Mutations in Phosphoinositide Metabolizing Enzymes and Human Disease

Phosphoinositides are implicated in the regulation of a wide variety of cellular functions. Their importance in cellular and organismal physiology is underscored by the growing number of human diseases linked to perturbation of kinases and phosphatases that catalyze interconversion from one phosphoinositide to another. Many such enzymes are attractive targets for therapeutic interventions. Here, we review diseases linked to inherited or somatic mutations of these enzymes.

Phosphatidylinositol (PtdIns), a membrane phospholipid, can be reversibly phosphorylated at the 3, 4, and 5 positions of the inositol ring to generate seven phosphoinositides [PI(3,4,5)P3, PIP2, PIP3, PI(4,5)P2, PI(3,4)P2, and PI(3,5)P2] (FIGURE 1A). The importance of this metabolism in cell regulation was first established in the context of studies on stimulus-secretion coupling. It was found that many stimuli that trigger secretion also trigger enhanced turnover of PtdIns and phosphoinositides (42). Subsequently, it became clear that phospholipase C-dependent hydrolysis of PI(4,5)P2 generates the second messenger molecules diacyl glycerol and Ino(1,4,5)P3 (IP3), a mechanism through which many cell surface receptors, including many receptors that stimulate secretion, transduce their signals (10). Diacyl glycerol binds and regulates protein kinase C and a variety of other effectors, whereas IP3 triggers calcium release from the endoplasmic reticulum (10, 42). In another signal transduction pathway, PI(4,5)P2 is cleaved by phospholipase A2 to generate arachidonic acid, a precursor of many signaling molecules.

More recently, PI(4,5)P2 and the other phosphoinositides, which are all concentrated on the "cytosolic" leaflet of membrane bilayers, have been found to be important in their own right (24). Their phosphorylation and their cytosolic localization at the plasma membrane is ensured by a combination of "dual key" mechanisms (7), including a coincident localization at the plasma membrane, a major function of PI4P is to act as a precursor of actin nucleation (104). Its selective localization at the plasma membrane is ensured by the concentration of PI4P-5-kinases (type I PIP kinases) in this membrane and by the tight coupling between endocytosis and its diphosphorylation by inositol 5-phosphatases (19, 25, 53, 86, 101).

The site of synthesis of PtdIns in the endoplasmic reticulum, from which this phospholipid is exported to other membranes either via membrane traffic or via cytosolic phospholipid transfer proteins. Phosphorylation of PtdIns to PIP occurs primarily in the Golgi complex and at the plasma membrane. In the Golgi complex, PIP plays an important role in the biogenesis of transport vesicles via the recruitment of coat proteins and of their accessory factors (7, 21, 98). At the plasma membrane, a major function of PIP is to act as precursor of PI(4,5)P2, a phosphoinositide predominantly localized in this membrane. PI(4,5)P2 binds and regulates a wide array of proteins that function at the cell surface and serves as a precursor of second messengers. In addition to these functions, PI(4,5)P2 contributes to the secretory machinery by recruiting clathrin coats and other endocytic factors (111), and PI binds actin regulatory proteins, thus functioning as a cofactor for actin nucleation (104).

The seven phosphoinositides, which are heterogeneous in the control of nuclear function and nucleic acid biology have also been reported (32, 33, 103, 105).

