PHOSPHOINOSITIDES: TINY LIPIDS WITH GIANT IMPACT ON CELL REGULATION

Tamas Balla

Section on Molecular Signal Transduction, Program for Developmental Neuroscience, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland

Balla, T. Phosphoinositides: Tiny Lipids With Giant Impact on Cell Regulation. *Physiol Rev* 93: 1019–1137, 2013; doi:10.1152/physrev.00028.2012.—Phosphoinositides (PIs) make up only a small fraction of cellular phospholipids, yet they control almost all aspects of a cell’s life and death. These lipids gained tremendous research interest as plasma membrane signaling molecules when discovered in the 1970s and 1980s. Research in the last 15 years has added a wide range of biological processes regulated by PIs, turning these lipids into one of the most universal signaling entities in eukaryotic cells. PIs control organelle biology by regulating vesicular trafficking, but they also modulate lipid distribution and metabolism via their close relationship with lipid transfer proteins. PIs regulate ion channels, pumps, and transporters and control both endocytic and exocytic processes. The nuclear phosphoinositides have grown from being an epiphenomenon to a research area of its own. As expected from such pleiotropic regulators, derangements of phosphoinositide metabolism are responsible for a number of human diseases ranging from rare genetic disorders to the most common ones such as cancer, obesity, and diabetes. Moreover, it is increasingly evident that a number of infectious agents hijack the PI regulatory systems of host cells for their intracellular movements, replication, and assembly. As a result, PI converting enzymes began to be noticed by pharmaceutical companies as potential therapeutic targets. This review is an attempt to give an overview of this enormous research field focusing on major developments in diverse areas of basic science linked to cellular physiology and disease.

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I. INTRODUCTION

It is hard to define the research interest of people who study polyphosphoinositides (PPIs). Naturally, PPIs are lipid molecules, yet many researchers who study PPIs did not initially have a primary interest in lipids. Many of us have gotten interested in PPIs when these lipids became known as the source of second messengers in transducing signals from cell surface receptors. The spectacular progress in the 1980s in defining the pathways by which G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs) activated phospholipase C (PLC) enzymes had a major impact on many scientists who showed interest in transmembrane signaling. However, cell biologists also developed immense interest in PPIs because of the importance of PPIs in shaping the membranes and controlling vesicular trafficking and organelle physiology. The attention of scientists who study ion channels also turned toward PPIs as it became obvious that many channels or transporters require PPIs for their activity or control. The discovery of phosphatidylinositol 3-kinases (PI3Ks) has set the stage to widen research interest in PPIs: association of PI3K with oncogenic as well as RTKs and their strong ties with cancer biology has won over cancer researchers, while the importance of PPIs in immune cell functions, chemotaxis, and secretion brought immunologists to the field. If this had not been enough, researchers working with infectious diseases noted that many pathogenic organisms possess enzymes essential for their pathogenic nature that act upon PPIs to invade cells or use the host cells’ PPI machinery to evade natural defense mechanisms or reprogram cells to produce the pathogen. Neuroscientists also discovered that synaptic vesicle exocytosis and recycling requires phosphoinositides at multiple steps and that brain development, including neurite outgrowth and axon guidance, is highly dependent on PPIs. Even the invertebrate photo-sensing and signal transduction is dependent on PPIs, further extending the group of scientists showing interest in PPIs. This selected and probably incomplete list increases every day as more and more cellular processes are linked to these universal lipid regulators.
Such an ever-expanding list of processes regulated by PPIs begs an answer to the fundamental question of how and why these lipids gained such a pivotal role in eukaryotic cell regulation during evolution? What structural and functional features make these molecules so widely used and so adaptable to support the functions of a variety of signaling complexes? We have only begun to ask, let alone answer these questions for which evolution may give us some clues. Although PIs have been detected in mycobacteria, their appearance in evolution coincides with the development of internal membranes and organelles. Remarkably, PI kinases surfaced earlier in evolution than tyrosine kinases (190, 986) with common ancestors being a group of serine-threonine kinases, called the PI-kinase related kinases (190, 669). The latter enzymes are all functionally linked to DNA damage control and repair (190, 1350, 1422). PtdIns is unique among phospholipids in that it is a rich phosphorylation target at the cytoplasmic surface of any cellular membrane. In their phosphorylated forms, PPIs can serve as critical reference points for a great variety of proteins to find their docking destinations and/or change their conformation. This is true for cytosolic proteins that are recruited to the membrane by PPIs, as well as for peripheral or integral membrane proteins whose membrane adjacent regions or cytoplasmic “tails” show interaction with PPIs.

With the spectacular expansion of the PI field, it has become impossible to cover all aspects of PPI regulation at great depth in a comprehensive review. In the following overview I will attempt to describe the most basic features of the enzymes that synthesize and degrade PPIs and focus on aspects of this diverse research field that highlight general principles that govern PI-mediated regulation of the many different processes. For a more comprehensive analysis and deeper understanding of the details of the individual processes and their PPI regulation, the reader is referred to the many excellent reviews that have been published over the years and ones that are likely still in preparation. Soluble inositol phosphates partly liberated from inositol lipids and further phosphorylated to highly charged inositol polyphosphates also represent a research topic of great interest but will not be discussed here. The interested reader will find excellent reviews on that topic (29, 1079, 1390). Another aspect of PtdIns function not covered here is related to PtdIns serving as an anchor for a selected group of proteins found on the cell surface linked to the 6-position of the inositol ring through glycan linkages. More about the biology of these GPI-anchored proteins can be found in several comprehensive reviews (473, 1200).

II. THE BASICS: PHOSPHOINOSITIDE STRUCTURES AND ENZYMATOLOGY

Polyphosphoinositides are phosphorylated derivatives of PtdIns generated by a number of kinases and phosphatases that act upon their membrane-bound lipid substrates (FIGURE 1). Phosphorylation occurs in one of the -OH groups of the inositol ring of PtdIns that is linked to the diacylglycerol (DG) backbone via a phosphodiester linkage utilizing the -OH group of the ring at the D1 position. PtdIns contains myo-inositol that assumes a “chair” conformation with five of its six -OH groups being equatorial and the one at position 2 being axial. The best visual representation of the myo-inositol structure and ring numbering was introduced by Bernard William Agranoff in 1978, who likened the “chair” structure of myo-inositol to a turtle whose body represents the inositol ring and the numbering starts at the right front flipper and proceeds counterclockwise through the head and the other appendages (9) (FIGURE 1A). Since the turtle is taken through its right flipper by the DG backbone, it leaves five hydroxyls for phosphorylation, but only three of these (positions -3, -4, and -5) are actually phosphorylated in naturally occurring PPIs according to current knowledge. The combination of these phosphorylations gives rise to the seven known PPIs (FIGURE 1B). A distinctive feature of PPIs is their enrichment in arachidonic acid at the sn-2 position of their glycerol backbone. The majority of the PPIs is the 1-stearoyl-2 arachidonyl form, and it has been a puzzling question where this enrichment takes place and what role deacylation-reacylation cycles play in determining this composition. It has been suggested that enzymes responsible for PtdIns synthesis and phosphorylation may show preference to the form(s) of lipids esterified with arachidonic acid at the sn-2 position (405). Arachidonate-rich phosphoinositides are also believed to be the source of PLA2-mediated arachidonate release for the synthesis of prostaglandins and leukotrienes.

The amounts of PPIs within cells have been estimated in different cells and tissues (1121, 1703). These estimates and measurements show significant variations. PtdIns represents ~10–20% (mol%) of total cellular phospholipids, whereas PtdIns(4)P and PtdIns(4,5)P2 constitute ~0.2–1%. Based on long-term [3H]inositol labeling, PtdIns4P and PtdIns(4,5)P2 have about 2–5% of the labeling relative to PtdIns (e.g., Ref. 87). Recent estimates of PtdIns(4,5)P2 density in the plasma membrane (PM) ranged between 5,000–20,000 molecules/μm2 (421). The other PPIs contribute even smaller amounts, PtdIns(3,4,5)P3 being about 2–5% of PtdIns(4,5)P2 and PtdIns3P about 20–30% of PtdIns4P. It is noteworthy that these ratios show great tis-

FIGURE 1. Phosphoinositide basics. A: Agranoff’s turtle demonstrating the orientation of the hydroxyl groups in myo-inositol. B: interconversions between various phosphoinositides and the enzymes catalyzing these reactions. The yeast enzymes are listed in parentheses. Where there is some ambiguity it is indicated by “??”. *It is worth pointing out that contrary to their designation, PIP5K2s are 4-kinases that act on PtdIns5P.
Agranoff’s turtle

myo-inositol

PtdIns

**Kinases**
1. PI4KA, PI4KB, PI4K2A, PI4K2B (Stt4, Pik1, Lsb6)
2. PIP5K3/PIKfyve ?
3. PI3KC3, (Vps34), PI3KC2A
4. PIP5K1A, PIP5K1B, PIP5K1C (Mss4)
5. PIP5K2A*, PIP5K2B*, PIP5K2C*
6. PIP5K3/PIKfyve (Fab1)
7. PI3KCA, PI3KCB, PI3KCG, PI3KCD

**Phosphatases**
8. SAC1M1L, (Sac1), SYNJ1/2 ?
9. SYNJ1, SYNJ2, OCRL, INPP5B, INPP5E, INPP5F, (Inp51, Inp52, Inp53)
10. TMEM55 ??
11. MTMs, MTMRs
12. INPP4A, INPP4B
13. SAC3 (Fig4)
14. PTEN
15. SHIP1, SHIP2, INPP5E, INPP5J, INPP5K

**B**

PtdIns

PtdIns4P

PtdIns5P

PtdIns3P

PtdIns(4,5)P2

PtdIns(3,4)P2

PtdIns(3,5)P2

PtdIns(3,4,5)P3
PtdIns is synthesized in the endoplasmic reticulum from CDP-DAG and myo-inositol by a PtdIns synthase (PIS) enzyme (11) (see sect. IV) and is then distributed throughout the cell presumably by several PI transfer proteins (PITPs) (277, 689) and possibly via vesicular trafficking. Our recent studies identified the PIS enzyme in an ER-derived highly mobile “organelle” that may serve as a dynamic PtdIns distribution device (796). Early studies already detected the phosphorylation reaction generating two “polyphosphoinositides” that had been previously described in the brain and determined to be PtdIns4P and PtdIns(4,5)P₂ (186), thereby postulating PtdIns kinase (PI-kinase) and PtdInsP kinase (PIP kinase) activities associated with membranes (1044). Although these enzymatic activities were associated with various membrane fractions in fractionated tissues, and they showed even some unique features (like sensitivity to different detergents), the general consensus that emerged from these studies was that PI- and PIP-kinase activities were primarily present in the PM, serving what has become known as the signaling pool of PPIs (see sect. III). However, by now it is apparent that multiple isoforms of almost all of the kinase and phosphatase enzymes act upon PPIs, and they do so in different cellular compartments. This explains why in most cases these enzymes are not functionally redundant even if they catalyze the same biochemical reaction. The mechanism(s) that determine the intracellular localization and regulation of the PI kinase and phosphatase enzymes became a central question for each family of these proteins. Generally speaking, most PtdIns mono-phosphorylations (by the PI4Ks and Class III PI3K) occur in endomembranes, such as the endosomes and the Golgi/trans-Golgi network, whereas the phosphorylation of PtdIns4P to PtdIns(4,5)P₂ by PIP 5-kinases and further to PtdIns(3,4,5)P₃ by class I PI3Ks occurs primarily at the PM.

The two main routes of PPI elimination are through dephosphorylation by PPI phosphatases and hydrolysis by phosphoinositide-specific phospholipase C enzymes (PLCs). Some of the PPI phosphatases are quite specific for the position of the phosphate group that they remove, while others, mainly the ones that dephosphorylate monophosphorylated PPIs, are more promiscuous and their functional specificity lies in their localization. The diversity of the PPI phosphatases parallels, in fact, exceeds that of the kinases (FIGURE 1B), and several human diseases have been traced to the dysfunction of PPI phosphatases (see sect. VI). The diversity of phosphoinositide-specific PLCs is also remarkable (see sect. VII). The preferred in vivo substrate of the mammalian PLC enzymes is believed to be PtdIns(4,5)P₂, although this question has not been satisfactorily answered in whole cell studies and purified PLC enzymes can also hydrolyze PtdIns4P and PtdIns in vitro.

