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## New EMBO Member's Review

# Function and dysfunction of the PI system in membrane trafficking



**Mariella Vicinanza, Giovanni D'Angelo, Antonella Di Campli and Maria Antonietta De Matteis***

Department of Cell Biology and Oncology, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy

**The phosphoinositides (PIs) function as efficient and finely tuned switches that control the assembly–disassembly cycles of complex molecular machineries with key roles in membrane trafficking. This important role of the PIs is mainly due to their versatile nature, which is in turn determined by their fast metabolic interconversions. PIs can be tightly regulated both spatially and temporally through the many PI kinases (PIKs) and phosphatases that are distributed throughout the different intracellular compartments. In spite of the enormous progress made in the past 20 years towards the definition of the molecular details of PI–protein interactions and of the regulatory mechanisms of the individual PIKs and phosphatases, important issues concerning the general principles of the organisation of the PI system and the coordination of the different PI-metabolising enzymes remain to be addressed. The answers should come from applying a systems biology approach to the study of the PI system, through the integration of analyses of the protein interaction data of the PI enzymes and the PI targets with those of the 'phenomes' of the genetic diseases that involve these PI-metabolising enzymes.**

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## Introduction

The phosphoinositides (PIs) derive from reversible phosphorylation in three of the five hydroxyl groups of the inositol headgroup of the 'parent' PI, phosphatidylinositol (PtdIns). This process operates through the large repertoire of PI kinases (PIKs) and PI phosphatases that are present in practically all cell compartments (Figures 1 and 2). The combined activities of the various isoforms of these PIKs and PI phosphatases provide a dynamic equilibrium between the seven distinct, but interconvertible, PI species (Figure 1). It is now clear that all of these different PIs are 'active' in their own right, rather than many just serving as intermediates in the synthesis of the higher phosphorylated species.

Although they are quantitatively minor components of cell membranes, the PIs regulate many fundamental processes in the cell, including membrane trafficking, cell growth, cytoskeleton remodelling and nuclear events. These regulatory actions are mainly due to their ability to control the subcellular localisation and activation of various effector proteins that possess PI-binding domains, such as the PH, FYVE, PX, ENTH, PH-GRAM, FERM and GLUE domains (Lemmon, 2008).

Here, we will focus on the role of the PIs in membrane trafficking, where they can function as 'local' organisers of membrane domains and controllers of membrane sorting and deformation machineries, and as integrators of membrane trafficking within other cell function modules, such as the cytoskeleton, signalling, lipid metabolism and energy control. Although we refer to excellent recent reviews for an update on the enormous progress made towards the definition of the roles, regulation and mechanisms of action of the PIs (Di Paolo and De Camilli, 2006; Engelman *et al*, 2006; Gamper and Shapiro, 2007; Krauss and Haucke, 2007; Strahl and Thorner, 2007; Yeung and Grinstein, 2007; Marone *et al*, 2008; Michell, 2008), we will highlight in the following the many open questions that still dominate the field. We will also attempt to extract the general principles of the functioning of the PI system, and finally, we will illustrate how a systems biology approach to the study of the genetic defects of the PI-metabolising enzymes can provide important lessons for the understanding of the PI system itself.

## The roles of the PIs in membrane trafficking: organisers of molecular machineries and regulators of membrane lipid composition

The main role of the PIs in membrane trafficking involves the spatially and temporally controlled activation, recruitment and/or assembly of the molecular machineries (see Table I) involved in membrane bending and fission, and in vesicle

*Corresponding author. Department of Cell Biology and Oncology, Consorzio Mario Negri Sud, 66030 Santa Maria Imbaro (CH), Italy. Tel.: +39 0872570346; Fax: +39 0872570412;
E-mail: dematteis@negrisud.it

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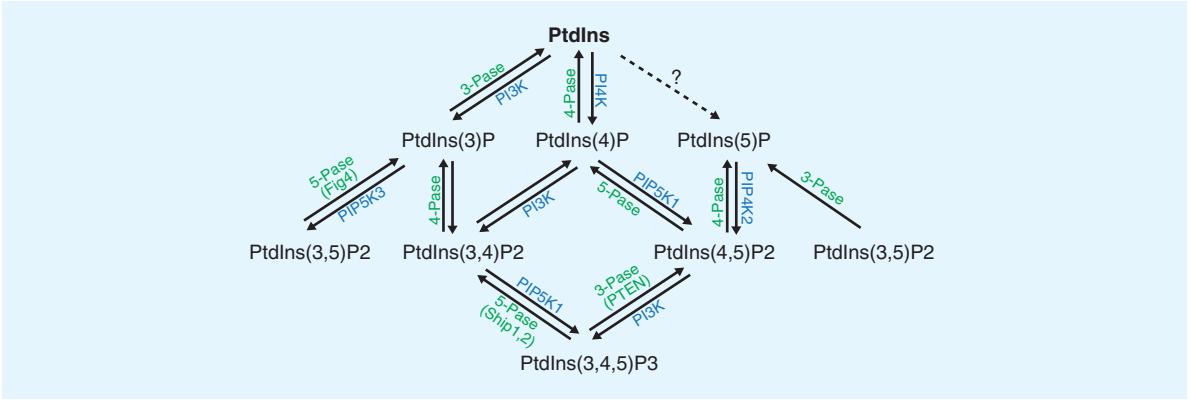
movement, tethering and fusion. The components of these machineries are mainly peripheral proteins that are targeted to their correct sites of action through their binding to a specific PI species. These PI–protein interactions usually occur with relatively low affinities, and additional stabilising binding sites are often required to engage either membrane-resident proteins or specific-organelle-associated small GTPases (Lemmon, 2008). A similar combinatorial recognition system increases the individuality of the identity code for each organelle and membrane domain, and offers an