Within the physiological context, the predominant roles of these enzymes are generally growth factor signaling and cell survival. In addition to these functions, PI(4,5)P2 contributes to the secretory machinery by recruiting clathrin coats and other endocytic factors (111), and 3′/5′-adenosine monophosphate (cAMP) is a ligand for several members of the superfamily, to mediate specificity of membrane interactions. In many cases, they function as co-receptors together with membrane proteins in the recruitment of cytosolic proteins. This ensures, via a coincidence detection mechanism (dual key mechanism), that pairing of a membrane protein with a cytosolic protein only occurs when the membrane protein reaches the compartment defined by the presence of a specific phosphoinositide (7, 24, 99). Phosphoinositide levels are tightly regulated spatially and temporally by the action of numerous kinases and phosphatases, which add or cleave phosphate groups at specific positions of the inositol ring, as well as by phosphoinositases (FIGURE 1B). The differential localization of each of these enzymes on specific membranes ensures maintenance of the heterogeneous distribution of phosphoinositides despite the continuous membrane flow from one compartment to another (FIGURE 2, A AND B).
Within the plasma membrane, PI(4,5)P2 can be further phosphorylated by PI3-kinases to PI(3,4,5)P3, another phosphoinositide with key signaling functions (13, 20, 50). A major role of PI(3,4,5)P3 is to stimulate cell survival and proliferation (13). Levels of PI(3,4,5)P3 are generally low but can undergo a rapid surge on growth factor stimulation. This increase is rapidly terminated by inositol 3- and 5-phosphatases with different signaling outcomes. The inositol 3-phosphatase PTEN reverses the reaction and regenerates PI(4,5)P2 (57). In contrast, inositol 5-phosphatases convert PI(3,4,5)P3 into a phosphoinositide, PI(3,4)P2, which contributes to propagation of the signals initiated by PI(3,4,5)P3 (58). PI(3,4)P2 is then further dephosphorylated in the endocytic pathway by inositol 4-phosphatases to PI3P, the signature PI of early endosomes and a ligand for a large number of endosomal proteins (85). The bulk of PI3P, however, is generated directly on endosomes by the phosphorylation of PI at the three positions (66, 83, 107). Subsequently, phosphorylation of PI3P to PI(3,5)P2 on endosomes is thought to generate docking sites for the recruitment of cytosolic factors that control outgoing traffic from early endosomes (63). The localization of PI5P, a low abundance PI species that can be generated by multiple pathways (FIGURE 1B), remains unclear (71).

The importance of proper phosphoinositide metabolism in cell function is emphasized by the many diseases that have been shown to result from mutations in genes encoding phosphoinositide metabolizing enzymes (FIGURE 1B). Additionally, mutations in several enzymes that have not yet been linked to human disease show striking phenotypes in animal models. The key role of these enzymes is further underscored by the existence of bacteria whose genomes encode phosphoinositide metabolizing enzymes that are injected into the cytoplasm of the host cell and are required for pathogenicity (37, 41). Human diseases resulting from germline or somatic mutations in genes encoding these enzymes are discussed below.

**Lowe Syndrome and Dent Disease**

Lowe Syndrome (also known as Oculocerebrorenal Syndrome of Lowe) is caused by mutations in an inositol-5-phosphatase, which was named OCRL after the initials of the name of the syndrome (3). Lowe Syndrome is an X-linked disorder seen in ~1 in 200,000 births. Affected boys have bilateral congenital cataracts, mental retardation, neonatal hypotonia, and renal Fanconi Syndrome, a disorder characterized by reabsorption defects in the kidney proximal tubule. Mutations in OCRL were also recently shown to be responsible for a subset of cases of Dent Disease, another human X-linked renal disorder characterized by reabsorption defects similar to those observed in Lowe Syndrome (43). Dent Disease patients with mutations in OCRL exhibit none of the neurological or ophthalmological symptoms of Lowe Syndrome. In addition, although the renal manifestations of Dent Disease and Lowe Syndrome are similar, they are not identical. There are no obvious differences in the pattern of mutations that cause Lowe Syndrome and those that cause Dent Disease, although, so far, no...
single mutation has been shown to be responsible for both conditions (43). It remains to be seen whether patterns will emerge from the analysis of additional mutations or whether a patient’s genetic background determines which disorder is present. The importance of genes that compensate for OCRL function in either specific tissues or in the entire organism is underscored by the finding that, although mutations in OCRL cause Lowe Syndrome in human patients, OCRL knockout mice do not have an apparent pathological phenotype (46). INPP5B, a close homolog of OCRL, may compensate for the absence of OCRL since a double knockout of both proteins in mouse results in embryonic lethality (46).