Although individual groups of PPI metabolizing enzymes will be featured in more details below, a few important general questions are worth highlighting here. The first is related to their substrate recognition. Most, if not all, of the PPI kinase and phosphatase enzymes are loosely membrane-associated peripheral membrane proteins that utilize a substrate that is part of the membrane with the inositol headgroup facing the cytoplasmic leaflet of the membrane. When enzyme activities of these proteins are measured in vitro, the assay usually contains the lipid substrate in some form of lipid micelle, and the type and amount of detergent yielding optimal activity greatly differ for each of these enzymes. For example, the in vitro activity of PI 4-kinases depends on the presence of detergents such as Triton X-100, while those of the class I PI 3-kinases are inhibited by detergents and the activity of class III PI 3-kinases are usually assayed in the presence of Mn²⁺ instead of the usual Mg²⁺. Overexpression in cells of some of the PI kinases (such as the type III PI 4-kinases) hardly yields an increase in the phosphorylation of their endogenous lipid substrates. This indicates that the mechanism(s) that ensure recruitment of the enzymes to the membrane and their access to the membrane-bound PtdIns substrate are major determinants of their in situ activities. Few studies have been designed to understand the exact nature of the lipid substrate PI kinase interactions, and most of the solved structures of the kinase enzymes do not resolve the activation loop (a mobile part of the molecule that is critical for substrate presentation) within their catalytic center. Similarly, the lipid substrate in these structures is either missing or, if present, was provided in the form of a soluble inositol-phosphate headgroup. Therefore, there is a major gap in our understanding of how these enzymes work in their natural membrane environment.

Phosphoinositides affect cellular functions by interacting with molecules that either reside in the membrane, such as ion channels and transporters, or get recruited to the membrane by reversible mechanisms. Several signaling molecules are recruited to the membrane through interaction with PPIs via inositol-binding protein modules (see TABLE 1). The first such protein module was identified in pleckstrin (574), and ever since, the homologous modules have been termed pleckstrin homology (PH) domains. PH domains are present in a large number of regulatory molecules (296). It is important to note, however, that not all PH domains bind lipids and probably all PH domains also bind proteins (880). Often simultaneous protein and lipid binding are required for the membrane recruitment or conformational...
change of PH domains; hence, these (and other PI binding modules) are called coincidence detectors (214). Most frequently the protein input for PH domains come from interaction with small GTP binding proteins. Other domains, such as the FERM domains link the actin cytoskeleton to the PM (255). Both FERM and GLUE-domains contain a structural fold similar to that of PTB (phosphotyrosine binding) or PH domains (1062, 1416). EHD domains (1120) and BAR domains also bind anionic lipids including inositol lipids and also sense and/or generate membrane curvatures (466). This list is ever expanding and now also includes PDZ domains (1825) and the KA1 domain, a novel fold found at the COOH terminus of a range of proteins and which binds PtdIns(4,5)P2 but also other anionic phospholipids such as PS (1083). A recently described PtdIns(3,4,5)P3 binding domain found at the COOH termini of some IQ domain containing GAP proteins has a structure reminiscent of the integral fold of C2 domains (363). In addition, several proteins contain polybasic stretches that do not amount to a characteristic domain, but also interact with acidic phospholipids with electrostatic interaction. Good examples are the MARCKS proteins (1670) and the K-Ras COOH terminus (601), but many other proteins show membrane association using this mechanism. Importantly, many of these targeting sequences can use PtdIns4P as well as PtdIns(4,5)P2 as their membrane anchor lipid (559).

### III. HISTORICAL OVERVIEW

Although one can argue that a newcomer to a field can benefit from not being biased by existing dogmas, ignorance of the history of a research field and lack of understanding of the milestones and breakthroughs are making it difficult, if not impossible, to put any new research findings into perspective. One also has to know and respect the contribution of the scientists whose findings serve as the foundation upon which new knowledge can be built. Therefore, I give a short overview of the field that is certainly biased by my experiences, but can still give some ideas about the major milestones as this research field has evolved. Several reviews and recollections have been published on the historical aspects of this huge research field, and they are highly recommended for interested readers (10, 634, 701, 1039).

Inositol had already been discovered as a component of muscle by the end of the 19th century and its structure was established as a hexa-hydroxyl-cyclohexane (990). Nine different stereoisomers of inositol were described in the 1940s, and myo-inositol was found to be the main eu-karyotic isomer (1234). The importance of inositol was recognized in the era of vitamins when it was realized that inositol was an important dietary ingredient for rodents, especially when animals were kept in germ-free conditions. Animals kept on inositol-free diets developed alopecia and “fatty liver” (reviewed in Ref. 642). Subsequently, it was found that mammalian cells required myo-inositol to grow properly in culture (394). In the fungus neurospora, lack of inositol caused a defect in lysosomal membrane integrity and autolysis (1006). The notion that inositol was a component of lipid membranes was first recognized in mycobacteria (the lipid was phosphatidylinosi-

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**Table 1. Inositide binding domains**

<table>
<thead>
<tr>
<th>Name of Domain</th>
<th>Lipid Species</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleckstrin homology (PH) domains</td>
<td>PtdIns(4,5)P2, PtdIns4P</td>
<td>574, 882</td>
</tr>
<tr>
<td></td>
<td>PtdIns(3,4,5)P3</td>
<td>478, 1252, 1627</td>
</tr>
<tr>
<td>FYVE domains</td>
<td>PtdIns3P</td>
<td>1457</td>
</tr>
<tr>
<td>FYVE domains (named after the four proteins in which they were first identified: Fab1,YOTB/ZK632, Vac1p, and EEA1)</td>
<td>PtdIns3P</td>
<td></td>
</tr>
<tr>
<td>Phox homology (PX) domains</td>
<td>PtdIns3P</td>
<td>1721</td>
</tr>
<tr>
<td>ENTH (epsin NH2-terminal homology)</td>
<td>PtdIns(4,5)P2</td>
<td>877</td>
</tr>
<tr>
<td>ANTH (AP180 NH2-terminal homology)</td>
<td>PtdIns(4,5)P2</td>
<td>877</td>
</tr>
<tr>
<td>FERM domains (named after the first proteins in which they were found: 4.1-ezrin-radexin-moiesin)</td>
<td>PtdIns(4,5)P2</td>
<td>255</td>
</tr>
<tr>
<td>PTB domain</td>
<td>PtdIns(4,5)P2</td>
<td>1062</td>
</tr>
<tr>
<td>GLUE domain</td>
<td>PtdIns(3,4)P3</td>
<td>1416</td>
</tr>
<tr>
<td>Eps15 homology (EHD) domains</td>
<td>PtdIns(4,5)P2, PtdIns4P</td>
<td>1120</td>
</tr>
<tr>
<td>BAR (Bin–Amphiphysin–Rvs)</td>
<td>PtdIns(4,5)P2, other lipids</td>
<td>1211, 1607</td>
</tr>
<tr>
<td>PDZ domain</td>
<td>PtdIns(4,5)P2</td>
<td>1825</td>
</tr>
<tr>
<td>KA1 (kinase-associated 1) domain</td>
<td>PtdIns(4,5)P2</td>
<td>1083</td>
</tr>
<tr>
<td>Acidic phospholipids, PS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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tol mannoside (96) and a phosphoinositide was also described in soybean (803).

A. Identification of Phosphoinositides and Their Metabolic Fate

The real beginning of the “modern” era of PI research was marked by a series of ground-breaking studies in the 1940s by Jordi Folch who identified inositol in the ethanol-insoluble phospholipid fraction of bovine brain and determined that it contained phosphates and inositol in a molar ratio of 2:1 (450). This lipid, termed diphosphoinositide or DPI, was found primarily in myelin in tight association with proteins (“neurokeratin”) (449) and showed rapid metabolic labeling when guinea pigs were injected with \(^{32}\text{P}\)-phosphate (328, 329). Subsequent work mainly by three groups (led by Clinton Ballou, Rex Dawson, and Tim Hawthorne) identified the structures of mono-, di-, and tri-phosphoinositides (abbreviated at the time as MPI, DPI, and TPI, respectively) as glycerophospholipids with an inositol ring linked to an \(sn\)-1,2-DG backbone via the D1-OH group of myo-inositol, and containing a phosphate at the 4- and both the 4- and 5-positions, in DPI and TPI, respectively (359, 581, 1563). Although these lipids had been isolated and identified primarily from brain, where they are most abundant, it had become evident by the early 1960s that they were present in small amounts in all eukaryotic tissues (1651).

To understand the meaning of their \(^{32}\text{P}\) labeling, it was essential to understand the synthetic and degradation pathways of PPIs. From the pioneering work of Eugene Kennedy and his colleagues on the synthesis of glycerolipids, it had been understood that cytidine nucleotide intermediates (such as CDP-choline and CDP-ethanolamine) donate the headgroups to the \(sn\)-1,2-DG backbone during phosphatidylcholine and -ethanolamine synthesis. However, Bernard Agranoff and his colleagues found that this was not the case for PtdIns. Here, the lipid backbone itself was “activated” by the cytidine nucleotide in the form of CDP-DG (11). Since the precursor of this intermediate is phosphatidic acid (PtdOH), which is produced by phosphorylation of \(sn\)-1,2-DG by a DG kinase using the terminal phosphate of ATP, described by Hokin and Hokin (640), an alternative name of CMP-PtdOH was suggested, to indicate that the phosphate was carried over to PtdIns not from CTP but ATP. PtdIns synthesis was found primarily associated with “microsomal” fractions and hence attributed to the ER, but CDP-DG is also a precursor of the mitochondrial lipids phosphatidylglycerol and cardiolipin (324), suggesting an important compartmentalization of these metabolic pathways.

Several studies then indicated that DPI was a phosphorylation product by PtdIns kinase activities associated with various membrane fractions (283, 636, 1044), including the PM (1043) and, importantly, similar activities were also present in red blood cell membranes where it was possible to show generation of PtdIns4P and PtdIns(4,5)P\(_2\) by presumed sequential phosphorylations of PtdIns (636). These observations, together with the notion that PPIs were highly enriched in myelin sheets that are essentially rolled up plasma membranes, gave strong support to the idea that PPIs were primarily associated with the PM. The metabolism of DPI and TPI was also explored in early studies. Sloane-Stanley identified a phospholipase C (PLC) activity (although not called it PLC yet) capable of hydrolyzing brain phosphoinositides (1420), and Rodnight found this activity increased by Ca\(^{2+}\) (1276). These early observations were followed by the realization that TPI is metabolized in two different ways: one route with dephosphorylation to DPI and PtdIns and another, via hydrolysis to InsP\(_3\) and diacylglycerol (what is now known as PLC) (1554).

The very rapid labeling kinetics of PtdIns(4,5)P\(_2\) and PtdIns4P in erythrocyte membrane and in intact cells relative to the much slower labeling kinetics of PtdIns and other phospholipids suggested high turnovers of the phosphomonoester groups due to rapid “futile” phosphorylation-dephosphorylation cycles (reviewed in Ref. 385). This also indicated that dephosphorylation of the monophosphate groups and rephosphorylation can all take place in the PM. PLC activities were then found in several tissues both in soluble and membrane fractions (464, 775), and association of PLC activity with the PM was also described (852, 1047). The distribution of PLC activities between soluble and membrane fractions and the enzymatic characteristics of the activities associated with different fractions showed significant variations between various laboratories (e.g., see Ref. 702). These discrepancies are now better understood knowing how many PLC enzymes and membrane-recruitment mechanisms exist (see sect. VII).