**A**




**B**

| | Gene | Domain organisation | Human disease | KO mice phenotype |
|---|---|---|---|---|
| PI3K | *PIK3CA, B, D* | RSBD, Kinase | Cancer (*A, B*) | Embryonal lethality (*A, B*), reduced immune response by B and T cells (*D*) (–/–) |
| | *PIK3CG* | RSBD, RBD, C2, HD, Kinase | – | Reduced macrophages and neutrophil function |
| | *PI3KC2A, B, G* | RBD, C2, HD, Kinase, C2 | – | – |
| | *PIK3C3* | C2, HD, Kinase | Bipolar disorder | – |
| PI4K | *PI4KA* | PRD, NLS, LKU, PH, Kinase | Bipolar disorder | – |
| | *PI4KB* | PRD, LKU, SRD, Kinase | – | – |
| | *PI4K2A, B* | PRD, Kinase, CRD, PRD, Kinase | – | – |
| | *PIP4K2A, B, G* | Activation loop, Kinase | Bipolar disorder, schizophrenia (*A*) | Hypersensitive to insulin and reduced body weight (*B*) |
| | *PIP5K1A, B, C, L1* | Activation loop, Kinase | Lethal contractual syndrome type 3 (*C*) | Myocardial developmental defects, neural tube closure defects (*C*), postnatal lethality (–/–), infertility (–/–) |
| | *PIP5K3* | FYVE, DEP, TCP1, SPEC, Kinase | Francois–Neetens fleck cornea dystrophy | - |
| 3-Pase | *PTEN* | C2, Phosphatase | Cowden and Bannayan–Zonana, cancer, macrocephaly/autism | Embryonic lethality (–/–), tumorigenesis (+/–) |
| | *MTM1, R1, R2* | PH-GRAM, Phosphatase, Coiled-coil | X-linked myotubular myopath (*MTM1*), Charcot–Marie–Tooth 4B1(*MTMR2*) | Progressive myopathy (*MTM1*), polyneuropathy (*MTMR2*) |
| | *MTMR3, 4* | PH-GRAM, Phosphatase, Coiled-coil, FYVE | – | – |
| | *MTMR6, 7, 8* | PH-GRAM, Phosphatase, Coiled-coil | – | – |
| | *MTMR9, 10, 11, 12* | PH-GRAM, Inactive phosphatase, Coiled-coil | – | – |
| | *SBF1, 2* | DENN, PH-GRAM, Inactive phosphatase, Coiled-coil, PH | Charcot–Marie–Tooth 4B2 (*SBF2*) | Peripheral neuropathy (*SBF2*) |
| 4-Pase | *INPP4A* | C2, Phosphatase | – | Neuronal loss (Weeble mutant mice) |
| | *INPP4B* | C2, Phosphatase | – | - |
| | *TMEM55A, B* | Phosphatase | – | - |
| | *SACM1L* | LZ, SAC, TM | – | - |
| 5-Pase | *SKIP* | Skich, Phosphatase | – | - |
| | *PIB5PA* | Skich, PRD, Phosphatase, PRD | – | - |
| | *INPP5B* | ASH, Phosphatase, Rho-GAP, CAAX | – | - |
| | *OCRL1* | ASH, Phosphatase, Rho-GAP | Lowe syndrome | Asymptomatic (–/–) |
| | *INPP5D* | SH2, Phosphatase, PRD | Acute myelogenous leukemia | Increased sensitivity to infections, severe osteoporosis |
| | *INPP5E* | PRD, Phosphatase, CAAX | – | – |
| | *INPPL1* | SH2, Phosphatase, PRD, SAM | Diabetes type II, metabolic syndrome | Increased sensitivity to insulin, resistance to high-fat-diet-induced obesity |
| | *SYNJ1, 2* | SAC, Phosphatase, PRD | Bipolar disorder (*SYNJ1*) | Lethal (*SYNJ1*) (–/–) |
| | *INPP5F* | SAC | – | – |
| | *FIG4* | SAC | Charcot–Marie–Tooth 4J | Massive neurodegeneration (Pale tremor mice) |

opportunity for signal integration (Itoh and De Camilli, 2004; Behnia and Munro, 2005). Various examples of such integration are known: many adaptors (e.g. the clathrin adaptors AP1 and AP2) share binding to the same coat protein (clathrin) and the same sorting signals in the cargo proteins (YXXΦ), but they are recruited to different intracellular 'districts' (trans-Golgi network (TGN) endosomes for AP1, and plasma membrane (Bai and Chapman) for AP2), which is here probably due to their different PI-binding selectivities (Table I).

The PIs also operate at the interface between membranes and the cytoskeleton, a key site for fundamental membrane trafficking events, such as membrane deformation and fission, and vesicle movements. The importance of actin-based machineries in endocytic processes is well established both in yeast and in mammals (Engqvist-Goldstein and Drubin, 2003). In particular, actin assembly accompanies the internalisation of coated pits at the plasma-membrane (PM) in yeast (Engqvist-Goldstein and Drubin, 2003), and a burst of localised actin polymerisation accompanies the cycling of synaptic vesicles. This cycling also correlates with the cycling of PtdIns 4,5-bisphosphate (PI45P2) production/consumption, and PI45P2 does indeed have an active role in coordinating endocytic and cytoskeleton events (Di Paolo and De

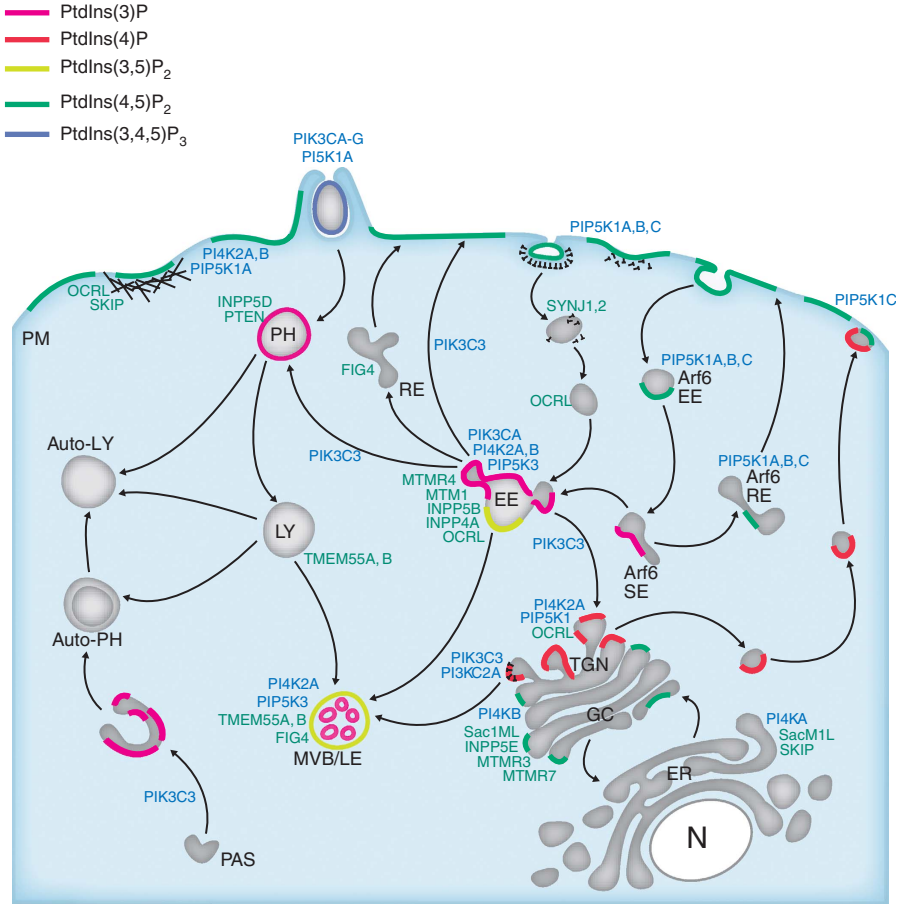



**Figure 2** Subcellular distribution of the PIs and PI-metabolising enzymes. The localisation of the different PIKs (blue) and PI phosphatases (green), as well as the predominant PI species (as visualised by PI-binding protein domains) in the different cell compartments. It is noted that many of the PI-metabolising enzymes are present in more than one cellular compartment, and their overall distributions do not completely fit with the PI map, as indicated using the PI-binding protein probes (see text for details). The PIKs and PI phosphatases are indicated according to the nomenclature given in Figure 1. PM, plasma membrane; EE, early endosome; SE: sorting endosomes; RE, recycling endosome; LY, lysosome; MVB/LE, multivesicular body/late endosome; PAS, pre-autophagosomal structure; PH, phagosome; TGN, trans-Golgi network; GC, Golgi complex; ER: endoplasmic reticulum; N, nucleus.