OCRL has multiple localizations in cells, being concentrated in the Golgi complex, on endosomes, at endocytic clathrin coated pits, and at plasma membrane ruffles (17, 28, 32, 34, 44, 68, 94). Its preferred substrates in vitro are PI(4,5)P₂ and PI(3,4,5)P₃, the two phosphoinositides predominantly localized at the cell surface (82, 109). Accordingly, levels of PI(4,5)P₂ are higher in fibroblasts of Lowe Syndrome patients than in controls (100). One of the proposed functions of OCRL is to couple endocytosis to the dephosphorylation of these two phosphoinositides, although OCRL may have an additional function in preventing accumulation of 5-phosphorylated phosphoinositides on internal membranes (32). Disease-causing mutations abolish protein expression, impair catalytic activity (missense mutations cluster primarily in the 5-phosphatase domain) (32), or abolish role in targetin...
endosomes, at plasma mem-

brane PI(3,4,5)P3, the two

phosphorylation of

or abolish protein-protein interactions that play a

role in targeting the protein to its sites of action (muta-
tions in the COOH-terminal region) (61).

The mechanisms through which a defect in OCRL

function produces the phenotypic manifestation of

Lowe Syndrome remain unclear. An attractive working

hypothesis, supported by the interaction of OCRL with

many endocytic proteins such as clathrin, the endocyt-

otic clathrin adaptor AP-2, Rab5, and the adaptor protein

APP1L, is that defective OCRL function may result in a

defect in endocytosis and membrane recycling (32, 44, 94).

For example, APP1L, via an interaction with the

endocytic adaptor GIPC, links OCRL to endocytosis of the

TrkA receptor in brain (which could account for mental

retardation) and to endocytosis of the scar-

evenger receptor megalin in the kidney and brain (which

could account for the abnormal reabsorption of low

molecular weight proteins in kidney, a defect present

in Lowe Syndrome and Dent Disease, and mental

retardation) (32). Interestingly, patients with Donnai-

Barrow Syndrome and Facio-oculo-acoustico-renal

Syndrome, two syndromes that share some features

with Lowe Syndrome, are caused by mutations in

LRP2, the gene that encodes megalin (49).

Since OCRL can dephosphorylate PI(3,4,5)P3, a

potential abnormality of PI(4,5)P2 signaling in Lowe

Syndrome should also be explored. Further insight

into the role of OCRL may come from studies of the

other gene implicated in Dent Disease, the CHS3 gene

(45). CHS3 encodes a chloride channel whose function

is thought to be critical for protein sorting in endo-

somes (70), thus supporting the hypothesis that Lowe

Syndrome and Dent Disease result from abnormal

traffic in the endocytic pathway.

Lethal Congenital

Contractions

An inactivating mutation in PIP5K1C, the gene encod-
ing PIP kinase type 1γ (PIPK1γ), was recently found to

be responsible for lethal contractual syndrome type 3

(LCCS3) (65). Of the three type 1 PIP kinases encoded

by the human genome, i.e., the three PIP4K 5-kinases

that account for the bulk of PI(4,5)P2 production (27),

PIPK1γ is the one expressed at highest concentration

in the nervous system (25, 101). Lethal congenital con-

tractions syndromes are a severe form of arthrogrypo-

sis multiplex congenita (AMC), a group of diseases

that share the common feature of congenital progres-
sive joint contractures. LCCS3, an autosomal rece-

sive LCCS, is characterized by severe multiple

joint contractures with muscle wasting and atrophy

(65). Those patients that were carried to term died of

respiratory failure within minutes to hours after birth.

PIPK1γ accounts for the bulk of PI(4,5)P2 production

in brain and plays a critical role in neuronal function

and synaptic transmission. However, it remains to be

confirmed that nervous system dysfunction plays a

primary role in the LCCS3 phenotype, since PIPK1γ

also plays important roles outside the brain, for exam-

ple in cell adhesion, cell-cell interaction, and cell

migration (26, 28, 54, 90). It also remains to be estab-

lished why a homozygous disrupting mutation of

PIPK1γ in mouse leading to absence of the protein did

not produce similar joint contractures and muscle

wasting, although even in this species it produced

early postnatal lethality (25). Note that another PIPK1γ

KO mouse generated by random insertional mutagen-

esis exhibits embryonic lethality at midgestation, the

reason for this discrepancy is not known (97).