B. Agonists Stimulate Phosphoinositide Metabolism

In 1953 Mabel and Lowell Hokin (638) reported that \(^{32}\text{P}\)phosphate incorporation into a phospholipid fraction was strikingly increased in the exocrine pancreas when the tissue was stimulated with secretagogues that induced protein secretion. In a series of subsequent work (635), the Hokins found that the increased \(^{32}\text{P}\)-labeling was limited to the two lipids, “DPI” and PtdOH that were identified by Dawson as acutely \(^{32}\text{P}\)-labeled in the brain slices (328). The Hokins also found that this increase was a general phenomenon observed in various cells associated with a stimulated secretion response (635). They suggested that the primary response was an increased phosphoinositide-specific PLC-catalyzed hydrolysis of PtdIns with production of DG, which was then converted to PtdOH (a step where the \(^{32}\text{P}\) incorporation took place) and then back to PtdIns to complete a cycle that was dubbed the “PL cycle” (639) (FIGURE 2A). These studies...
implicated PIs in secretion, but how increased inositide labeling was linked to any specific biochemical process in secretion remained elusive. In fact, more and more studies indicated that the increased PI turnover could be dissociated from secretion: it was observed in cells and with stimuli that did not evoke secretion, such as in postganglionic neurons (632) or lymphocytes (441). Also, the increased PI turnover was preserved in Ca$^{2+}$-free medium, whereas secretion was

**FIGURE 2.** The phosphoinositide cycle as originally perceived (A) and the updated version also showing the polyphosphoinositides, PtdIns4P and PtdIns(4,5)P$_2$ (B). The primary event in triggering the cycle is the agonist-induced PLC activation. Note that all of the products of PtdIns(4,5)P$_2$ hydrolysis are recycled. Diacylglycerol (DG) is converted to phosphatidic acid (PtdOH) by one of many DG-kinase enzymes (DGK). PtdOH then has to be transferred from the PM to the endoplasmic reticulum by a mechanism that has not been identified. In the ER, one of two CDP-DG synthase (CDS) enzymes conjugates PtdOH with CTP, and the CDP-DG is then conjugated with myo-inositol to phosphatidylinositol (PtdIns). PtdIns synthesis takes place mainly in a highly dynamic subcompartment of the ER. Much of the inositol used for PtdIns synthesis is derived from the sequential dephosphorylation of inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$], the other product of PLC-mediated PtdIns(4,5)P$_2$ hydrolysis. Several of the dephosphorylation steps are inhibited by Li$^+$, including the final dephosphorylation of inositol monophosphates by the enzyme inositol monophosphatase (IMP). The newly synthesized PtdIns has to reach the PM by a still obscure mechanism, perhaps mediated by PtdIns/PtdCho transfer proteins (PITPs).
eliminated under those conditions (633). Moreover, increased PI labeling needed higher concentrations of agonists than those for secretion (637), and it was not evoked by some agonists that increased secretion via cAMP (1337). In the meantime, a series of important experiments linked the PI turnover to cell proliferation. Fisher and Muller (442) found that lymphocytes stimulated with the mitogen phytohemagglutinin increased 32P- or [3H]inositol labeling of PtdIns and a rapid appearance of PtdOH, consistent with increased turnover of PI. A close correlation between cell proliferation and specifically inositol lipid turnover was found in cells subjected to various stimuli, including transformation with viruses, such as Rous sarcoma or SV40 (358). A dramatic drop in PtdIns turnover was shown to correlate with the transition from proliferation to differentiation during lens development in chicken embryos (1791).

These studies prepared the field for the notion that the PI turnover was somehow related to receptor action, although the link was not clearly defined (338, 392). This prompted Michell and Lapetina to consider that the PI response was a critical intermediate during stimulation of cell surface receptors that did not act via cAMP. They suggested that a product of PLC action on PtdIns, such as 1,2-cyclic inositol monophosphate, perhaps acted as a second messenger (852, 853). In parallel developments, cytoplasmic Ca2+ increase and, in many instances, cGMP elevations were increasingly recognized as possible triggering signals not only in excitable tissues, such as muscle cells, but in secretory cells as well. Intriguingly, the kind of receptors and agonists that evoked the Ca2+ responses were almost always identical to those that elicited a PI response. A theory was then introduced by Bob Michell in his seminal 1975 review (1040), where he proposed that the increased PI turnover was an early receptor-triggered event initiated by PLC activation characteristic of receptors that used Ca2+ or cGMP as second messengers. There were, however, some cracks in this picture that hampered its immediate endorsement and prompted further research. One of these points was related to the “speed” of the response relative to that of Ca2+ elevations, and the other was due to a few reports that found the enhanced PI labeling dependent on the presence of Ca2+ (21, 276) or perhaps Ca2+-controlled (32). This latter, together with the Ca2+ sensitivity of PLC activity appeared to support the views that the PI response was secondary or parallel to Ca2+ elevations (1174). Most studies, however, found the response independent of Ca2+, arguing against this idea (1041). Finally, Fain and Berridge (417) presented clear evidence that inositol lipid turnover was upstream of calcium fluxes during stimulation with 5-hydroxytryptamine in the blowfly salivary gland.

Another question raised was whether PtdIns or its phosphorylated derivatives (“DPI or TPI”) were the primary subject of PLC-mediated hydrolysis. Durrell (393) noted first that inositol-bisphosphate was one of the products that accumulated during stimulation, which can be derived only from hydrolysis of PtdInsP or PtdInsP2. Other reports described earlier that radiolabeled PtdInsP and PtdInsP2 were rapidly decreased upon stimulation with acetylcholine in avian salt gland (1339) and in iris smooth muscle (1), but the latter response was found Ca2+ sensitive and its connection to the increased PI labeling was not all that clear. The time must have been right for clarifying this question in the early 1980s when three groups reported that PtdIns(4,5)P2 was the initial target of PLC-mediated hydrolysis yielding Ins(1,4,5)P3 as the primary water-soluble hydrolytic product (131, 132, 299, 800, 1046). This was almost instantly followed by the recognition that Ins(1,4,5)P3 released Ca2+ from nonmitochondrial Ca2+ stores, finally unequivocally linking inositol lipid hydrolysis and Ca2+ signaling (1481). The other product of PtdIns(4,5)P2 hydrolysis, sn-1,2-DG, also found its targets when a DG-regulated phospholipid-dependent kinase family, named protein kinase C (PKC) was identified by Nishizuka. These findings opened a whole new research direction for the characterization and functional analysis of the PKC enzymes (1141, 1142). An extended view of the “PI cycle” has then been established by the mid 1980s (FIGURE 2B).

C. Expansion of Phosphoinositides

With these developments, the phosphoinositide-Ca2+ signaling paradigm became consolidated, and the efforts were concentrated on isolating and characterizing the enzymes that catalyzed these reactions. Particularly important was to identify the PLC enzymes that were activated by the Ca2+ mobilizing receptors. A significant achievement of this time was the purification (763, 1319, 1521) and subsequent molecular cloning (402, 762, 1493) of several forms of PLC pursued in the laboratories of Sue Goo Rhee, Matilda Katan, and Tadaomi Takenawa. It was also an important revelation that two major routes of PLC activation exist: one via the α and βγ subunits of heterotrimeric G proteins, in the case of PLCβ enzymes (207, 866, 1194, 1195), and the other via association and activation by RTKs, in the case of PLCγ (786, 995). Similar developments occurred in the inositide kinase (167, 362) and phosphatase (801, 1065, 1775) fields, and the former also produced some unanticipated and surprising results that were forerunners of an entirely new research field. These began with the discovery that several transforming oncogenes such as the Rous sarcoma virus, polyoma middle T antigen, or avian src were associated with PtdIns kinase activities (963, 1486, 1713), and it was simultaneously noted that more than one kind of PtdIns kinase was present in mammalian cells (403, 1712). Since all previous studies assumed that PtdInsP was a 4-phosphorylated PtdIns and hence PtdIns kinases were, by default, believed to be 4-kinases, it came as a surprise when the lipid product of the so-called type I PtdIns kinase was identified as a 3-phosphorylated PtdIns (1462, 1711). Soon it was also shown that growth factor or GPCR stimulation
evoked the production of novel phosphoinositides PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃ (66, 1572), while PtdIns3P was constitutively present in cells and did not respond to similar stimuli (1462).

Interest in PI 3-kinases was rapidly propelled by the association of PI 3-kinase activities with growth factor and oncogenic signaling, ultimately unraveling the various subclasses and primary structures of these enzymes and their adaptors (192, 614, 1178, 1367, 1465, 1476, 1638). Moreover, PI 3-kinase research was greatly aided by the discovery that the fungal drug wortmannin (Wm) was a potent PI 3-kinase inhibitor (52, 1761) and was further motivated by the revelation, with the help of large-scale genomic sequencing, that oncogenic mutations within the catalytic subunit of PI 3-kinase class I is found in a large number of human cancers (1334). However, PI 3-kinase research had a large impact on the overall PI field even beyond its close association with cancer. The fact that PtdIns(3,4,5)P₃ was a poor substrate of PLC enzymes (1384) has directed research toward the notion that the lipid itself within the membrane must function as a signaling entity and that molecules must exist that respond to these lipid changes (1467). This concept has been proven with the discovery of the Akt kinase (also known as protein kinase B, PKB) as a major signaling route downstream of PI 3-kinases and PtdIns(3,4,5)P₃ (202, 461). This, together with the identification of pleckstrin homology domains (1014, 1584) as protein modules that directly bind phosphoinositides (768, 881, 882), exposed the side of phosphoinositides as membrane-bound regulatory molecules as opposed to just being precursors of the two messengers Ins(1,4,5)P₃ and DG. This has been a monumental paradigm shift that hugely expanded the functional versatility of PPIs and gave new meaning to the diversity of the PI kinases and phosphatases. These enzymes turned out to be regulators of a whole range of cellular functions with a still poorly understood contribution to the increased metabolic labeling of inositides described in early studies. More detailed description of how the PI3K field was established and expanded can be found in (1559, 1612).

D. Soluble Inositol Phosphates

Another direction to which the expansion took place was marked by the discovery that eukaryotic cells contained water-soluble inositol phosphates other than those generated by PLC-mediated hydrolysis of the PPIs and their dephosphorylated metabolites. This started when Robin Irvine’s group discovered that the majority of the InsP₃ increase after stimulation was not due to the active Ins(1,4,5)P₃ isomer but another form, Ins(1,3,4)P₃ (707) that was inactive as a Ca²⁺ mobilizing agent (706). The source of this isomer was soon clarified with the discovery of the “inositol tris/tetrakisphosphate pathway” whereby Ins(1,4,5)P₃ was phosphorylated by a 3-kinase yielding Ins(1,3,4,5)P₄, which was then converted to Ins(1,3,4)P₃ (705). A long quest has followed to find a messenger function for Ins(1,3,4,5)P₄ with suggestions that it would link Ins(1,4,5)P₃-sensitive Ca²⁺ pools with sustained Ca²⁺ entry (235), and led to the isolation of an Ins(1,3,4,5)P₄-binding protein identified as a Ras-GAP1 protein family member (306). However, the biological functions (if any) of Ins(1,3,4,5)P₄ still remain elusive. In the meantime, additional, highly phosphorylated inositols, such as InsP₄ and InsP₅ have been described by HPLC analysis of myo-[³H]inositol-labeled cells (610) and a new pathway was identified by which Ins(1,3,4)P₃ could be converted to Ins(1,3,4,6)P₄ and then to InsP₅ (91, 92, 1391). Although there were significant agonist-induced changes observed in the newly discovered InsP₄ isomers, InsP₅, and even InsP₆ (86, 93, 1032), the significance and functions of these inositol phosphates remained unknown for a period of time until the discovery of a pathway in yeast that took Ins(1,4,5)P₃ all the way to InsP₆ with a functional link to messenger RNA export (1777). This was the beginning of a new era in inositol polyphosphate research with the discovery of pyro-phosphorylated inositol polyphosphates (954, 1031) and possible roles of several of these compounds (1068) in cell regulation. These new developments will not be detailed further in this review as they deviated from the PPI lipids themselves. However, these exciting findings deserve attention and have been reviewed recently elsewhere (1375, 1389, 1390).