**Figure 1** The PI kinases and phosphatases and their genetic defects. (**A**) Schematic representation of the PI metabolic cycle with the PIKs indicated in blue, and the PI phosphatases in green. (**B**) Listing of the different isoforms of the PIKs and PI phosphatases, together with their domain organisation and their corresponding genetic disease and knockout or knockdown phenotypes in mice. RSBD, regulatory subunit-binding domain; C2, conserved region 2; HD, helical domain; PRD, proline-rich domain, NLS, nuclear localisation signal; LKU, lipid kinase unique domain; PH, pleckstrin-homology domain; SRD, serine-rich domain; CRD, cysteine-rich domain; FYVE, Fab1 YOTB Vac1 EEA1; DEP, domain present in dishevelled, EGL-10 and pleckstrin; TCP1, tailless complex polypeptide-1; SPEC, spectrin repeat; PH-GRAM, pleckstrin homology glucosyltransferases, Rab-like GTPase activators and myotubularins; DENN, differentially expressed in normal versus neoplastic; LZ, leucine zipper; TM, transmembrane domain; ASH, abnormal spindle-like microcephaly-associated protein (ASPM), *C. elegans* centrosomal protein (SPD-2), hydrocephalus-associated protein (Hydin); RhoGAP, Rho-GTPase-activating protein; SAC, yeast suppressor of actin 1; Skitch, SKIP carboxyl homology domain; SH2, phosphotyrosine-binding module 2; SAM, sterile alpha-motif domain.

**Table I** PI-binding proteins belonging to membrane trafficking machineries

| | | PI targets | PI | PI-binding module | Membrane trafficking pathway | References |
|---|---|---|---|---|---|---|
| Small GTPases accessory proteins | Arf-GEF | ARNO | PI45P2, PI345P3, PI34P2 | PH | Endocytosis | Manna *et al* (2007) |
| | | Cytohesin-1 | PI345P3 | PH | Phagocytosis | Venkateswarlu *et al* (1999) |
| | | EFA6 | PI45P2 | PH | Endocytosis | Macia *et al* (2008) |
| | Arf-GAP | ARAP1 | PI345P3 | PH (multiple) | Endocytosis | Miura *et al* (2002) |
| | | ARAP2 | PI345P3 | PH (multiple) | Endocytosis | Yoon *et al* (2006) |
| | | ARAP3 | PI345P3 | PH (multiple) | Endocytosis | Krugmann *et al* (2002) |
| | | Centaurin 1α | PI345P3 | PH (multiple) | Endocytosis | Tanaka *et al* (1997) |
| | | ACAP1 | PI45P2, PI34P2, PI35P2, PIP3 | PH | Sorting at endosomes | Shinozaki-Narikawa *et al* (2006) |
| | | ACAP2 | PI45P2 | PH | Sorting at endosomes | Jackson *et al* (2000) |
| | | ASAP1 | PI45P2, PIP3 | PH | Sorting at endosomes | Kam *et al* (2000) |
| | | AGAP1 | PI45P2 | PH | Endo-lysosomal trafficking | Nie *et al* (2002) |
| | | AGAP2 | PI45P2 | PH | Endo-lysosomal trafficking | Nie *et al* (2005) |
| | | GIT1 | PI345P3 | ND | Endocytosis | Vitale *et al* (2000) |
| | | ArfGAP1 | PI45P2 | ND | Vesicle budding at the Golgi | Vitale *et al* (2000) |
| | | ArfGAP3 | PI45P2 | ND | | Liu *et al* (2001) |
| | Rab-GEF | HERC1 | PI45P2 | RLD1 | Coatomer assembly Endocytosis | Garcia-Gonzalo *et al* (2004) |
| | Rab-GAP | Rabphilin3A | PI45P2 | C2 | Secretion | Chung *et al* (1998) |
| Coats and adaptors | | AP-1γ | PI4P | Positively charged patch | TGN–endosome trafficking | Wang *et al* (2003) |
| | | EpsinR | PI4P | ENTH | Sorting at endosomes | Hirst *et al* (2003) |
| | | AP-2α, AP-2μ | PI45P2 | Positively charged patch | Endocytosis | Rohde *et al* (2002) |
| | | AP3 | PI45P2 | ND | Endo-lysosomal trafficking | Lee *et al* (1997) |
| | | AP180/CALM | PI45P2 | ANTH | Endocytosis | Ford *et al* (2001) |
| | | CLINT | PI4P, PI35P2, PI45P3, PIP3 | ENTH | Formation of clathrin-coated vesicles | Kalthoff *et al* (2002) |
| | | HIP1/HIP1R | PI45P2, PI34P2, PI35P2 | ENTH | Endocytosis | Hyun *et al* (2004) |
| | | GGAs | PIP4 | GAT | Sorting at TGN | Wang *et al* (2007) |
| | | Dab2 | PI45P2 | PTB | Endocytosis | Mishra *et al* (2002) |
| | | ARH | PI45P2 | ND | Endocytosis | Mishra *et al* (2002) |
| | | Epsins 1–3 | PI45P2 | ENTH | Endocytosis | Itoh *et al* (2001) |
| | | β-Arrestins | PI45P2 | ND | Endocytosis | Gaidarov *et al* (1999) |
| | | Hrs | PI3P | FYVE | Sorting at endosomes | Raiborg *et al* (2001) |
| Sorting | Sorting nexins | SNX1 | PIP3, PI35P2, PI3P | PX | Sorting at endosomes | Cozier *et al* (2002) |
| | | SNX2 | PI3P>PI4P>PI5P | PX | Sorting at endosomes | Zhong *et al* (2002) |
| | | SNX3 | PI3P | PX | Endosomes TGN trafficking | Xu *et al* (2001) |
| | | SNX5 | PI4P, PI5P, PI35P2 | PX | Sorting at endosomes | Liu *et al* (2006) |
| | | SNX9 | PI3P, PI34P2, PI35P2, PI45P2 | PX | Sorting at endosomes | Shin *et al* (2008) |
| | | SNX10 | PI3P | PX | Endocytosis | Qin *et al* (2006) |
| | | SNX13 | PI3P=PI5P>PI35P2, PI4P | PX | Sorting at endosomes | Zheng *et al* (2001) |
| | | SNX16 | PI3P | PX | Endo-lysosomal trafficking | Hanson and Hong (2003) |
| | | SNX17 | PI3P | PX | Sorting at endosomes | Czubayko *et al* (2006) |
| | ESCRT | ESCRTII | PI3P>PI34P2 | GLUE | Endo-lysosomal trafficking | Teo *et al* (2006) |
| | | ESCRTIII | PI3P, PI35P2 | ND | Endo-lysosomal trafficking | Lin *et al* (2005) |
| Fission | | Dynamin1, 2, 3 | PI45P2 | PH | Severing vesicles and tubules from the TGN and PM | Burger *et al* (2000) |
| | | Amphiphysin 2-Bin1 | PI45P2 | Exon10 | Endocytosis | Lee *et al* (2002) |
| Fusion | | EEA1 | PI3P | FYVE | Endosomes | Burd and Ernr (1998) |
| | | Rabenosyn5 | PI3P | FYVE | Endosomes | Nielsen *et al* (2000) |
| | | Synaptotagmin | PI45P2 | C2 | Regulated exocytosis | Bai and Chapman (2003) |
| | | SCAMP2 | PI45P2 | Hydrophobic region | Regulated exocytosis | Liao *et al* (2007) |
| | | VAMP8 | PI3P | ND | Phagocytosis | Dai *et al* (2007) |
| | | Exophilin4/Slp-2 | PI45P2 | C2 | Regulated exocytosis | Yu *et al* (2007) |
| | | SNAP25/Syntaxin1A | PI45P2 | ND | Regulated exocytosis | Aoyagi *et al* (2005) |
| Actin and MT-based motors | | KIF1A | PI45P2 | PH | TGN–PM and synaptic vesicles from transport | Klopfenstein *et al* (2002) |
| | | KIF16B | PI3P, PI345P3 | PX | Sorting at endosomes | Hoepfner *et al* (2005) |
| | | GAKIN/KIF13B | PI345P3 | FHA | Neuronal vesicles transport | Horiguchi *et al* (2006) |
| | | Myosin VI | PI45P2 | ND | Golgi–PM and endocytic vesicle transport | Spudich *et al* (2007) |
| | | Myosin 1C | PI45P2 | PH | Glut4 trafficking and Golgi–PM trafficking | Hokanson *et al* (2006) |
| | | Myosin X | PI345P3 | PH | Phagocytosis | Cox *et al* (2002) |
| | | Dynein/dynactin | PI345P3 | ND | ER–Golgi and endosome–TGN transport | Watson *et al* (2005); Toyoshima *et al* (2007) |

RLD1, RCC1-like domain; ENTH, epsin N-terminal homology; ANTH, AP180 N-terminal homology; GAT, GGA and Tom1; PTB, phosphotyrosine-binding domain; PX, Phox homology; FHA, forkhead associated.
PI-binding proteins belonging to membrane trafficking machineries are shown according to their specific roles, together with their PI-binding modules and their PI-binding preference.