Myopathy

The myotubular family of proteins (myotubularin and

myotubularin-related proteins, MTM and MTMR

proteins) are inositol 3-phosphatases that dephospho-

rylate PI3P and PI(3,5)P2. The myotubularin family

also comprises catalytically inactive members, which

are thought to help regulate the active members (72,

93). Several myotubularin family members have been

implicated in disease.

Mutations in myotubularin 1 (MTM1) cause X-

linked myotubular myopathy. This disease, which

affects 1 in 50,000 newborn males, is the most severe

form of centronuclear myopathy, a group of disorders

characterized by muscle weakness and muscle cells

with centrally located nuclei. Infants with myotubular

myopathy exhibit severe muscle weakness and hypo-

tonia, often requiring ventilatory assistance at birth.

Most die of respiratory failure within the first year of

life, but some survive for longer periods (48).

Recently, mutations in another 3-phosphatase,

hJUMPY, which is not considered a bona fide

myotubularin due to the lack of a GRAM domain, a

signature domain of myotubularins, were found in two

cases of centronuclear myopathy (92). Like MTM1,

hJUMPY dephosphorylates PI3P and PI(3,5)P2, and

patient mutations affect catalytic activity. However, it

is still unclear whether impairment in hJUMPY is a

direct cause or a modifier of the disease phenotype

(92). Regardless, the presence of mutations in this pro-

tein in myopathic patients supports the importance of

3-phosphatase activity for proper muscular function.

Charcot-Marie-Tooth Disease and

Amyotrophic Lateral Sclerosis

Mutations in two myotubularin family proteins,

MTM2, a catalytically active protein, and MTM1B, a

catalytically inactive protein, cause Charcot-Marie-

Tooth Disease type 4B1 and 4B2, respectively (4, 11,

84). Charcot-Marie-Tooth Disease refers to a group of

diseases involving peripheral neuropathy. Type 4B is a

severe autosomal recessive demyelinating neuropathy

(73). Misfolding of myelin sheaths is characteristic of

the disease. Patients usually develop leg weakness dur-
ing childhood and become unable to walk by the time they reach young adulthood. MTMR13 forms a complex with MTMR2, which helps explain how mutations in both a catalytically active and a catalytically inactive phosphatase can produce a similar phenotype (73).

Recently, a subset of patients with autosomal recessive Charcot-Marie-Tooth Disease were found to be compound heterozygous for mutations in the FIG4 gene. In these patients, the mutation of one allele prevents expression of a functional protein, whereas mutation of the other allele produces a protein with impaired function (18). The authors designated this form of the disorder, characterized by asymmetric neuronal degeneration (108), as Charcot-Marie-Tooth 4J (CMT4J). More recently, the same authors have identified heterogeneous disrupting mutations of the FIG4 gene in ALS patients (18a). Fig4 is an inositol phosphatase that acts on PI(3,5)P2 but is also part of a complex that includes, and is required for the activation of, PIKfyve (Fah in yeast). PIKfyve (which is encoded by the PIP5K3 gene) is a PI5-kinase that produces PI(3,5)P2 from PIP3 (18). As a result, lack of Fig4 results in abnormal PI(3,5)P2 metabolism and lower PI(3,5)P2 levels (18).

Although the importance of turnover of PIP3 and PI(3,4,5)P3 in disease is clear, the mechanisms by which defects in the metabolism of these phosphoinositides contribute to disease remain unknown. Given the predominant localization of PI3P and PI(3,5)P2 in endosomes, a defect in endosomal function is likely. It remains to be seen which of the many functions of endosomes (signalling, hub for intracellular traffic, pre-lysosomal compartment that controls the degradation of membrane proteins) is predominantly implicated in the pathogenetic mechanisms. Mouse studies may prove helpful in elucidating the exact mechanism. Disruption of the FIG4 gene in mice (pale tremor mouse) produces neurodegeneration (18), although the control of the other allele prevents expression of a functional protein, whereas mutation of the other allele produces a protein with impaired function (18).