IV. PHOSPHATIDYLINOSITOL SYNTHESIS

PtdIns synthesis takes place in the ER and, as recently suggested, in an ER-derived highly mobile subcompartment that may serve as a means to supply the lipid to other membranes (796). Since the increased PI synthesis after agonist stimulation is believed to be secondary to PPI breakdown that occurs in the PM, there have been speculations that PtdIns synthesizing activities might have separate ER and PM components and perhaps more than one phosphatidylinositol synthase (PIS) enzymes were responsible for them. Indeed, the whole PI synthesizing machinery was found in turkey erythrocyte membranes (1021, 1622), and PIS activities were found associated with PM as well as ER membranes after cell fractionation (694, 695, 1407). Molecular identification of the PIS enzyme was first achieved in yeast (1133, 1134) as a result of complementation cloning using a yeast strain that had been previously found defective in PIS enzyme activity (1135). Mammalian PIS was then cloned and characterized (957, 1528), showing that the same enzyme was responsible for both PtdIns synthase and myo-inositol exchange activities, two reactions that had been formerly attributed to distinct molecular entities (1522). There appears to be no other gene for PtdIns synthesis, so the association of the enzyme activity with PM fractions may be related to their contamination with ER or with the lighter mobile PIS membrane compartment (796). Two studies reported defects in PIS activity in zebrafish, one describing ER stress and hepatic steatosis (1547), while the other observed lens structural defects and reduced number of photoreceptors (1098).
The two substrates of PtdIns synthesis are myo-inositol and CDP-DG (FIGURE 2B). The latter is synthesized from CTP and PtdOH by CDP-DG synthase (CTP:phosphatidate cytidylyltransferase, or CDS for short) enzymes (11, 119). Early studies described two CDS activities: one associated with the outer surface of the ER (95) and another located in the matrix of mitochondria that was responsible for feeding into cardiolipin synthesis (1360). Mammalian CDS enzymes have been cloned (589, 1325, 1688), and two, highly similar but different genes, cds1 and cds2 are found in the human and other mammalian genomes (552, 1645). Both CDS proteins localize to the ER, but they do not enter the PIS positive mobile compartment (796). Importantly, none of the cloned CDS enzymes is found in the mitochondria (796) or showed the unique characteristics of the mitochondrial activity (1522). Therefore, the mitochondrial enzyme still remains elusive. A photoreceptor-specific isoform of Drosophila CDS was shown to be necessary for the maintenance of PtdIns(4,5)P₂ levels and hence for a sustained light response, and CDS mutant flies developed light-dependent retinal degeneration (1736). Recent studies also identified mutations in one of the CDS genes in zebrafish causing specific defects in blood vessel formation and angiogenesis, and these effects were attributed to the rundown of PtdIns(4,5)P₂ levels in the VEGF-stimulated endothelial cells (1188). These studies also concluded that CDS enzyme activities are necessary for maintaining the signaling pool of PtdIns(4,5)P₂.

The other substrate of PtdIns synthesis is myo-inositol, and cells have three ways of providing this substrate for the PIS enzyme. Some organisms and cells can synthesize myo-inositol de novo from glucose-6-phosphate via Ins3P. [Many studies use Ins1P to designate this product. This refers to the L-enantiomer, whereas we use the D-designation for all inositol phosphates. Accordingly, D-Ins1P corresponds to myo-inositol, and it shows higher expression in kidney medulla and MDCK cells (1188). These studies also concluded that CDS enzyme activities are necessary for maintaining the signaling pool of PtdIns(4,5)P₂. However, D-Ins1P is quickly converted to myo-inositol.] and its membrane insertion is regulated by activity in neurons (1590).

The question of how cells supply myo-inositol for PtdIns synthesis has attracted a lot of attention when it was discovered that treatment of rats with Li⁺ at doses that are used in the treatment of manic-depressive disorders caused brain inositol levels to drop with accumulation of inositol monophosphate (34, 35). Subsequent studies showed that Li⁺ inhibited the dephosphorylation of inositol monophosphates and that it was D-Ins1P that accumulates in the rat brains after prolonged Li⁺ treatment (1396). This latter finding was important because it showed that the accumulating inositol phosphate was not the isomer synthesized de novo by the cells (which is Ins3P or L-Ins1P) but it was originated from the PLC-liberated Ins-phosphates that yield Ins1P and Ins4P (87, 88). Indeed, Li⁺ was shown to enhance the accumulation of several inositol phosphate isomers upon stimulation by agonists that activate PLC enzymes (88, 133). Based on these findings it was suggested that the beneficial effects of Li⁺ treatment in manic-depressive disease are related to a deficient recycling of the inositol phosphates liberated by PLC activity, and this would affect neurons that display the highest activity (134). These observations and the proposed theory draw attention to the question of why brain cells are unable to supply inositol from the CSF and brought the inositol uptake pathways into the focus of intense research. Many studies have since been devoted to study the effects of mood-stabilizing drugs on myo-inositol uptake and inositol phosphate generation and recycling (60, 350, 351, 1258).

In light of these observations, it was a remarkable finding that SMIT1 knockout mice develop normally but die from congenital central apnea due to abnormal respiratory rhythmogenesis (138, 195). These mice have more than 90% reduction in brain and more than 80% in whole body myo-inositol content. Yet, their brain PtdIns levels are completely normal (137, 195). These findings cast some doubts about the “inositol-depletion hypothesis” of Li⁺ action and suggested that myo-inositol uptake by SMIT1 serves a different purpose, perhaps contributing to osmotic control. These findings also showed that PtdIns synthesis could go on undisturbed even at greatly reduced myo-inositol levels. However, Li⁺ treatment combined with strong PLC activation can drive down PtdIns levels in cultured cells (87, 727), suggesting that without sufficient external inositol it is possible to deplete cellular myo-inositol levels to the point where PtdIns synthesis will suffer. Concerning Li⁺ effects, it has also been hypothesized that some of the accumulating metabolites, such as CDP-DG or PtdOH, exert an inhibitory effect on signaling from PLC-coupled receptors (87, 90, 727). There are a number of other effects of Li⁺ unrelated to PtdIns synthesis, such as the inhibition of the GSK3β enzyme (739), but those are not subject to this review.
V. PHOSPHOINOSITIDE KINASES

As mentioned above in the historical overview, PI kinases were described as early as the 1960s, and it was already understood that separate activities converted PtdIns to PtdIns4P and PtdIns4P to PtdIns(4,5)P₂, defining these two activities as “PI-kinases” and “PIP kinases.” This historical fact determines the terminology of these enzymes and their classification. Some of the enzymes can phosphorylate both PtdIns and phosphorylated PPIs in vitro, but in the following sections they will be discussed according to what is (or was) believed to be their primary enzymatic function within the intact cells. This may be a deviation from other reviews that discussed the enzymes only according to the position of the inositol ring that they can phosphorylate.

A. PtdIns Kinases

1. PtdIns 4-kinases

PtdIns 4-kinases (PI4Ks) were initially believed to be the only PtdIns kinases. Only after discovering that type I PI kinases were actually PI 3-kinases, had the PI4Ks been so named. That is why PI4Ks were left with the type II and type III PI4K designations. Initial reports on PI4K activities suggested that the enzymes were associated with the PM (1043), and consistent with this notion, they were also present in red blood cell membranes (636). However, distinct activities present in ER membranes showing differential sensitivities to detergents such as cutscum were already noted by early studies (280, 283, 577, 1043). Purification of these activities from a variety of membranes was then carried out (525, 651, 1233, 1658, 1705). This required solubilization of the membranes with detergents yielding an activity of ~55 kDa termed type II PI4K. This tightly membrane-bound activity could be reactivated after separation and extraction from SDS gels and showed high affinity for ATP (Kₐ ≈ 10−50 µM), potent inhibition by adenosine (Kᵢ ≈ 10−10−70 µM), stimulation by detergents, and inhibition by Ca²⁺. Another PI4K activity was described in cholate extracts of bovine brain with larger size, lower ATP affinity, and lower adenosine sensitivity, and it was termed type III PI4K (403). A soluble, PI4K activity was also identified based on its sensitivity to PI 3-kinase inhibitors (1112), which showed the enzymatic properties of type III PI4Ks (386) and was then separable to a larger (210–230 kDa) and smaller (110 kDa) entity (89). The first PI4Ks were cloned from Saccharomyces cerevisiae and were designated as Pik1p and Str4p (444, 1778). The mammalian homologs of these enzymes cloned from various species (89, 504, 1036, 1106, 1107, 1725) were then identified as PI4K type IIβ and -α, respectively. Although the type II PI4Ks were known first with several attempts for isolation, their successful purification and cloning happened only later when two groups independently cloned PI4K type IIα (108, 1058) followed by the identification of another closely related form, PI4KIIβ (83, 1058). The structural features of PI4K enzymes are shown in Figure 3.

2. PI4KIIIa/PI4KA

Most of what we know about this enzyme was derived from studies in S. cerevisiae (1479). The yeast ortholog STT4 was initially identified in a screen that sought staurosporine-sensitive mutants (1778). Deletion of STT4 results in an osmo-remediable phenotype with defects in cell wall integrity (64, 1778). The connection to PKC1 was first revealed in that Str4p was found to be essential to supply the Mss4p PIP 5-kinase with its substrate, PtdIns4P, to synthesize the PM pool of PtdIns(4,5)P₂ (63, 64). The PM pool of PtdIns(4,5)P₂ anchors Rom2p, an exchange factor that activates the Rho1p GTPase, which, in turn, is an activator of Pkc1p (63). Pkc1p kinase is a well-documented hub for several signaling pathways that are essential for cell wall integrity (892). Synthetic genetic array analysis of Str4p revealed a strong connection to sphingolipid metabolism, again via the control of PM PtdIns4P pools and recruitment of Slm1p and Slm2p proteins (1510). Further defect related to an insufficient supply of the PM with PtdIns4P in temperature-sensitive Stt4 alleles was the failure of the actin cytoskeleton to properly organize (64). Such cells are also unable to recruit the p21-activated kinase, Cla4p, a direct downstream effector of Cdc42, to the sites of polarized growth (1717) and to assemble septin at the bud neck (140).

However, in addition to the roles of Str4p in supplying the PM with PtdIns4P, several observations suggest that the kinase also affects processes linked to internal membranes. First, Strt4p generates a pool of PtdIns4P that is degraded by the ER resident Sac1p phosphatase (455). Although recent elegant studies showed that Sac1 in the ER still can access the PM PtdIns4P pool in trans (1454), it is likely that Strt4p is also involved in the synthesis of additional PtdIns4P pools found in the ER membranes. This possibility is supported by studies in which Strt4p could rescue defects in aminophospholipid transfer between the Golgi and the ER (1580) and that temperature-sensitive Stt4p mutants also display lysosomal defects (64). Moreover, Strt4p function was genetically linked to some of the Sec14 homologs (SFHs), which are lipid transfer proteins (1303), some of them connected to aminophospholipid transport (1737). Interestingly, Strt4p is also the major target of Wm in yeast, since neither the yeast PI 3-kinase, Vps34p, nor the other PI 4-kinase, Pik1p is particularly sensitive to this inhibitor (311).

As far as regulators of Strt4p are concerned, temperature-sensitive alleles of Str4p can be rescued by overexpression of Sfk1 (suppressor of four kinase 1), a multispanning membrane protein with yet unidentified functions (63). Intriguingly, two other proteins, Ypp1p (79, 1792) and Efr3p, were described as regulators of Str4p and organizers of the kinase into special signaling domains at contact zones between the ER and the PM (79, 980). Ypp1 also targets the
Parkinson-related protein, A30P α-synuclein to the vacuole for degradation in yeast, again pointing out the possible role of Stt4p at internal membranes.

The biology of Stt4p orthologs (PI4KA or PI4KCA) in higher eukaryotes is much less understood. This enzyme also seems to be primarily responsible for the generation of the PM pool of PtdIns4P in cultured cells (82, 84), even though its primary location was originally described at the ER/Golgi interface (1107, 1726). Recent studies, however, showed that PI4KA was localized to the PM with the aid of the TTC7 and EFR3 proteins, which are homologs of the yeast Ypp1 and Efr3 proteins (1114). Importantly, this feature was linked to the presence of an NH2-terminal short sequence in PI4KA that has been missed in previous studies. Morpholino-induced downregulation of PI4KA in zebrafish embryos caused massive and complex developmental defects especially affecting the brain and were characterized by lack of pectoral fins. This latter defect could be traced to impaired Fgf signaling and was phenocopied by PI 3-kinase inhibitors consistent with a decreased supply of PtdIns(4,5)P2 at the PM (959). It is notable that PI4KA shows the highest expression in the brain both during development and in adult rodents (1107, 1828). At the cellular level, the enzyme was also found associated with nucleoli, but the biological significance of this finding is not known (748). A series of recent studies using RNAi screens to search for cellular host factors required for the infection cycle of hepatitic C virus (HCV), identified PI4KA as a critical protein for HCV replication in the liver (122, 166, 1513, 1577, 1597). Since HCV-infected cells develop a special replication organelle, the “membranous web,” it is possible that the PI4KA is needed for the formation of this organelle (1082). This is supported by the finding that PI4KA interacts with the HCV
viral protein NS5A (13), which by itself can generate membrane structures resembling the “membranous web” (1082). Since PI4KA has also been linked to COPII positive ER exit sites (154, 425), it is a possibility that the “membranous web” originates from the ER. Studies are underway in several laboratories to untangle this process and to test whether inhibition of the kinase is a viable strategy to combat HCV infections.