Camilli, 2006). The targets involved in this coordinating activity of PI45P2 during endocytosis belong to different classes of proteins. These include the actin network growth machinery proteins, including Cdc42, Arp2/3 and Abp1; some of the so-called endocytic accessory proteins, such as HIP1/HIP1R, which is recruited into growing coated structures and which connects the clathrin coat with actin filaments (Chen and Brodsky, 2005) and the actin-based myosin I and VI motors (Krendel *et al*, 2007; Spudich *et al*, 2007), which are believed to provide the required pulling force to drive membrane deformation and fission (Table I). The PIs are also involved in the subsequent cytoskeleton-driven endocytic steps, such as microtubule-dependent motility of endosomes, where the PIs act on the motors (e.g. KIF16B; Hoepfner *et al*, 2005) that mediate the plus-end-directed motility of early endosomes, and that are required for recycling and degradation pathways. Furthermore, the PIs appear to coordinate the cytoskeleton and membrane trafficking not only at the PM and endosomes but also at the Golgi complex, where they are involved in the assembly of a spectrin-based actin skeleton (De Matteis and Morrow, 1998), in synergising with Cdc42, NWASP and cortactin in Arp2/3-mediated actin nucleation, and in the control of the Golgi pool of myosin VI (Egea *et al*, 2006).

A further mechanism through which the PIs can affect the properties of cell membranes and have an impact in membrane trafficking has emerged recently with the demonstration that they can control the synthesis of the sphingolipids. This control is mediated through a family of lipid-transfer proteins that share a common domain organisation, and includes CERT and FAPP2 (De Matteis *et al*, 2007). Thus, these possess a closely homologous PH domain at their N terminus, and a distinct additional lipid-binding/transfer domain at their C terminus. Their homologous PH domains interact with PtdIms4-phosphate (PI4P) and the small GTPase Arf, and are responsible for the association of these lipid-transfer proteins with the Golgi complex. Their distinct lipid-transfer domains bind ceramide and glucosylceramide (GlcCer), for CERT and FAPP2, respectively (D'Angelo *et al*, 2008).

CERT mediates the non-vesicular transport of ceramide from the endoplasmic reticulum (ER), its site of synthesis, to the Golgi complex, where it is converted into sphingomyelin (SM). Consequently, defects in CERT inhibit SM synthesis (Hanada *et al*, 2003). Lowering the levels of PI4P also inhibits SM synthesis, through its impact on CERT recruitment/activity (Toth *et al*, 2006; D'Angelo *et al*, 2007).

FAPP2 is a GlcCer-transfer protein that is required for complex glycosphingolipid (GSL) synthesis (D'Angelo *et al*, 2007; Halter *et al*, 2007) due to its ability to transfer GlcCer from the cytosolic side of the early Golgi compartments to the late-Golgi compartments, where the GSL synthetic enzymes reside. This activity of FAPP2 is regulated by PI4P, and interfering with PI4P production inhibits GSL synthesis (D'Angelo *et al*, 2007).

## The PIs as integrators of signalling and membrane trafficking

The PIs have recognised roles in membrane trafficking also as transducers of PM receptor activation and of the general nutritional and stress status of the cell.

Well-known examples of the ability of the PIs to mediate the effects of PM receptor-initiated signalling cascades on membrane trafficking include clathrin-mediated endocytosis (Irie *et al*, 2005), phagocytosis (Stephens *et al*, 2002), macropinocytosis (Lanzetti *et al*, 2004), translocation of GLUT4 (Thong *et al*, 2005) and degranulation of mast cells (Ito *et al*, 2002). A more recent demonstration of how PM receptors regulate trafficking through the PIs relates to the involvement of the PI 4-phosphatase Sac1 in the stimulation of anterograde trafficking in the Golgi complex in response to growth factors (Blagoveshchenskaya *et al*, 2008). Sac1 accumulates at the Golgi complex in quiescent cells, where it 'consumes' the Golgi pool of PI4P, and by doing so, it downregulates anterograde trafficking. After stimulation by mitogens, Sac1 is phosphorylated and undergoes a transition from an oligomeric to a monomeric state of aggregation. As a monomer, Sac1 is relocated to the ER, resulting in an increase in PI4P levels at the Golgi complex, and consequently in the promotion of anterograde trafficking (Blagoveshchenskaya *et al*, 2008).

Local pools of the PIs can undergo significant changes in relation to nutrient availability and cell stress. In yeast, nutrient availability appears to strictly control the pool of PI4P at the Golgi complex, which is a key determinant in anterograde trafficking through and out of the Golgi complex (Strahl and Thorner, 2007). Interestingly, nutrient deprivation reduces the Golgi pool of PI4P (thus inducing a sort of quiescent state of the organelle) by two parallel but synergistic mechanisms: by releasing the PI 4-kinase (PI4K) Pik1p (the major source for PI4P at the Golgi complex) from the Golgi complex (Demmel *et al*, 2008); and by promoting the translocation of the PI 4-phosphatase Sac1p from the ER to the Golgi complex (Faulhammer *et al*, 2007).

With the PI response to cell stress, in their seminal paper identifying PtdIms 3,5-bisphosphate (PI35P2) as a novel endogenous PI species, Dove *et al* (1997) reported that PI35P2 levels increase by up to 30-fold in yeast cells subjected to hyperosmotic stress. This increase is mainly sustained by an increased production of PI35P2 through the PIP5K Fab1p (Cooke *et al*, 1998), with PI35P2 having a major role in controlling the size and shape of the vacuole (Dove and Johnson, 2007) and in mediating its fragmentation in response to hyperosmotic stress (Bonangelino *et al*, 2002). A remaining open question concerns the nature of the stress sensors that lead to this increase in PI35P2 levels. An intriguing possibility is that the PIP5K Fab1p might itself function as a stress sensor through its chaperonin-like domain (Dove and Johnson, 2007).

## The principles behind the organisation of PI metabolism: compartmentalisation and tight spatio-temporal control

A distinctive feature of the organisation of PI metabolism is its regionalisation, as opposed to the centralisation of most lipid biosynthetic pathways in the ER (and in the Golgi complex). For the PIs, the ER is just the site of synthesis of the common precursor, PtdIns, as all the subsequent steps of phosphorylation (and dephosphorylation) occur in practically all the other cell compartments through the PIKs and PI phosphatases (Figure 2). This regionalised organisation and the isoform specialisation of PI metabolism have manifold implications.