François-Neetens Mouchetée Fleck Corneal Dystrophy

Mutations in PIP5K3, the gene encoding PIKfyve (see above), are found in patients with François-Neetens Mouchetée Fleck Corneal Dystrophy (51), an autosomal dominant disease. Affected patients exhibit small white flecks in the stroma of the cornea. They are usually asymptomatic with normal vision, so the disorder is typically an incidental finding at routine examination (51). PI(3,5)P2 participates in budding from endosomes, and a defect in its synthesis results in enlarged endosomes and abnormal multivesicular bodies (63, 67, 76). Corneal flecks are thought to represent swollen keratocytes filled with vesicles containing lipids and mucopolysaccharides (51), which could reflect an abnormal endosomal maturation. Based on studies in model organisms, lack of PIKfyve is expected to result in embryonic lethality (75). The dominant nature of the heterozygous mutation may be due to a dominant negative effect of the truncated protein or to haploinsufficiency.

Cancer

PI(3,4,5)P3 is a major regulator of cell survival, cell proliferation, and cell growth. Accordingly, genetic manipulations that enhance PI(3,4,5)P3 signaling can often cause cancer. The protein phosphatase and tensin homolog deleted on chromosome 10 (PTEN), i.e., the inositol 3-phosphatase that acts on PI(3,4,5)P3 (57, 102), is a potent tumor suppressor. It is mutated in many human cancers including glioblastoma, melanoma, prostate cancer, thyroid cancer, colon cancer, endometrial cancer, breast cancer, lung cancer, cancer of the uterus, and lymphoma (15, 55, 69, 79, 106). Furthermore, decreased levels of PTEN correlate with resistance of glioblastomas to inhibition of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR), a receptor that acts upstream of PI(3,4,5)P3 signaling (62).

Although germline homozygous mutations of PTEN produce embryonic lethality (23, 91), heterozygous mutations predispose to cancer as a result of loss of heterozygosity in somatic cells. Autosomal dominant hereditary cancer syndromes that have also been reported in humans may be due to a dominant negative effect of the truncated protein or to haploinsufficiency. Based on studies in model organisms, lack of PIKfyve results in abnormal PI(3,5)P2 metabolism and lower PI(3,5)P2 levels (18).

An interesting study by the authors has demonstrated that elevated SHIP2 levels can increase the resistance of glioblastomas to anticancer drugs that inhibit PI3-kinases and Akt. The authors suggest that this may be due to the selective expression of SHIP2 in glioblastomas and the elevated activity of SHIP2 in glioblastomas. The authors also report that SHIP2 expression is elevated in glioblastomas and that this expression is correlated with a longer survival time. The authors suggest that this may be due to the selective expression of SHIP2 in glioblastomas and the elevated activity of SHIP2 in glioblastomas. The authors also report that SHIP2 expression is elevated in glioblastomas and that this expression is correlated with a longer survival time.
A positive correlation exists results in the development of multivisceral cancers, including the following: thyroid, breast, esophageal, and gastric cancers, as well as glioblastoma (6, 31, 69). The importance of PI3-kinases in cancer is underscored by the anti-cancer effect of drugs that inhibit PI3-kinases, such as wortmannin and LY294002, or downstream effectors of PEK/Akt signaling, such as a rapamycin, an mTOR inhibitor (1, 69, 106). Developing drugs that affect PI3-kinases is a major goal of anticancer pharmacology (79, 106). Developing drugs that affect PI3-kinases is a major goal of anticancer pharmacology (79, 106).

In addition to being a substrate for the inositol 3-kinase PTEN, which reverts PI(3,4,5)P 3 to its precursor PI(4,5)P 2, PI(3,4,5)P 3 can be also converted to PI(3,4)P 2 through the action of insulin-sensitive 5-phosphatases. Although PI(3,4)P 2 retains some ability to bind and activate PI(3,4,5)P 3, effectors such as the protein kinase AKT (36), even this pathway downregulates via its effects on the PI3K/AKT signaling pathway. PTEN completely turns off the PI(3,4,5)P 3 signal at the cell surface, SHIP2 generates PI(3,4)P 2, a phosphoinositide with its own signaling functions, which may also act on endosomes.