3. PI4KIIβ/PI4KB

The yeast homolog of PI4KB, called Pik1p, was the first PI4K purified and cloned in the Thorner laboratory (444, 445). Interestingly, the same gene was cloned using a monoclonal antibody that was raised against NUO135, a protein part of the nuclear pore complex (493). The PIK1 gene is essential, which clearly demonstrates that Sec4p and Pik1p assume nonredundant functions in yeast. Pik1p inactivation by temperature-sensitive alleles causes ~50% decrease in cellular PtdIns4P and PtdIns(4,5)P2 levels (64, 1655). Pik1ts strains show greatly distorted and exaggerated Golgi membranes, vacuole fragmentation, and defective actin polarization at the budding pole (64, 1655). Certain Pik1ts alleles also show cytokinesis defects (493, 1655). Pik1p localizes to the Golgi and the nucleus (493, 1478, 1655) and regulates trafficking in the late secretory pathway (64, 555, 1655). Pik1p is localized to the Golgi in the yeast by interaction with Fpr1p, the yeast ortholog of the small Ca2+ binding protein frequenin (also called NCS-1 in mammalian cells) (reviewed in Ref. 615) (599). Fpr1p is essential for Pik1p Golgi localization (1478), and the Fpr1p binding site was mapped between residues 125–169 of Pik1p, a region adjacent and downstream of the lipid kinase unique (L Ku) domain characteristic of PI3Ks and type III PI4Ks (672). The Golgi recruitment of Pik1p is also controlled by Arf1, and the enzyme also interacts with the Arf1 exchange factor Sec7 (512). Pik1p shuttles between the nucleus and the cytoplasm, and both the nuclear and Golgi localizations of the enzyme are required for viability (1478). Phosphorylated Pik1p interacts with 14-3-3 proteins, and this interaction stabilizes the protein in its active conformation (578). Mammalian PI4KB also shuttles between the cytoplasm and the nucleus, but the nuclear function(s) of the enzyme remains to be determined (332).

Recent studies reported that PI4KB is a key host enzyme in the replication cycle of small RNA viruses that reorganize the host cell ER-Golgi membrane structure to establish a replication platform (656). Intriguingly, some HCV strains also require this enzyme for replication and inhibitors of PI4KB potently slow down viral replication (656). This identifies PI4KB as a potential therapeutic target in antiviral strategies.

No studies have been published with targeted deletion of PI4KB in mammalian organisms. Disruption of the gene Fwd encoding PI4KB in Drosophila results in male infertility due to a cytokinesis defect during spermatogenesis (182). Since PtdIns(4,5)P2 is important in the formation of the cleavage furrow during cytokinesis (436, 1728), it seems that Fwd is the only PI4K that can supply the PtdIns4P for cytokinesis during spermatogenesis in Drosophila. Gene disruption of both PI4KB (AtPI4Kβ1 and its sister, AtPI4Kβ2) in Arabidopsis thaliana manifests in a defect in root hair growth (1237). These enzymes facilitate budding of vesicles.
from the TGN and associate with the TGN-localized RabA4b protein (a Rab11 homolog of the plant) at the region corresponding to the Rab11 interaction site in PI4KIIIβ. This process seems to be essential for polarized secretion and hence for root hair extension. Similarly to yeast and mammalian cells, the Arabidopsis homolog of frequenin interacts with the NH2-terminal domain of AtPI4Kβ1 to add Ca2+ regulation to the enzyme (1237).

4. Type II PI 4-kinases/PI4K2s

Early biochemical studies using mammalian tissues characterized what has become known as the type II PI 4-kinases. However, cloning of the genes for this group of proteins had to wait for a while (108, 1058). Two forms of the enzymes exist in vertebrates, the type IIα (PI4K2A) and type IIβ (PI4K2B) enzymes, with very similar features. Little information unique to PI4K2B is available as of to date; therefore, the two isoforms will be discussed together. Yeast and other lower organisms only have one form of the protein.

Type II PI4Ks are tightly membrane-bound proteins, due to the palmitoylation of a conserved stretch of cysteines within their catalytic domains (108, 109). In spite of a similar palmitoylation sequence within the two isoforms, a larger fraction of PI4K2B than PI4K2A is found in the cytosol (83, 1690) and the cytoplasmic fraction of PI4K2B is not palmitoylated (743). Based on early studies in which the type II PI4K activity was found in PM fractions, and in red blood cell membranes, it was logically assumed that the type II PI 4-kinase produces PtdIns4P in the PM. Therefore, it was surprising to find the majority of PI4K2A and PI4K2B enzymes in intracellular membranes, mostly associated with the TGN and endosomes by immunocytochemical analysis (83, 1060, 1680). It has been shown that PtdIns4P in the Golgi/TGN is critical to the membrane recruitment of various clathrin adaptors, such as the heterotetrameric AP-1 (1680) and monomeric GGAs (1509, 1671) and that PI4K2A, rather than the type III PI4KB, was the important enzyme in this process (1671, 1680). PI4K2A also shows association with a vesicular pool rich in the adaptor protein AP-3 (1330), and it directly interacts with AP-3 via a sorting motif found in the enzyme, which then acts both as a regulator and a cargo (298). Both the catalytic activity of the kinase and the direct interaction with AP-3 are important in supporting AP-3-mediated trafficking (298). An additional role of PI4K2A in EGF receptor trafficking was indicated by studies showing that EGFR targeting and degradation in lysosomes were found to be impaired after RNAi-mediated knock-down of the enzyme (1060). Similarly, PI4K2A was found important for the lysosomal trafficking of the glucocerebrosidase enzyme (741). Undoubtedly, type II enzymes are also present in the PM, either under unstimulated conditions (PI4K2A) or after stimulation with PDGF (PI4K2B) (1690). Based on mass measurements, ~50% of the total cellular PtdIns(4,5)P2 pool is synthesized via Wm-sensitive PI4Ks (1615), but what fraction of this is found in the different membranes has yet to be determined.

Regulation of the type II PI4Ks is poorly understood. An important feature of the type II PI4Ks is their association with cholesterol and sphingolipid-rich membrane domains (1685). The cholesterol content of these membranes has a big impact on both enzyme activity and interaction of the protein with regulatory factors (1059, 1686). Recent studies found that the palmitoylation of the PI4K2A enzyme in the Golgi is regulated by cholesterol (949). Calcium inhibits both type II PI4Ks, and membrane association increases PI4K2B activity (1690). Remarkably, the activity of type II PI4Ks varies between the isoforms, the PI4K2A enzyme being most active, while the PI4K2B form has much lower activity (83). The enzyme activity shows good correlation with palmitoylation and membrane association. In addition to palmitoylation, membrane attachment of these enzymes is also determined by the COOH-terminal part of the catalytic domain (109). The yeast ortholog LSB6 (also termed PIK2) (562, 1392) shows only very moderate PI4K activity and makes minor contribution to the overall PtdIns4P production of yeast cells under normal growth conditions. It also lacks most of the cysteines that are palmitoylated in the mammalian enzymes (562, 1392). Importantly, inactivation of LSB6 causes only a mild alteration in the trafficking of the endocytosed mating factor receptor. This defect is rescued by a construct that does not contain the catalytic domain but requires regions that interact with Las17p, the yeast homolog of WASP, a protein that is important for regulation of actin polymerization (230). It is possible that LSB6 acts as a scaffold and regulates the movements of vesicles; the lipid kinase activity of LSB6 may not be crucial for normal functions in the yeast.

A gene-trapped mouse with a truncated PI4K2A has been thoroughly investigated. The homozygous mice develop and are born normal in spite of the lack of detectable PI4K2A protein, but they succumb to late-onset spinocerebellar degeneration (1408). These animals develop severe lipofuscin-like depositions in the cerebellum associated with gliosis, and lose most of their Purkinje cells. They also show massive axonal degeneration in the spinal cord and die prematurely (1408). However, a screening study on patients suffering from autosomal recessive hereditary paraplegia, a human disease highly similar to the pathologies of PI4K2A-deficient mice, failed to identify pathogenic changes in the PI4K2A gene (270). Little is known about type II PI4Ks in other organisms. Zebrafish already contain both isoforms of the type II enzymes (959), while there is only one gene found in Drosophila (110). Arabidopsis contains eight genes encoding proteins with homology to the type II PI4Ks, six of which contain ubiquitin within their coding sequences (1092). Since ubiquitination is an important means of tagging proteins, including EGF receptors destined for endocytosis and degradation (547), this observa-
tion is a further hint that type II PI4Ks are regulators of endocytosis and they may direct endocytosed proteins for degradation.

5. **Class III PI 3-kinase/PI3KC3**

These enzymes are usually discussed with the PI 3-kinases, but because of their substrate restriction they are, in fact, PtdIns kinases and, therefore, will be discussed in this paragraph. The yeast enzyme, VPS34, was the first cloned PtdIns 3-kinase (1367). As indicated by its name, this protein had been initially identified in screens for *S. cerevisiae* mutants that develop vesicular sorting defects (Vps) (101, 1300). Only after the realization that the COOH-terminal half of Vps34p shows very high sequence homology with the mammalian type I PI 3-kinase 110 kDa catalytic subunit (614) was it understood that Vps34p is a PI 3-kinase (1367). Yeast cells with deleted Vps34p lack PtdIns 3-kinase activity and the lipid PtdIns3P, indicating that this enzyme is the sole source of this lipid in yeast (1367, 1446). The enzyme is unique among PI 3-kinases in that it can only phosphorylate PtdIns but not further phosphorylated PPIs (1446). Cells with Vps34 mutations or deletions have morphologically intact vacuoles and a normal secretory pathway, but fail to sort certain cargoes, such as the enzyme carboxypeptidase Y (CPY), to the vacuole (604). Vps34p tightly associates with a serine/threonine kinase, Vps15p, that greatly stimulates its kinase activity (1445), and Vps15 mutants display vacuolar sorting defects very similar to those described in Vps34p mutants (605). The production of PtdIns3P by Vps34p is important for transport from the Golgi to a prevacuolar compartment and subsequently to the vacuole (1445). Vps34p is also important for the membrane invaginations in multivesicular bodies (MVB) (70, 71, 765) and for various forms of autophagy (779). In each of these functions, different proteins are associated with the Vps34p-Vps15 complex (779). Accordingly, PtdIns3P exploits its effects by recruiting a variety of proteins that contain PPI binding domains that are attracted to the membrane by this particular PPI species. These include FYVE domains (584, 766, 836), PX domains (239), and perhaps others that are less characterized (486, 1739). The specificity of these interactions is not solely determined by the interaction with PtdIns3P but also by the protein-protein interactions that are unique for the functionally distinct signaling complexes. It is noteworthy that Vps34p is less sensitive to the PI 3-kinase inhibitor Wm (IC50: ~3 μM) than the mammalian PI 3-kinases (~2–5 nM), especially the class I enzymes (1446). Vps34p not only regulates the trafficking of proteins to the vacuole but is also important for autophagy (74, 751), a starvation-induced protein-degradation process targeting cytosol and organelles to the lysosome (1073). Vps34p serves the secretory and autophagy pathways in two different protein complexes: both complexes I and II contain Vps34p, Vps15, and Vps30p, but complex I also has Atg14p and complex II has Vps38p. Complexes I and II regulate autophagy and CPY sorting to vacuoles, respectively (779).