First, the kinds, levels and activities of the PIKs and PI phosphatases are different in each cellular compartment, meaning that at steady state, each of the seven PI species can maintain different concentrations across these compartments. This non-homogeneous distribution of the PI species in the cell has been 'visualised' using PI-binding protein modules that have distinct PI-binding profiles (De Matteis and Godi, 2004). With due caution deriving from an awareness of the limits of these tools (Roy and Levine, 2004; Lemmon, 2008), which can only detect the free pools of the PIs and which can have protein as well as PI targeting determinants (Godi *et al*, 2004; Lemmon, 2008), a distribution map of the PIs in the cell has been constructed. In this map, PI45P2 is enriched at the PM, PI4P at the Golgi complex, PI3P at the early endosomes, and PI3P and PI35P2 in the late endosomes. However, many 'deviations' from this main distribution map have been described as a more assorted series of tools have become available (Roy and Levine, 2004; Lemmon, 2008) and more accurate methods of detection are applied (Downes *et al*, 2003). Thus, it has been possible to obtain direct visualisation and functional data in favour of the presence of PI45P2 in the Golgi complex (Watt *et al*, 2002), of PI4P at the PM (Roy and Levine, 2004) and at the micropexophagy-specific membrane apparatus (Yamashita *et al*, 2007), of PI3P at the PM (Falasca *et al*, 2007; Lodhi *et al*, 2008) and of PI35P2 in secretory granules (Osborne *et al*, 2008).

A question raised by this apparent spatial segregation of the PI species into different and distant compartments is how the global PI homoeostasis is maintained across the cell. Different possibilities can be envisaged here. One is that the PI homoeostasis is maintained due to intense communication of the different PI pools through vesicular trafficking. This would establish a sort of bidirectional profitable relationship, where the PIs are used as organisers of membrane trafficking, and membrane trafficking serves PI metabolism by ensuring the delivery of PI substrates generated in a given compartment to their metabolic enzymes located in a distant, but communicating, one. Another possibility is that in spite of hosting an apparently predominant PI species, each compartment is in fact self sufficient in sustaining an autonomous phosphorylation–dephosphorylation PI cycle and thus contains different PI species at the same time at steady state.

These two possibilities are not mutually exclusive and are indeed both pursued with the differently located PI enzyme isoforms that are involved in the generation of the same PI product in a given cell compartment depending on the cell or environmental conditions. This has been shown recently for the origin of PI4P as a precursor for PI45P2 at the PM: this is different under steady-state and stimulated conditions, whereby for the former it arises from the Golgi-complex-localised PI4Ks, PI4KIIα and IIIβ, whereas in angiotensin-II-stimulated cells it arises mainly from PI4KIIIα (Balla *et al*, 2008). Interestingly, although it cannot be excluded that a small fraction of PI4KIIIα relocalises to the PM upon receptor stimulation, the majority of this PI4K isoform resides in the ER. This prompts the speculation that at least under these circumstances, the generation of PI4P at the PM might occur at the sites of close apposition between the ER and the PM (the ER–PM contact sites; Levine and Loewen, 2006). This is an additional and intriguing possibility for PI metabolic reactions that has been shown to occur for other lipid metabolic pathways, such as phosphatidylserine–phosphatidylethanolamine conversion at the level of the ER–mitochondria membrane contact sites (Shiao *et al*, 1998).

The ability of the PIs to serve the multiple and dynamic functions in membrane trafficking mentioned above is due to the tight spatial and temporal control of their generation/consumption, that is, of the PIKs and PI phosphatases. These are usually cytosolic enzymes that are timely and precisely recruited to sites that are actively involved in trafficking events, through their interactions with key components of the molecular machineries that control or carry out specific transport steps. These include the small GTPases, coat/adaptors and the fissioning machinery in particular (Table II). Interestingly, these same classes of molecules are also often targets of the PIs (Table II). Thus, the interactions of the PI-metabolising enzymes with components of the trafficking

**Table II** Interaction between PI-metabolising enzymes and components of membrane trafficking machineries

| | Trafficking machinery components | PI enzymes | References |
|---|---|---|---|
| Small GTPases | Rab5 | PI3KC2A, B, G<br>PIK3C3<br>INPP4A<br>INPP5B<br>OCRL1 | Christoforidis *et al* (1999)<br>Shin *et al* (2005)<br>Hyvola *et al* (2006) |
| | Rab7 | PIK3C3 | Stein *et al* (2003) |
| Accessory proteins | ARF1 | PIP5K1<br>PI4KB<br>OCRL | Godi *et al* (1999)<br>Lichter-Konecki *et al* (2006)<br>Santarius *et al* (2006) |
| | ARF6 | PIP5K1A, B, C<br>OCRL | Lichter-Konecki *et al* (2006)<br>Santarius *et al* (2006) |
| Coats and adaptors | Clathrin | PI3KC2A, B | Gaidarov *et al* (2001); Wheeler and Domin (2006) |
| | AP2 | PIP5K1C | Krauss *et al* (2006) |
| | AP3 | PI4K2A | Craige *et al* (2008) |
| Sorting | SNX9 | PIP5K1A, B, C | Shin *et al* (2008) |
| Fission | PKD | PI4KB<br>PIP4K2A<br>PIK3C3<br>PIP5K1<br>PI4K2 | Nishikawa *et al* (1998)<br>Hausser *et al* (2005)<br>Hinchliffe *et al* (2006) |

Components of molecular machineries involved in different steps of membrane trafficking that control the localisation and/or activity of the PI-metabolising enzymes.

machinery constitute a way not only for recruiting these enzymes to specific cellular compartments but also to effectively channel specific PIs to their effectors and to sustain positive or negative feedback if the recruited enzymes produce or consume, respectively, the PI species that interacts with the effector. The best studied examples of GTPases that control the PI-metabolising enzymes are those of Rab5 and the Arfs. Rab5 seems to be a key controller and coordinator of the PIKs and PI phosphatases in the endocytic pathway, as it binds and stimulates type III PI3K, type I PI3Kβ, type I PI 4-phosphatase and the INPP5B and OCRL 5-phosphatases (Shin *et al*, 2005), whereas both Arf1 and Arf6 can recruit and stimulate PIP5K (Santarius *et al*, 2006), with Arf1 also recruiting and activating PI4KIIIβ on the Golgi complex (Godi *et al*, 1999).

## The open questions on substrate channelling, functional redundancy and the general coordination of the PI-metabolising enzymes

A peculiar feature of the PI system is the coexistence of pathways that are both divergent (where the same PI species is subjected to different and alternative modifications) and convergent (where the same PI species is produced through different routes). These features pose two important questions for which only partial answers are at present available: (i) what are the mechanisms through which a given PI species is channelled towards one of its different possible products? and (ii) to what extent are the alternative pathways leading to the same end product redundant or 'dedicated' to different functions?

The answer to the issue of substrate channelling towards a given product will need to come from a consideration of the regionalised distribution of the different PI-metabolising enzymes that determines that the same PI species can have distinct destinies in different locations (Figure 2). This could thus explain, for instance, why PtdIns is mainly converted to PI4P in the Golgi complex and into PI3P in the endosomes, whereby PI4K and PI3K are differentially enriched in these two compartments.

However, in many instances, different enzymes acting on the same substrate coexist in the same compartment (Figure 2), and together with the highly diffusible nature of the PIs as substrates; this means that the spatial segregation argument cannot be applied here.