"PI3-kinase is a major effector of the insulin receptor, and PI(3,4,5)P 3 mediates many of the actions of insulin via its effects on the PI3K/AKT signaling pathway."

**Psychiatric Diseases**

Several genes involved in synthesis and degradation of phosphoinositides are located on chromosome regions to which schizophrenia or bipolar disorder has been mapped. These include the genes PIK3C2 (a member of the PI3-kinase family), PIK3CA (PI3-kinase type III alpha/Strik), PIP5K2A (a PIP4-5-kinase), and SYN1 (a polyphosphoinositide phosphatase), which are located at 18q, 22q11, 10p12, and 21q22, respectively (77, 78, 87–89). Studies of these genes in psychiatric patients and healthy controls found variations and polymorphisms (77, 78, 87–89). However, further evidence is necessary to confirm a relationship to disease. A link of genes that control insulin phospholipid metabolism to bipolar disorder is supported by the therapeutic effect of lithium on such disorders. One action of lithium is to reduce insulin levels by its inhibitory action on insulin monophosphatase (9), although other targets for the action of this drug have also been identified (39).

**Down Syndrome and Alzheimer’s Disease**

Recently, it was suggested that genetic perturbation of synaptophin 1, a polyphosphoinositide phosphatase predominantly concentrated in neurons, may have a role in the early onset of Alzheimer’s Disease that is associated with Down Syndrome (95). Synaptophin 1 accounts for the bulk of the PI(4,5)P 2 phosphatase activity in brain and plays a critical role in synaptic transmission (19, 24, 38). Alzheimer’s Disease peptide Aβ42 stimulates PI(4,5)P 2 cleavage and inhibits hippocampal long-term potentiation in mouse brain slices, suggesting a potential role of abnormal PI(4,5)P 2 metabolism in Alzheimer’s Disease (8). The gene encoding synaptophin 1, like the gene encoding the Aβ peptide precursor APP, is located in the region of chromosome 21 whose triplication is responsible for Down Syndrome. Accordingly, levels of synaptophin 1 are increased in the cerebral cortex of Down Syndrome patients.
patients (2, 16) and in mouse models of this condition (95), whereas levels of PI(4,5)P2 are correspondingly decreased (95). Conversely, levels of PI(4,5)P2 in brain are increased not only in the brain of synaptojanin 1 KO mice (which die perinatally) but also, to a lower extent, in the brain of mice that lack one copy only of the synaptojanin 1 gene and that do not display any obvious phenotype due to this haploinsufficiency (18, 95). Interestingly, synaptojanin haploinsufficiency antagonizes AP2's effect on PI(4,5)P2 levels and long-term potentiation (88). An attractive possibility is that the early Alzheimer's disease observed in Down syndrome patients may result from a synergy between overexpression of APP, the precursor of the Aβ peptide, and overexpression of synaptojanin 1, which results in decreased levels of PI(4,5)P2 and thus greater sensitivity to the disrupting effects of the Aβ peptide (8).

Conclusion

The wide array of diseases known to be caused by perturbation of genes encoding phosphoinositide metabolizing enzymes emphasizes the importance of inositol phospholipid regulation in cell and organismal physiology. It can be predicted that the number of such conditions will greatly increase as the identification of disease genes expands. In many cases, the mechanistic link between the metabolic defect due to the mutation and the phenotypic manifestations of the disease remains poorly understood. Each enzyme not only catalyzes a specific reaction, but also acts on specific phosphoinositide pools. Thus, to fully understand the mechanisms of action of each phosphoinositide metabolizing enzyme, these studies will both advance fundamental aspects of cell physiology and help identify new potential therapeutic targets. Given the broad importance of phosphoinositide metabolism in cell function, it can be anticipated that drugs resulting from these studies will both expand fundamental mechanistic knowledge and provide new targets for rational drug design.

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