The mammalian ortholog of Vps34p, called hVps34, PtdIns-specific PI 3-kinase or class III PI3K and its adaptor protein p150 have been also cloned (1189, 1642). The mammalian class III PI3K also has a role in endocytic sorting, and its hVps34/p150 complex is recruited to Rab5 positive endosomes (254, 1100) where it produces PtdIns3P, which attracts proteins that contain FYVE domains and PX domains (509, 752, 1409, 1750). hVps34 is also central to autophagy in mammalian cells (1532, 1631), but there is less distinction between the roles of complex I and complex II in this process. This is due to multiple interactions of the hVps34-associated Beclin-1 (the homolog of yeast Vps30p) with distinct protein complexes that regulate autophagy as well as different steps of autophagy (709, 1011, 1818). One of these proteins is UVRAG (functionally related to Vps38) (709), which promotes autophagy either by recruiting curvature-sensing BAR-domain containing proteins (317, 1517) or by enhancing fusion of autophagosomes with lysosomes (908). An additional Beclin 1-interacting protein, Rubicon, is a negative regulator of autophagy that competes with Arg14L (the mammalian Arg14p) for binding Beclin 1 (1011, 1818). In addition, hVps34 is also involved in mediating mTOR activation during amino acid starvation (203). The mammalian enzyme is also less sensitive to Wm than the Class PI3Ks (1464). The structural basis of this difference as well as the potential for isoform specific inhibition of the enzyme has been highlighted in the recently published structure of the protein (1054).

**B. PtdInsP Kinases**

As mentioned above, PtdInsP kinase (PIPK) activities associated with membranes including the red blood cell membrane have been detected by early studies. These activities were thought to produce PtdIns(4,5)P2 from PtdIns4P in the PM as part of the PI signaling cycle, but comprised of two clearly distinguishable activities (type I and type II) with unique immunological and catalytic properties (114, 361, 1504). One of the distinctive features of the type I PtdInsP 5-kinase activity was its strong stimulation by PtdOH (726). It was also a curious early observation that type II PtdInsP kinase was ineffective against native membrane PPIs even though it was active against PtdInsP4 substrates presented as micelles (although with higher Km for the lipid) (114). The first PtdInsP kinase cloned was the type II form (167, 362) (called C-isofrom in Ref. 362) followed by the type I enzymes with a high degree of sequence similarity between the two forms (933). Only later was it discovered that the type II enzymes actually use PtdInsP as substrate adding a phosphate to the 4-position to yield PtdIns(4,5)P2 and that their “5-kinase activity” was due to contamination of the commercial PtdInsP4 preparations with PtdInsP (1254). This explains why the type II enzymes were unable to make PtdIns(4,5)P2 from PtdInsPs of natural membranes.
PIP kinases are soluble peripherally membrane-bound proteins that show a highly conserved catalytic core and differ only in their COOH termini (Figure 4). The substrate specificity of the enzymes is determined by the activation loop within their catalytic domains and can be reversed by swapping activation loops between the type I and type II forms (832). The first reports on the cloning of the mammalian type II enzymes also identified two homologous sequences in the S. cerevisiae genome, Mss4 and Fab1 (167, 362). Mss4p is important for actin organization and membrane morphogenesis (348, 644) while Fab1p is essential for normal vacuolar morphology and functions (498, 1757). Since Fab1, as well as its mammalian homolog PIKfyve (1404), turned out to phosphorylate PtdIns3P to PtdIns(3,5)P2, these enzymes were classified as type III PIP kinases.

1. Type I PIP-kinases

These enzymes are the true PtdIns4P 5-kinases, and several forms have been described in mammalian tissues encoded by three genes (PIPK1α, -β, and -γ) and a number of splice forms within PIPK1γ. Although all three major forms are widely expressed, the relative tissue distribution and the cellular localization of the various isoforms are unique, and

![Figure 4](http://physrev.physiology.org/)
From this description it is clear that Mss4 controls 

2. PIPKι/PIP5K1C

PIPKι is the most versatile PIP 5-kinase, as it comes in several splice forms: PIPKι87, -γ90 (708), and a neuronal-specific -γ93 (510, 1741) (FIGURE 4). Aditonal forms of 700 and 707 amino acid forms have been found in human (1359) and another 90-kDa form in the rat (1741). Since the exon structure differs between the rodent and human genes, and different splice forms have similar apparent molecular weights, the designation of these splice forms becomes somewhat complicated (1359, 1741). The various PIPKι forms are now named PIPKι-v1–6 in human and PIPKι-v1,2,3,6 in the rat (1359, 1741) (it is not clear if the 1y-4,5 forms exist in rodents). In most tissues the 87-kDa form (PIPKι-v1) is prevalent, but brain expresses mostly the 90-kDa (PIPKι-v2) variant. Both the 87- and 90-kDa forms localize to the PM, but the 90-kDa form also localizes to focal adhesions via interaction with talin (353, 913). The interaction with talin is controlled by a balance of Tyr and Ser phosphorylations at the COOH terminus of the 90-kDa form of the kinase (875, 914). PIPKι93 (PIPKι-v3) is also localized to the PM, but the 707 amino acid human splice form (PIPKι-v5) shows both PM and intracellular vesicular localization (511, 1359), and the 700 amino acid form (PIPKι-v4) is found in nuclear speckles (1359). These localization patterns cover all the compartments where the α and β forms of type I PIPKs are found. Substrate (PtdIns4P) binding is a major factor in the PM localization of PIPKι (as well as α and -β) (832), although there are other protein-protein interactions that are suspected to contribute to membrane localization (511). For example, Rac binding makes a significant contribution to the PM binding of the PIPKιβ (553). Functionally, PIPKι has been linked to endocytosis via interaction with the AP-2 adaptor and clathrin (80, 1550) and by promoting actin polymerization at the PM (398). A similarly important role of the enzyme was found in establishing adherent junctions via regulation of E-cadherin trafficking (912) and for EGF-driven directional migration (1498). Both the invasion and proliferation of breast cancer cells was found highly dependent on PIPKι (1499). PIPKι is the major PtdIns(4,5)P2 synthesizing activity in the brain and the synapse in particular (1704), and it plays critical roles at several steps in the synaptic vesicle exocytosis-retrieval cycle (352). Outside the brain, expression of PIPKι90 increases the pool of rapidly releasable dense core vesicles in chromaffin cells (1056), while elimination of the enzyme decreases the same pool and causes a defect in vesicle priming (520). Finally, PIPKι87 was found uniquely responsible for the generation of the PtdIns(4,5)P2 pools for agonist-stimulated Ins(1,4,5)P3 increases and Ca^{2+} signaling in HeLa cells (1679), and mast cells lacking PIPKι (both the 87- and 90-kDa forms) showed greatly reduced response to IgE receptor cross-linking (1621).

The activity of PIPKι is regulated by the small GTP-binding protein Arf6 (824) and by binding to AP-2 adaptor
complexes (80, 1113). Stimulation of the enzyme by PtdOH has also been well documented, but it needs further studies to clarify if this regulation occurs in a physiological setting. PIPK1γ knockout mice develop normally but die soon after birth probably because of lack of suckling due to generalized neuronal defects (352). Interestingly, in another study, in which PIPK1γ was inactivated by a gene-trap method, the mouse exhibited embryonic lethality at E11.5 and showed cardiovascular and neural tube closure defects (1677). The lack of this enzyme also impairs anchoring the membrane to the cytoskeleton in megakaryocytes and platelets (1678). A mutation abrogating the kinase activity of PIPKI and unable to recycle to the PM (189). There have been reports, however, on changes specific to particular isoforms. For example, PIPKI expression manifests in actin comet formation (1305), increased number of stress fibers (1759), increased membrane ruffling (646) and forced the appearance of internal vesicles that are coated with actin fibers (1759), increased membrane ruffling (646) and forced the appearance of internal vesicles that are coated with actin fibers (1759), increased number of stress fibers (1759), and - mediated phagocytosis (172). Here, PIPKI was shown to localize at the trailing appendage of differentiated HL60 cells and knockdown of PIPKI interfered with polarization and movement of these cells during chemotaxis (845). Another special case of highly coordinated membrane activity where specific PIP5Ks are important is FcγR-mediated phagocytosis (172, 289). Here, PIPKIα is recruited to the phagocytic cup, and a kinase dead version of the enzyme blocks the process as well as the PtdIns(4,5)P₂ accumulation associated with it (172, 289). It has been suggested that PtdIns(4,5)P₂ is required for the actin polymerization during the pedestal formation to engulf and initiate ingestion of the phagocytic particle (172), but PtdIns(4,5)P₂ needs to be eliminated for closure of the phagocytic cup (1370, 1766). However, recent studies suggest a more complicated mechanism, where PIPKIγ-deficient bone marrow-derived macrophages (BMM) showed the presence of highly polymerized actin and impaired attachment of the opsonized particles. In contrast, BMMs from PIPKIα-deficient mice showed no attachment defect, but instead a problem with particle ingestion (989). Remarkably, PIPKIγ-deficient cells showed increased Rac activity and decrease Rac1 ratio remedied the attachment defect (989). PIPKIα-deficient BMMs, on the other hand, had problems extending their pseudopods due to impaired WASP and Arp 2/3-dependent actin polymerization. These studies clearly showed that distinct PtdIns(4,5)P₂ pools delivered by distinct kinases had functionally unique roles in a highly orchestrated process of membrane remodeling.

Not all of the effects of type I PIP5Ks are linked to actin polymerization. PIP5Kβ promotes transferrin receptor endocytosis in HeLa cells unrelated to its effects on actin polymerization by increasing the membrane association of AP-2 adaptor protein complexes (1185). Conversely, PIP5Kβ knockdown by RNAi (but not the other forms) inhibits the same process (1185). In another study, PIPKα was found to regulate EGF receptor endocytosis (106) and an N-terminally truncated PIPKIα was described as a potent inhibitor of the removal of colony stimulating factor-1 receptors from the cell surface (326). As mentioned above, it is the PIPKIγ isoform that regulates clathrin-mediated endocytosis in neurons (80, 824).

In most of these actions, PIP5Ks are working tightly coupled with small GTP-binding proteins, which can act both upstream and downstream of the PIP5K. RhoA was found to mediate the effects of PIP5K on stress fibers (1759) but was also found to act via a PIP5K (1010). Similarly, both PIPKIγ and -β were found to associate with Rac1 regardless of the GTP-GDP status of the latter, but only PIPKβ association regulated actin assembly (1560). On the other hand, the effects of PIP5K on ezrin membrane localization were found to be Rac1 dependent (68). Arf6 was also found to recruit both PIPKIα and -β to the PM and stimulate PIPKIβ activity (646), and constitutively active Arf6 mimicked many of the effects of overexpressed PIP5K (189). Arf6 also can indirectly regulate PIP5K by recruiting PLD2 to membrane ruffles where production of PtdOH by PLD can further activate PIP5K activity (1415). Type Iβ PIP kinases are also regulated by phosphorylation. Oxidative stress decreased PtdIns(4,5)P₂ levels by releasing PIPKIβ from the PM (244, 554). This effect was due to Tyr phosphorylation of PIPKIβ by a Src family kinase (554) that was later identified as Syk and the phosphorylated residue as Y105 (244). Syk also phosphorylates PIPKIγ at Thr87 (but not the α form) during phagocytosis (989). In another paradigm, hypertonic osmotic stress increases PIPKIβ activity by Ser/Thr dephosphorylation (1758).

In addition to being associated with membrane dynamics, an exciting role of PIPKIα in the nucleus has recently been revealed. The existence of a nuclear PI system has been appreciated and studied for quite some time (see sect. X), but the exact processes that are regulated by the PI lipids and their modifying enzymes have been elusive. The presence of endogenous PIPKIα in nuclear speckles at the sites of pre-mRNA processing has been described earlier (168). Recent studies described the association of a noncanoni-
PIPKI\(\alpha\) knockout mice (lacking the mouse PIPKI\(\beta\)) appear normal and develop to adulthood, although the number of offspring is lower than expected by the Mendelian ratio (1676). Interestingly, the platelets of these mice showed a defect in thrombin-induced InsP\(_3\) increases and aggregation that could be overcome at high agonist concentrations (1676). Because of the lack of any major abnormalities, it is likely that the other type I PIPK can assume the membrane-associated functions normally performed by PIPKI\(\alpha\), and as for nuclear functions, the recently described PIPK\(\gamma\) 700 amino acid form found in nuclear speckles (1359) may be able to substitute for the PIPKI\(\alpha\) enzyme. PIPKI\(\beta\) knockout mice (lacking mouse PIPKI\(\alpha\)) are also born and develop normally but show highly enhanced degranulation response of their mast cells after FceR-I crosslinking and enhanced cutaneous and systemic anaphylaxis (1344). This effect is explained by a defect in the stability of the actin cytoskeleton.