A solution that appears to be pursued in some cases is the preassembly of multi-enzyme complexes, which are generally centred on regulatory/scaffold components. One of these complexes controls the turnover of PI3P at the endosomes, and includes a PI3K (Vps34), a PIP 3-phosphatase (MTM1) and a regulatory component (Vps15) (Cao *et al*, 2007). Another complex controls PI35P2 synthesis and turnover in MVBs and contains the PIP5K PIKfyve, the PIP 5-phosphatase Sac3 (a Fig4 homologue), and the regulatory component ArPIKfyve (homologue of Vac14) (Sbrissa *et al*, 2007). Other examples of multi-enzyme complexes in this context include a complex isolated from platelets that contains PIP 5-phosphatases and 4-phosphatases and PI 3-kinases (Munday *et al*, 1999), and a complex orchestrated by Rab5 that includes type I PI3K, PIP 4-phosphatase and PIP 5-phosphatase (Shin *et al*, 2005).

Interestingly, this latter complex has been shown to sustain one of the two convergent pathways that leads to the production of PI3P in the endosomal membranes: one which proceeds through the two-step dephosphorylation of PI345P3 in positions 5 and 4, as opposed to the one which is based on the direct phosphorylation of PIs by type III PI3K (Munday *et al*, 1999; Shin *et al*, 2005). The production of PI45P2 can also proceed through two convergent pathways: either by 5-phosphorylation of PI4P (by PIP5K1; Figure 1) or by 4-phosphorylation of PI5P (by PIP4KII; Figure 1).

A question that arises from the existence of convergent pathways and of different enzyme isoforms (Figure 1) is to what extent these pathways or enzyme isoforms are functionally redundant or generate 'specialised' and distinct pools of the PIs. This is a question that has not been systematically and quantitatively addressed, and for which the solution will be of extraordinary importance to exploit this, even if limited, functional redundancy as a target in the treatment of diseases linked to genetic defects of single PI-metabolising enzymes (see below).

An aspect that has remained little explored to date relates to the coordination of the PI enzymatic activities at both the local and the global cellular levels. Locally, the small GTPases have key roles, as they function as recruiters and timers for the PI-metabolising enzymes (Table II), and in selected cases (such as for Rab5), they can also coordinate multiple enzymatic activities. Another level of local homoeostatic control appears to be intrinsic to the structures of the enzymes themselves (Figure 1), as in addition to their catalytic domain, some of these possess additional PI-binding domains with good affinities for their substrate. This is the case, for instance, of the 3-phosphatase MTMR4, which has a FYVE domain that binds PI3P and that contributes both to its recruitment and to its release once the substrate has been removed by the enzyme itself (Lorenzo *et al*, 2006), or of MTMR2, which is recruited, through its PH-GRAM domain, to PI35P2-containing vesicles (Berger *et al*, 2003). A special case is seen with PI35P2 synthesis in the vacuole in yeast: here, Fig4 not only has 5-phosphatase activity towards PI35P2 but also appears to activate the PIP5K Fab1 kinase that synthesises PI35P2 from PI3P (Duex *et al*, 2006). Thus, Fig4 regulates both the turnover and the synthesis of PI35P2. A similar dual regulation might be very effective in ensuring that PI35P2 undergoes continual production that is balanced by continual consumption, and thus promoting an elevated flux of PI35P2 through a phosphorylation–dephosphorylation cycle. An elevated PI35P2 flux is an elegant way to increase the local availability (without raising the absolute levels) of PI35P2 and to reduce its diffusion.

Another way to exploit the coordination of the activities of the PIKs and PI phosphatases is to subject them to common regulation through phosphorylation–dephosphorylation cycles through shared protein kinases and phosphatases. This is the case for PI45P2 synthesis and turnover at the synapse, which are both inhibited through Cdk5-mediated phosphorylation and inactivation of PIP5KC and synaptojanin, and are activated by calcineurin-mediated dephosphorylation of both PIP5K and synaptojanin (Lee *et al*, 2004).

At present, we have scattered examples of the mechanisms that coordinate the local activities of the different

PI-metabolising enzymes. However, we have no real clues as to the existence of a more general plan for the coordination and specific combination of the expression and/or interactions in the cell of given PI-metabolising enzyme isoforms that function along the same or alternative PI-metabolising branches. Deciphering such a plan will be important for the unravelling of the 'real' physiological roles of apparently equivalent pathways that can operate through the coupling of different enzyme isoforms. Similarly, this will enable us to determine the tissue specificities of the different pathways, and to identify the pathopathways underlying the diseases that derive from genetic defects in single PI-metabolising enzymes and the drug targets for the treatment for these diseases (see below).

Help towards the answering these questions may well arise from the application of a systems biology approach to the study of the PI-metabolising enzymes. By combining and completing the data for the expression profiles and protein interactions of these enzymes (and of the PI targets), this approach should arrive at the definition of the 'interactome' of the PI system. An example of an analysis of co-expression profiles of PI-metabolising enzymes is given in Figure 3. By centring the analysis on 3-phosphatases and 5-phosphatases and selecting the genes for PI-metabolising enzymes that are among the most significantly co-expressed genes, a number of PI-enzyme clusters emerge. It turns out, for instance, that the *OCRL* gene, which is responsible for Lowe syndrome (see below), is part of a highly interconnected gene cluster that comprises *PIP5K1A*, *INPP5A*, *PI3KC2A* and *MTMR1*. Thus, the expression of two 5-phosphatases, OCRL and INPP5A (a type I 5-phosphatase acting exclusively on soluble inositol phosphates and involved in inositol 1,4,5-trisphosphate removal), seems to be coordinated with that of a PI45P2-synthesising enzyme, specifically *PIP5K1A*. This co-expression profile might represent the basis for homoeostatic control of PI45P2 and inositol 1,4,5-trisphosphate levels, and might provide an explanation for the calcium-signalling imbalance that occurs when PI45P2 levels increase due to a mutation in *OCRL* (Suchy *et al*, 2005). Furthermore, the significant co-expression of *OCRL* with *MTMR1* suggests a possible role for OCRL in the control of the endocytic PI3P pool.

Although limited, the example given shows the power of such an approach for the uncovering of unsuspected couplings between specific isoforms of the PI-metabolising enzymes, for the provision of candidate interactors and for the delineation of novel pathways of communication

