda, R., Naoe, T., Akao, Y., Ohno, R. and Ohnishi, K. (2003) Leukemia 17, 1–8.
[29] Giuriato, S., Blero, D., Robaye, B., Bruyns, C., Payrastre, B. and Erneux, C. (2002) Biochem. Biophys. Res. Commun. 296, 106–110.
[30] Clement, S., Krause, U., Desmedt, F., Tanti, J.F., Behrends, J., Pesesse, X., Sasaki, T., Penninger, J., Doherty, M., Malaisse, W., Dumont, J.E., Le Marchand-Brustel, Y., Erneux, C., Hue, L. and Schurmans, S. (2001) Nature 409, 92–97.
[31] Marion, E., Kaisaki, P.J., Pouillon, V., Gueydan, C., Levy, J.C., Bodson, A., Krzentowski, G., Daubresse, J.C., Mockel, J., Behrends, J., Servais, G., Szpirer, C., Kruys, V., Gauguier, D. and Schurmans, S. (2002) Diabetes 51, 2012–2017.
[32] Laporte, J., Hu, L.J., Kretz, C., Mandel, J.L., Kioschis, P., Coy, J.F., Klauck, S.M., Poustka, A. and Dahl, N. (1996) Nat. Genet. 13, 175–182.
[33] Taylor, G.S., Maehama, T. and Dixon, J.E. (2000) Proc. Natl. Acad. Sci. USA 97, 8910–8915.
[34] Blondeau, F., Laporte, J., Bodin, S., Superti-Furga, G., Payrastre, B. and Mandel, J.L. (2000) Hum. Mol. Genet. 9, 2223–2229.
[35] Kim, S.A., Taylor, G.S., Torgersen, K.M. and Dixon, J.E. (2002) J. Biol. Chem. 277, 4526–4531.
[36] Buj-Bello, A., Laugel, V., Messaddeq, N., Zahreddine, H., Laporte, J., Pellissier, J.F. and Mandel, J.L. (2002) Proc. Natl. Acad. Sci. USA 99, 15060–15065.
[37] Laporte, J., Blondeau, F., Buj-Bello, A., Tentler, D., Kretz, C., Dahl, N. and Mandel, J.L. (1998) Hum. Mol. Genet. 7, 1703–1712.
[38] Bolino, A., Muglia, M., Conforti, F.L., LeGuern, E., Salih, M.A., Georgiou, D.M., Christodoulou, K., Hausmanowa-Petrusewicz, I., Mandich, P., Schenone, A., Gambardella, A., Bono, F., Quattrone, A., Devoto, M. and Monaco, A.P. (2000) Nat. Genet. 25, 17–19.
[39] Berger, P., Bonneick, S., Willi, S., Wymann, M. and Suter, U. (2002) Hum. Mol. Genet. 11, 1569–1579.
[40] Laporte, J., Liaubet, L., Blondeau, F., Tronchere, H., Mandel, J.L. and Payrastre, B. (2002) Biochem. Biophys. Res. Commun. 291, 305–312.
[41] Senderek, J., Bergmann, C., Weber, S., Ketelsen, U.P., Schorle, H., Rudnik-Schoneborn, S., Buttner, R., Buchheim, E. and Zerres, K. (2003) Hum. Mol. Genet. 12, 349–356.
[42] Dressman, M.A., Olivos-Glander, I.M., Nussbaum, R.L. and Suchy, S.F. (2000) J. Histochem. Cytochem. 48, 179–190.
[43] Zhang, X. and Majerus, P.W. (1998) Semin. Cell Dev. Biol. 9, 153–160.
[44] Zhang, X., Hartz, P.A., Philip, E., Racusen, L.C. and Majerus, P.W. (1998) J. Biol. Chem. 273, 1574–1582.
[45] Ungewickell, A.J. and Majerus, P.W. (1999) Proc. Natl. Acad. Sci. USA 96, 13342–13344.
[46] Suchy, S.F. and Nussbaum, R.L. (2002) Am. J. Hum. Genet. 71, 1420–1427.
[47] Adam, T., Arpin, M., Prevost, M.C., Gounon, P. and Sansonetti, P.J. (1995) J. Cell Biol. 129, 367–381.
[48] Mengaud, J., Ohayon, H., Gounon, P., Mege, R.-M. and Cossart, P. (1996) Cell 84, 923–932.
[49] Shen, Y., Naujokas, M., Park, M. and Ireton, K. (2000) Cell 103, 501–510.
[50] Ireton, K., Payrastre, B., Chap, H., Ogawa, W., Sakaue, H., Kasuga, M. and Cossart, P. (1996) Science 274, 780–782.
[51] Ireton, K., Payrastre, B. and Cossart, P. (1999) J. Biol. Chem. 274, 17025–17032.
[52] Mansell, A., Khelef, N., Cossart, P. and O'Neill, L.A. (2001) J. Biol. Chem. 276, 43597–43603.
[53] Norris, F.A., Wilson, M.P., Wallis, T.S., Galyov, E.E. and Majerus, P.W. (1998) Proc. Natl. Acad. Sci. USA 95, 14057–14059.
[54] Niebuhr, K., Jouihri, N., Allaoui, A., Gounon, P., Sansonetti, P.J. and Parsot, C. (2000) Mol. Microbiol. 38, 8–19.
[55] Terebiznik, M.R., Vieira, O.V., Marcus, S.L., Slade, A., Yip, C.M., Trimble, W.S., Meyer, T., Finlay, B.B. and Grinstein, S. (2002) Nat. Cell Biol. 4, 766–773.
[56] Takeshita, S., Namba, N., Zhao, J.J., Jiang, Y., Genant, H.K., Silva, M.J., Brodt, M.D., Helgason, C.D., Kalesnikoff, J., Rauh, M.J., Humphries, R.K., Krystal, G., Teitelbaum, S.L. and Ross, F.P. (2002) Nat. Med. 8, 943–949.
[57] Nystuen, A., Legare, M.E., Shultz, L.D. and Frankel, W.N. (2001) Neuron 32, 203–212.
[58] Wanner, M., Celebi, J.T. and Peacocke, M. (2001) J. Am. Acad. Dermatol. 44, 183–187.
[59] Helgason, C.D., Damen, J.E., Rosten, P., Grewal, R., Sorensen, P., Chappel, S.M., Borowski, A., Jirik, F., Krystal, G. and Humphries, R.K. (1998) Genes Dev. 12, 1610–1620.
[60] Jänne, P.A., Suchy, S.F., Bernard, D., MacDonald, M., Crawley, J., Grinberg, A., Wynshaw-Boris, A., Westphal, H. and Nussbaum, R.L. (1998) J. Clin. Invest. 101, 2042–2053.