4. Type II PIP kinases PIP5K2s

Although the type II form was the first member cloned from the PIP kinase family (167, 362), the role(s) of these enzymes has remained largely elusive. Structurally, they are highly homologous to the type I enzymes (FIGURE 4) but, as pointed out above, they use PtdIns5P as substrate and convert it to PtdIns(4,5)P\(_2\) (1254). Understanding the function of the kinases is closely coupled to understanding the distribution, the pathway of production, and function(s) of the minor PI species PtdIns5P. There is much to be learned in this area, but the picture emerging from recent studies indicates that type II PIPKs (also called PISP 4-kinases) may be more important in controlling PtdIns5P levels than to contributing to PtdIns(4,5)P\(_2\) production (267, 737). Mammalian type II PIP kinases also exist in three forms: -\(\alpha\), -\(\beta\), and -\(\gamma\) (710, 934). Functional data suggest that PIPKI\(\alpha\) is enzymatically the most active, while -\(\beta\) has moderate and -\(\gamma\) very low catalytic activity using the standard in vitro assay conditions (197, 266). This is relevant as PIPKII enzymes form dimers, and it has been speculated that the less active forms may just serve as targeting devices to localize the active PIPKII\(\alpha\) enzyme to specific cellular locations (197, 263, 1672). The X-ray analysis of PIPKII\(\beta\) crystals also shows the protein in a dimeric form (1257). In fact, PIPKII\(\beta\) is localized both in the cytosol and to nuclear speckles, and it can bring PIPKI\(\alpha\) into the same nuclear location (197, 1672). PIPKII\(\gamma\), on the other hand, shows special tissue distribution being highly enriched in the kidney (266, 710) where it is mostly found in the epithelial cells of the thick ascending limb associated with intracellular vesicles (266). Notably, in other reports, the expressed enzyme was found ER-associated (710). The enzyme is also expressed in nervous tissues in neurons, again associated with endomembranes (265). Given the very low enzymatic activity of this form, it has been speculated that PIPKII\(\gamma\) is a scaffold that brings the active -\(\alpha\) enzyme to special membrane compartments (267).

Functionally speaking, there are very few studies addressing the cellular role(s) of PIPKII enzymes. RNAi-mediated knockdown and overexpression studies showed that PIPKII\(\alpha\)-mediated PtdIns(4,5)P\(_2\) formation was important for secretion in platelets (1307, 1308) and that the enzyme was also involved in platelet formation during development of the demarcation membranes in megakaryocytes (1368). Membrane recruitment of PIPKII\(\alpha\) could be mediated by interaction of the kinase with type I PIP kinases (620). A cytosolic and membrane-related role of PIPKII\(\beta\) was suggested by association of the protein with the TNF (223) and EGF (222) receptors. Deletion of PIPKII\(\beta\) in mice increases insulin sensitivity by enhancing Akt activation in muscle (848), and overexpression studies suggest that the enzyme attenuates insulin signaling (218). In agreement with these data, generation of PtdIns5P in the PM by the bacterial phosphatase IpgD enhances Akt activity (1206) presumably by altering EGF receptor trafficking and signaling (1255). The nuclear functions of PIPKII\(\beta\) have also been emerging. Ultraviolet irradiation increased nuclear PtdIns5P levels by a cascade of events that included p38 MAP-kinase mediated phosphorylation of PIPKII\(\beta\) at Ser236 leading to decreased activity. Decreased activity of the kinase led to the elevation of PtdIns5P, which, in turn, interacts with the ING2 protein via a PHD domain. PHD2, a nuclear adaptor protein controls p53 and histone acetylation, and PtdIns5P binding to PHD2 changes its association with the chromatin (737). In light of the recent discovery that the majority of the kinase activity associated with PIPKII\(\beta\) can be attributed to the -\(\alpha\) enzyme as part of a heterodimer (197, 1672), it was important to show that Ser236 phosphorylation of -\(\beta\) does not affect its association with -\(\alpha\), and it is likely that the decreased activity of the complex is due to an allosteric regulatory mechanism (197).

5. Type III PIP kinases/PIP5K3

Type III PIP kinases phosphorylate PtdIns3P to PtdIns(3,5)P\(_2\). The yeast ortholog, named Fab1, was first described based on a genetic screen for defects in nuclear segregation (1757). The cloning of the 257-kDa protein revealed a strong sequence similarity with the first cloned type II PIPK within its putative catalytic region (1757). Fab1 contains a PtdIns3P binding FYVE domain close to its NH\(_2\) terminus (FIGURE 4). Although FAB1 is not an essential gene, fab1 null or temperature-sensitive mutant cells grow slowly and show defective spindle orientation resulting in haploid and binucleated daughter cells (1757). Fab1 null cells also develop huge vacuoles and display a block in the transport of certain hydrolysates (such as CPY) to the vacuole (498). It is specu-
lated that the chromosomal segregation defect is secondary to the vacuolar defect possibly because of steric interference from the enlarged vacuoles (1757). That Fab1 is a PtdIns3P 5-kinase was revealed when it was found that osmotic stress led to a rapid increase in the level of a novel phospholipid, PtdIns(3,5)P2, in yeast (376), and it was shown that this increase was dependent on functional Fab1 (287). Simultaneously, it was demonstrated that Fab1 deletion or Fab1 mutant cells lacked PtdIns(3,5)P2 (498). Finally, in vitro Fab1 was found to phosphorylate PtdIns3P to PtdIns(3,5)P2 (287, 1017), and consistent with its roles in vacuole physiology, Fab1 was localized to the vacuolar membrane (162, 378, 498). Interestingly, deletion of two other genes, Vac7 and Vac14, also eliminates detectable PtdIns3P 5-kinase activity (162, 378, 498), suggesting that they are upstream regulators of Fab1. Counterintuitively, deletion of Fig4, the phosphatase that converts PtdIns(3,5)P2 back to PtdIns3P (390, 497, 1311), does not increase the level of PtdIns(3,5)P2. Quite to the contrary, it decreases both the basal and osmotic stress-induced increase in the level of this lipid (389, 1311). This curious finding is due to the fact that Vac14 acts as a scaffold that holds Fig4 and Fab1 in a signaling complex (that also contains Vac7 and Atg18) (734), and removal of Fig4 leads to the destabilization of this complex with impaired Fab1 activity (170). Functionally, all these proteins control the level of PtdIns(3,5)P2 as a multivesicular body (MVB) sorting pathway (378, 1159). However, this pathway is also important in autophagy via connection to the Atg18 protein (379) (see more on this in sect. X).

The mammalian type III PIP kinase is an ortholog of Fab1 and is named PIKfyve (1404). PIKfyve shows many similarities to Fab1 both structurally and functionally. It also has a PtdIns3P recognizing FYVE domain and interacts with several proteins that are homologues of the yeast proteins found in complex with Fab1. The mammalian version of the Vac14 scaffold is called ArPIKfyve that holds PIKfyve and Sac3 in one complex (734, 1351). Sac3 is the homolog of Fig4, the 5-phosphatase that converts PtdIns(3,5)P2 back to PtdIns3P (685). Intriguingly, the presence of Sac3 in the complex not only ensures that the kinase is balanced, but curiously, it also keeps PIKfyve in a highly active conformation (683). The biological advantage of this unique phenomenon that maintains a high turnover rate of PtdIns(3,5)P2 is not fully understood. Nevertheless, inhibition of PIKfyve function either by RNAi-mediated depletion (1316), expression of a dominant negative version (684), or pharmacological inhibition (with a selective PIKfyve inhibitor, YM2016636; Ref. 723) leads to massive vacuolization. These vacuoles represent swollen endocytic compartments and are associated with defects in fluid phase endocytosis (684) and in retrograde transport from the endosomes to the TGN (1316). Importantly, while the trafficking and degradation of transferrin and EGF receptors were found unaffected by knockdown studies (684, 1316), acute pharmacological blockade of PIKfyve did affect the degradation of EGF receptors with their accumulation in the membrane of the enlarged vesicles (336, 723). PIKfyve was also found functionally coupled to insulin-induced GLUT4 translocation and glucose uptake (618, 686), and PIKfyve inhibitors lead to the accumulation of the autophagy marker LC3-II in endocytic structures (336). This latter finding also indicates a defect in the fusion of prelysosomal compartments and MVB with lysosomes. PIKfyve-depleted or -inhibited cells also fail to properly acidify their endocytic compartments (723), but it is not clear whether PtdIns(3,5)P2 directly or indirectly affects the activity of the V-ATPases. This latter effect may be related to the recently described PtdIns3P dependence of the lysosomal cation channel, TRPML1 or mucolipin (371). Most recently, PtdIns3P and PtdIns(3,5)P2 generation were found to control the activation and localization of the mTORC1 complex via a direct association between Raptor and PtdIns(3,5)P2 (180). Other recent studies indicated that PIKfyve, together with some of the myotubularin phosphatases that remove the 3-phosphate from PtdIns(3,5)P2 (see sect. VI), are important for PtdIns5P generation in mammalian cells (1173, 1352, 1827). One of these studies also found that PtdIns5P generated via this pathway is a regulator of cell migration (1173).

PIKfyve inactivation in Caenorhabditis elegans (1129) and Drosophila (1315) also leads to swollen endocytic compartments. Germine deletion of PIKfyve in mice is embryonic lethal in the preimplantation stage, and MEF cells from heterozygotes show an increased sensitivity to the PIKfyve inhibitor YM2016636 (682). A debilitating mutation in one allele of PIKfyve causes Francois-Neetens mouchetée corneal fleck dystrophy in humans characterized by multiple focal vacuolization in the cornea (901). The evolutionary conservation of the tripartite PIKfyve/Fig4/Vac14 complex is required for proper PIKfyve function and is underscored by mouse and human genetics. The genetic abberation responsible for the “pale-tremor mouse” phenotype has been identified as an early transposon insertion into the mouse Fig4/Sac3 gene. This also resulted in a decrease rather than an increase in PtdIns(3,5)P2 levels and caused a prominent defect at the late-endosome to lysosome transition (and probably in many other trafficking steps) responsible for the pigmentation defect and a progressive neurodegeneration (253). Pathogenic mutations in the human FIG4 gene have also been identified in a novel form of autosomal recessive Charcot-Marie-Tooth disorder, CMT4J, characterized by motor and sensory neuropathy (253) and in a small percentage of patients with amyotrophic lateral sclerosis (ALS) (252). Similarly, a Vac14 gene-trap mouse showed early newborn lethality associated with rapid neurodegeneration with massive vacuolization and cell death in certain brain areas. Since the development of these animals appears to be normal and their other organs are not as severely affected, the lack of Vac14 only compromises but does not completely eliminate PIKfyve function (1810). It is
a fascinating question why only certain neurons mainly after birth are so dependent on PtdIns(3,5)P₂ function.