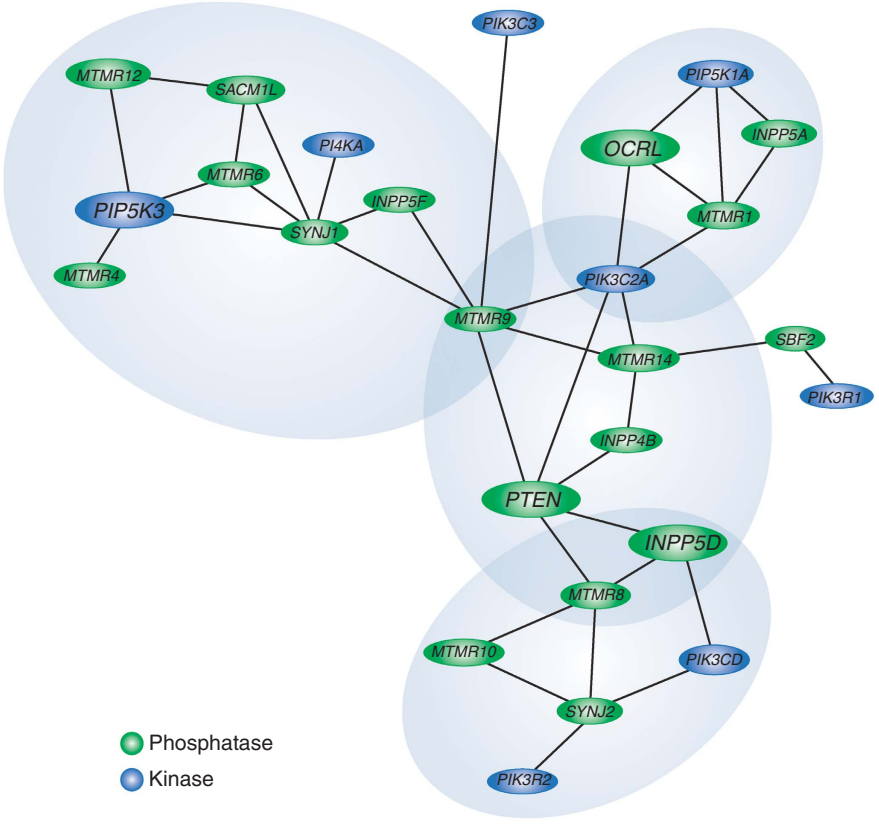



**Figure 3** Co-expression network of the PI-metabolising enzymes. Co-expression network of the PI-metabolising enzymes (PIKs, blue ellipses; PI phosphatases, green ellipses; as listed in Figure 1), focused on the 3- and 5-phosphatases. The analysis was performed using the COXPRESdb database of gene expression profiles from a variety of normal and pathological conditions (from a total of 123 experiments; Obayashi *et al*, 2008 no. 22). Here, using the 3- and 5-phosphatase genes as individual queries and analysing the most significantly co-expressed genes (according to the weighted Pearson's correlation coefficient between gene probes, and choosing 0.4 as a threshold; Obayashi *et al*, 2008), we selected specifically for the genes for PI-metabolising enzymes. Groups of genes extensively connected in the network represent co-expressed gene clusters that are likely to be involved in common cell functions. The 3- and 5-phosphatase genes responsible for genetic diseases (see Figure 1) are highlighted in bold. Four interconnected enzyme clusters emerge from the analysis, each containing a disease gene (see Figure 1). In particular, *OCRL* gene is part of a cluster that also comprises *PIP5K1A*, *INPP5A* and *MTMR1*.

between different branches of PI metabolism, for which their relevance can and will have to be explored experimentally.

## What we can learn from the genetic diseases of the PI system?

The importance of maintaining a tight balance between the activities of the various PI-metabolising enzymes is highlighted by the severe consequences arising from defects in PI metabolism. The pivotal roles of PI345P3 dysmetabolism in cancer, inflammation and diabetes are well established (Cantley, 2004; Wymann and Marone, 2005), to the point where the PI3Ks and the PI345P3 phosphatases PTEN and SHIP have become attractive targets for the development of novel pharmacological agents (Workman, 2004; Lazar and Saltiel, 2006; Ruckle *et al*, 2006; Zhao, 2007).

For monogenic diseases that have been linked to defects in PI-metabolising enzymes, these represent a heterogeneous group of conditions, many of which affect the nervous system (both central and peripheral; such as lethal contractural syndrome, Lowe syndrome and Marie–Charcot–Tooth), with others affecting muscle (myopathy), eye (fleck corneal dystrophy, Lowe syndrome) and kidney (Lowe syndrome) (Figure 1). In the majority of cases, we do not understand in depth the links connecting the genetic defects with the clinical manifestations of these diseases. A lot of help in this direction should come from consideration of pathological states with similar clinical pictures, as it has now been shown that diseases with overlapping clinical manifestations can be caused by mutations in different genes that are part of the same functional module. In such instances, the clinical overlap can be attributed to defects in individual genes that render the entire module dysfunctional. Analyses involving model organisms, and more recently humans, have also shown that direct and indirect interactions often occur between protein pairs that are responsible for similar phenotypes (Oti, 2007). Recently, this concept has been successfully applied and exploited to identify and experimentally confirm the relationships between genes involved in various inherited ataxias that all share a dysfunctional state of the Purkinje cells (Lim *et al*, 2006).

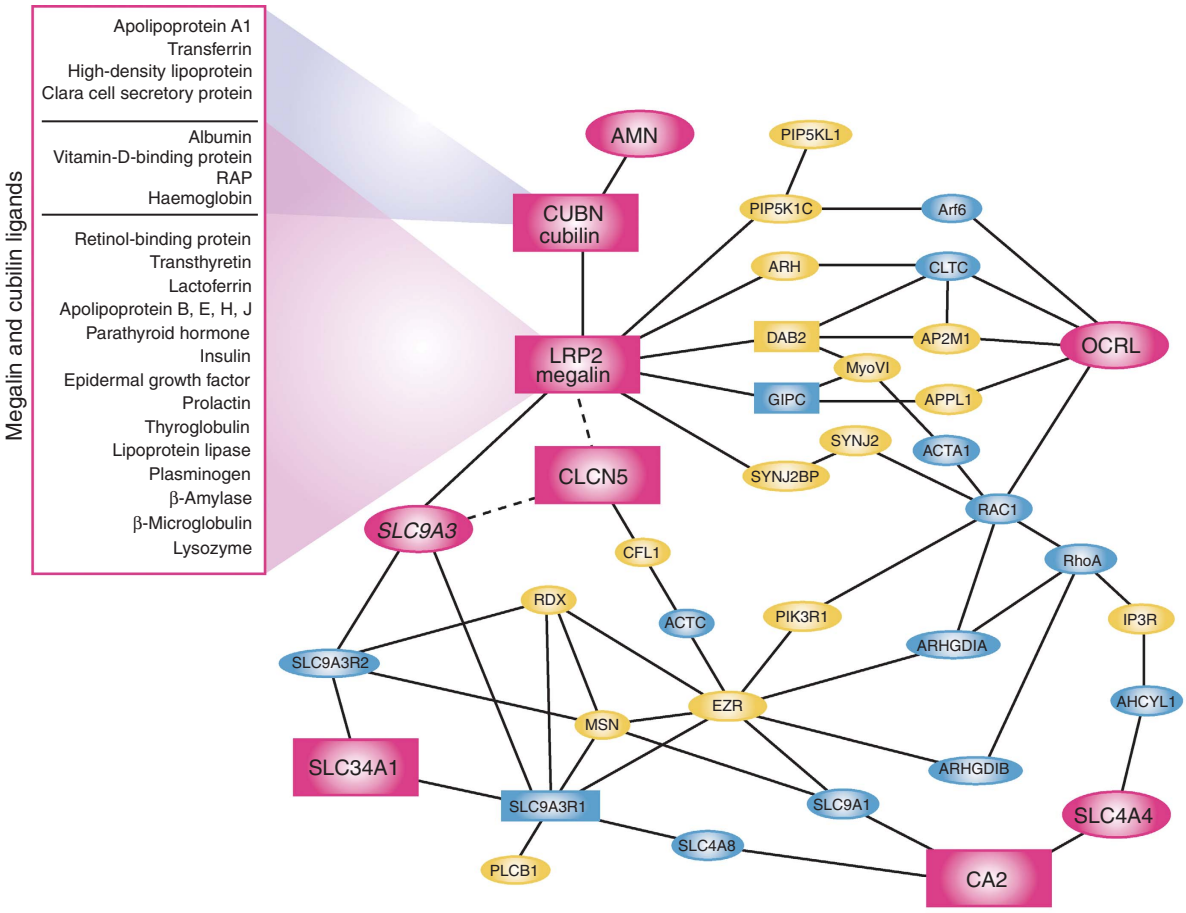
Figure 4 shows the results of a similar approach (based on the available data) as it might be applied to one of the main manifestations of Lowe syndrome: a dysfunction of the kidney proximal tubule cells (PTCs) that is responsible for the loss of salts (including bicarbonates) and low molecular weight (LMW) proteins with the urine (Igarashi *et al*, 2002; Christensen and Gburek, 2004). This proximal tubular acidosis and LMW proteinuria are pathognomic of the renal Fanconi syndrome, which is common to a series of genetic defects that involve intracellular chloride channels (ClC5 in Dent syndrome) and ion transporters or exchangers (SLC9A3, SLC34A1 and SLC4A4). In some cases, the extent of the overlap of clinical signs is such that the conditions cannot be distinguished: this is the case for patients who have been clinically diagnosed as affected by Dent disease, but have then been found to carry mutations in OCRL (Attree *et al*, 1992; Hoopes *et al*, 2005). Building up a comprehensive network including the mouse genes where a knockout causes protein and/or salt urinary loss (Figure 4, rectangles), and the interactors of the human gene products that show the defects causing renal Fanconi syndrome (Figure 4, large ellipses) highlight the molecular pathways that are involved in protein and salt reabsorption by PTCs and provide a range of testable candidates as possible OCRL interactors and/or PI45P2-binding proteins (yellow ellipses) with proven physiological roles in PTCs (Figure 4). A key node in the network illustrated in Figure 4 is megalin (LRP2), a member of the LDL receptor family that is highly expressed in the brush border of PTCs, and that functions together with cubilin (CBN) as a multiligand receptor that mediates the capture and resorption of the LMW proteins present in the ultrafiltrate (indicated as megalin ligands in Figure 4) (Igarashi *et al*, 2002; Christensen and Gburek, 2004). Megalin and cubilin continuously cycle between the apical PM, and the early/recycling endosomes, with delivery of luminal ligands to the lysosomal compartments, escorted by PI-binding proteins, such as Dab2 (Disabled-2) and ARH (autosomal recessive hypercholesterolaemia). A defect in megalin recycling has been shown to be at the origin of Dent disease (Piwon *et al*, 2000), and has been hypothesised to contribute to urinary protein loss also in Lowe syndrome (Norden *et al*, 2002; Lowe, 2005). This hypothesis has been experimentally reinforced recently by the demonstration that OCRL interacts with APPL1, which in turn binds GIPC, an interactor of megalin (Erdmann *et al*, 2007).

Finally, introducing a similar phenome analysis in our PI studies will not only allow us to delineate candidate pathopathways for pharmacological intervention in the genetic defects of the PI-metabolising enzymes, but when integrated with the interaction network of these PI-metabolising enzymes, it will also greatly improve our overall understanding of the global organisation of PI metabolism and of the real physio-pathological relevance of its numerous branches.

## Conclusions and perspectives

Through work carried out over the last decade on yeast and mammalian cell models in many laboratories, we have now reached a deep state of knowledge of the molecular details of the regulation and roles of several of the PI-metabolising enzymes. However, we are still missing the overall picture of the global functional organisation and coordination of the PI system. An answer to this problem should arise from the computational integration of phenotypic data (derived from genetic diseases and knockout mice) with a high-confidence interaction network of the PI-metabolising enzymes and their regulators and effectors; thus from a combined PI-phenome-interactome network.

Despite these many residual uncertainties, we are, however, also convinced that it is time to translate the many research discoveries of these past years towards the development of pharmacological treatments for genetic diseases that involve the PI-metabolising enzymes. The main considerations here are three-fold: first, powerful drug discovery technologies are now available; second, the diseases that are related to the PI-metabolising enzymes fulfil the general criteria for drug discovery as they originate from defects within molecular pathways where it is possible to identify 'drugable' targets and third, some of these PI-metabolising enzymes (e.g. PI3K, SHIP and PTEN) are indeed already drug targets and have inspired the development of highly specific inhibitors. The legitimate conclusion that we reach is that the




| Gene | OMIM |
|---|---|
| AMN (amnionless homologue) | #261100; megaloblastic anemia 1 with proteinuria |
| LRP2 (megalin) | #227920; Facio-oculo-acoustico-renal syndrome with proteinuria |
| CUBN (cubilin) | #261100; megaloblastic anemia 1 with proteinuria |
| CA2 (carbonic anhydrase 2) | *611492; osteopetrosis with renal tubular acidosis |
| CLCN5 (chloride channel 5) | *300008; Dent disease 1, hypercalciuria, hyperphosphaturia, proteinuria |
| OCRL (oculocerebrorenal syndrome of Lowe) | #309000; Lowe syndrome, Dent disease 2 |
| SLC4A4 (solute carrier family 4, member 4) | *603345; renal tubular acidosis, proximal, with ocular abnormalities and mental retardation |
| *SLC9A3* (solute carrier family 9, member 3) | *182307; candidate gene for autosomal dominant proximal renal tubular acidosis (Igarashi *et al*, 2002) |
| SLC34A1 (solute carrier family 34, member 1) | +182309; urolithiasis, hypophosphatemic, osteoporosis |

**Figure 4** Interaction map of the products of the genes responsible or candidate for disorders of kidney proximal tubule cells (PTCs). The genes responsible or strong candidate for diseases due to dysfunction of kidney PTCs are shown (large red ellipses), together with selected interactors, including genes for which the knock out causes proteinuria and tubular acidosis in mice (rectangles). The interaction map was generated using Osprey (powered by Human GRID, General Repository of Interaction Datasets). The components of the network that are active on or sensitive to PI45P2 (the substrate of the 5-phosphatase OCRL) are marked in yellow. LRP2 encodes for megalin, a multiligand receptor that, together with cubilin (CBN), is responsible for the resorption of the indicated LMW proteins by PTCs (Igarashi *et al*, 2002; Christensen and Gburek, 2004). ARH, autosomal recessive hypercholesterolaemia; DAB2, disabled homologue 2; GIPC, GAIP C terminus interacting protein; PIP5K1C, phosphatidylinositol-4-phosphate 5-kinase type I gamma; SYNJ2BP, synaptojanin 2-binding protein; CLTC, clathrin heavy chain 1; AP2M1, clathrin coat assembly protein AP50, Myo VI, myosin 6, APPL1, adapter protein containing PH domain, PTB domain and leucine zipper motif 1; Arf6, ADP ribosylation factor 6; PIP5KL1, phosphatidylinositol-4-phosphate 5-kinase-like 1; SYNJ2, synaptojanin-2; RAC1, Ras-related C3 botulinum toxin substrate 1; RhoA, transforming protein RhoA; IP3R, inositol 1,4,5-trisphosphate receptor type 1; AHCYL1, putative adenosylhomocysteinase 2; SLC9A1, Na/H exchanger 1 (NHE-1) (solute carrier family 9 member 1); ARHGDIA, Rho GDP dissociation inhibitor 1 (Rho GDI 1); ARHGDIB, Rho GDP dissociation inhibitor 2 (Rho GDI 2); PIK3R1, phosphatidylinositol 3-kinase regulatory alpha subunit; EZR, Ezrin; SLC4A8, solute carrier family 4, sodium bicarbonate cotransporter, member 8; SLC9A3R1, Na(+)/H(+) exchange regulatory cofactor NHE-RF) (NHERF-1); SLC9A3R2, Na(+)/H(+) exchange regulatory cofactor NHE-RF2 (NHERF-2); PLCB1, phospholipase C-beta-1; ACTA1, alpha actin 1; ACTC, alpha cardiac actin.

time for a pharmacological approach to genetic diseases involving the PI cycle is ripe. Therefore, greater efforts now need to be put into the exploitation of our basic knowledge for the identification and validation of drug targets.

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