C. PtdIns(4,5)P₂ Kinases

The first indication that PtdIns(4,5)P₂ can be further phosphorylated was the detection of novel lipids, PtdIns(3,4,5)P₃ and PtdIns(3,4)P₂, in 1989 by Taynor-Kaplan in human neutrophil cells (1572). In parallel studies it was discovered that type I PtdIns kinases could phosphorylate the 3-position of PtdIns (1711) and that the PI 3-kinase activity associated with oncogenes and stimulated growth factor receptors primarily produced PtdIns(3,4,5)P₃ and, in fact, was a PtdIns(4,5)P₂ 3-kinase (66). These early reports have marked the beginning of the explosion of the PI 3-kinase field that unraveled the existence of several classes of PI 3-kinase enzymes and their regulatory adaptor proteins (FIGURE 5). The 110-kDa catalytic domains of class I PI 3-kinases are encoded by four distinct but highly related genes in mammals (α, β, γ, and δ). In addition, five

FIGURE 5. The family of PI 3-kinase enzymes. Class I PI 3-kinases phosphorylate PtdIns(4,5)P₂ to PtdIns(3,4,5)P₃ and PtdIns(3,4)P₂. There are four different genes coding for the catalytic subunits of PI3Ks, called p110α, p110β, p110γ, and p110δ. They all have a conserved COOH-terminal catalytic domain preceded by lipid kinase unique (LKU, also called “helical”), C2 and Ras binding domains (Ras-BD). These enzymes have tightly associated regulatory subunits: p110α, p110β, and p110γ associate with p85 and p55/p50 regulatory subunits encoded by three different genes. Their association is mediated by an interaction between the very NH₂-terminal p85-binding region (p85BR) of the catalytic chains with the inter-SH2 (iSH2) region of the regulatory subunits. These enzymes are also called class IA enzymes. The catalytic p110γ differs from the previous forms (hence it is called class IB) in that it associates with either of two adaptors, p101 and p84/p87. These different adaptors interact with NH₂-terminal adaptor binding region (AdBR) of p110γ and lend regulation by G protein βγ subunits (p101) and perhaps by Ras (p84/p87) to the enzyme. Class II PI3Ks also come in three forms (α, β, and γ) and contain PH-domains (PX) and C2 domains placed COOH terminally to their catalytic domains. These enzymes can phosphorylate PtdIns and PtdIns4P in vitro, but their in vivo substrate preference is still debated. The enzymes most likely form PtdIns3P in the cell. The single class III PI3K and its yeast ortholog, Vps34p, phosphorylates PtdIns to PtdIns3P. These enzymes associate with a larger regulatory protein Vps15p in yeast and p150 in mammalian cells (not shown).
distinct genes encode tightly associated PI 3-kinase regulatory proteins, p85α (with several splice variants), p85β, p55γ, p101 and p84/p87, the latter two serving as adaptors for the p110γ enzyme. Class II PI 3-kinases also exist in three forms (α, β, γ). These are larger proteins (150–180 kDa) with relatively little known about their cellular regulation and functions. These enzymes were believed to phosphorylate PtdIns(4)P to PtdIns(4,5)P2 until recently, when increasing evidence suggests that their primary product in vivo is PtdIns3P (418, 1700) and, hence, they function as PtdIns 3-kinases rather than PtdIns4P or PtdIns(4,5)P2 kinases. However, because of the still lingering uncertainty about their in vivo substrate preference, these enzymes will be discussed here. The only class III PI 3-kinase, hVps34, is usually discussed with the PI 3-kinases, but because of its only substrate being PtdIns, it was already covered with the PtdIns kinases.

Class I PI 3-kinase activation leads to the production of PtdIns(3,4,5)P3 in the PM, and this molecule is the ultimate initiator of a great variety of cellular signaling responses and cascades of molecular events. A chief mediator of PtdIns(3,4,5)P3 actions is the protein kinase Akt (also called PKB) that is recruited to the PM by PtdIns(3,4,5)P3 via its PH domain where it gets phosphorylated on Thr308 and Ser473 by PDK1 (1081) and mTORC2 (1830), respectively, leading to its increased kinase activity. Interestingly, PDK1 also has a PH domain, and its recruitment to the membrane is also aided by PtdIns(3,4,5)P3 (816). Recent data suggest that mTORC2 may also be regulated by PtdIns(3,4,5)P3 (489, 1430). Activated Akt phosphorylates a whole range of substrates to regulate energy metabolism, survival, cell cycle progression, or differentiation depending on the tissue and the signaling context under which it is activated. These pathways will be detailed in section IX. Additional molecules regulated by PtdIns(3,4,5)P3 are guanine exchange factors (GEFs) for a range of small GTP binding proteins, such as Arfs (1627) and Rac/Rho/Cdc42 (608, 1699) or the Tec family tyrosine kinases such as Bruton’s tyrosine kinase (Btk) (1261) or Itk (44), key components of B- and T-cell activation, respectively. PtdIns(3,4,5)P3 gradients have a major role in the migrational response of cells to chemotactic stimuli (459), a response not only serving phagocytic cells, but also being critical for morphogenic patterning, axon extension, blood vessel formation, and for invasion and metastasis by cancer cells. The contribution of the individual isoforms and their regulatory subunits to these processes during development and to the functions of various cells and tissues is only beginning to unfold with the help of genetic deletion of the individual enzymes in whole animals or in selected tissues and with the availability of subtype-specific PI 3-kinase inhibitors.

Class I PI 3-kinases control several cellular processes such as cell energetics and metabolism or immune cell functions, and they are also key players in cancer development, propagation, and metastasis. These important roles have propelled PI 3-kinase research into ever-increasing heights. In light of the incredible progress made in this research area, it seems ironic that PI 3-kinases were initially deemed non-druggable targets because of their fundamental roles in supporting almost every aspect of a cell’s life. Contrast this with the fact that several subtype-specific PI 3-kinase inhibitors are now in development and some are being tested in clinical trials. Due to the enormity of the PI 3-kinase literature, its coverage in this review will be inevitably limited and focused only on fundamentals. Several excellent reviews have summarized various aspects of this topic in greater details in recent years (75, 221, 467, 814, 996, 1609, 1612, 1613, 1640, 1727, 1785).

1. Class I PI 3-kinases

PI3Kα/PI3KCA is a widely expressed and most studied member of the class I PI3K family. The 110-kDa protein contains a COOH-terminal catalytic domain that is highly homologous to those found in other PI 3-kinases, in type III PI 4-kinases (190, 505), and in PI kinase-related kinases (669). This group of catalytic domains is also highly conserved from yeast to human, although yeast has only a class III PI 3-kinase, Vps34. The domain architecture of these enzymes is shown in FIGURE 5. PI3Kα is tightly associated with a regulatory subunit encoded by the p85α gene with three splice forms: p85α, p55α, and p50α (468, 699, 1178). p85α has an NH2-terminal SH3 domain, a BH (BCR homology) domain, proline-rich regions, and two SH2 domains linked with a coiled-coil iSH2 (inter-SH2) region, which is one of the major sites of interaction with the NH2 terminus (1053) and C2 domains (660) of the catalytic p110α subunit. The shorter variants, p55α and p50α, lack the NH2 terminus of p85α including the SH3 and BH domains. The classical activation sequence of PI3Kα occurs during receptor tyrosine kinase activation, with recruitment of the p85/p110α complex via direct binding of the SH2 domains of p85 to the tyrosine phosphorylated motifs of the receptors (1435) such as the PDGF receptor (770) or the immunoglobulin family tyrosine-based activation motif (ITAM) of T- and B-cell receptors (943, 1078). In some cases, the recruitment is indirect, involving docking proteins, such as Grb2 and Gab1 for EGF receptors (1277, 1669) or IRS-1/2, in the case of insulin or IGF1 receptors (76, 1102). During this process, the p85 subunit also gets tyrosine phosphorylated and renders the catalytic subunit active via molecular rearrangements, the details of which are just beginning to unfold thanks to elegant structural studies (1053, 1807). Early reports showed that the iSH2 domain of p85 fused to the NH2 terminus of p110α yielded a constitutively active PI 3-kinase (657), but p85 interaction actually stabilizes the catalytic domain and keeps it in an inactive state (1781) as indicated by more recent structural studies (660, 978, 1807). As with all class I PI 3-kinases, the p110α subunit also has a Ras binding domain, and GTP-bound form of Ras is capable of directly activating PI3Kα.
(221, 657, 809, 1280). Several oncogenes like Src and polyoma middle T or the avian Rous sarcoma virus associate with the p85/p110α complex also leading to its strong activation (228, 292, 479, 760).

Homozgyous deletion of PI3Kα in mouse is embryonic lethal (143) and so is the homozygous replacement (“knock-in”) of the kinase with a catalytically inactive mutant, D933A (457). Mice heterozygous for this mutation showed reduced growth, hyperinsulinemia with glucose intolerance, hyperphagia, and adiposity, all signs of reduced insulin sensitivity, demonstrating that IRS-mediated recruitment and activation of PI3Kα is a major pathway in insulin/IGF1-mediated regulation of growth and metabolism (457). Targeted deletion of PI3Kα in endothelial cells showed that this isoform has a cell-autonomous function critical for angiogenesis, and the embryonic lethality of PI3Kα deficiency is likely caused by defects in vascular remodeling (524). The complete deletion of PI3Kα also changes the balance between the p85 and p110 subunits resulting in excess free p85 subunits that can associate with other proteins affecting their functions (899). The best example for this is the association of the free p85α subunit with IRS-1 after insulin stimulation, which competes with the recruitment of the p85/p110 heterodimer, therefore limiting insulin signaling (955). These findings also highlight the complex changes that can indirectly result from a complete deletion of a protein in a knockout animal.

Several studies reported on the knockout of the p85α regulatory subunit, either eliminating all three splice forms (469, 470, 1585) or either the long (85 kDa) (1503, 1543) or short (p55 and p50) (241) forms. These studies surprisingly found that all these mice showed increased insulin sensitivity and hypoglycemia. Moreover, the MEF cells isolated from these animals showed enhanced rather than decreased activity and hypoglycemia. Moreover, the MEF cells isolated from these animals showed increased insulin sensitivity, demonstrating that IRS-mediated recruitment and activation of PI3Kα is a major pathway in insulin/IGF1-mediated regulation of growth and metabolism (457). Targeted deletion of PI3Kα in endothelial cells showed that this isoform has a cell-autonomous function critical for angiogenesis, and the embryonic lethality of PI3Kα deficiency is likely caused by defects in vascular remodeling (524). The complete deletion of PI3Kα also changes the balance between the p85 and p110 subunits resulting in excess free p85 subunits that can associate with other proteins affecting their functions (899). The best example for this is the association of the free p85α subunit with IRS-1 after insulin stimulation, which competes with the recruitment of the p85/p110 heterodimer, therefore limiting insulin signaling (955). These findings also highlight the complex changes that can indirectly result from a complete deletion of a protein in a knockout animal.

to mitogens and in this case PI 3-kinase signaling is decreased rather than increased (470, 1503).

Mutations within the human PI3KCA (coding for PI3Kα) as well as in the PIK3r1 (coding for p85α) genes have been identified in a variety of cancers, at especially high percentage in colorectal cancers and glioblastomas, but also in other cancer forms (247, 716, 1198, 1219, 1334). It was also demonstrated that oncogenic mutants of PI3Kα are sufficient to promote cell proliferation and dissemination of cancer cells when injected into nude mice (541, 1190, 1333). It has been noted that the mutations cluster mostly in two regions that code for the helical and kinase domains of PI3Kα and some other ones within the NH2-terminal and C2 domains (1334). The X-ray structures of the p110α complexed with the iSH2 domain (660, 978) and the p110β/p85β complex (1807) has shed light on the possible mechanism of how these mutations lead to constitutive activation of the kinase. Most of these mutations are located in regions where the catalytic domain interacts with the iSH2 domain. These mutations probably weaken the interaction between the p85 and p110 subunits, therefore releasing the catalytic domain from the inhibitory effect exerted by the iSH2 domain. A unique role of the p85 regulatory subunits in stabilization of the PTEN phosphatase has been reported recently as well as mutations within these subunits causing destabilization and loss of PTEN in endometrial cancers (247).

2. PI3Kβ

The roles of PI3Kβ appear to be more restricted or specialized than those of PI3Kα, but this isoform has also been less studied. PI3Kβ forms a heterodimer with p85β and was expected to function in a similar manner as PI3Kα and thought to be functionally redundant with the latter. However, this idea was quickly repudiated when the embryonic lethality of the PI3Kα deleted homozygous animals was reported. An initial study reported early embryonic lethality in mice after deletion of PI3Kβ (142). Later studies using different strategies, either replacing the enzyme with a catalytically inactive (K805R) form (261) or deleting exons critical for catalytic activity (534), unexpectedly showed that these animals were viable and developed to adulthood. However, heterozygous mice in either case were born at a sub-Mendelian ratio, and some of the early embryos were small and moribund. Remarkably, the severity of the developmental defect of the embryos in one study correlated with the low expression levels of the kinase-dead PI3Kβ alleles and not with compensation from other forms of PI3K or regulatory subunits (261). This suggested a noncatalytic function of the protein that was attributed to a scaffolding role supporting clathrin-mediated endocytosis (261). Analysis of MEF cells from these animals revealed unexpected enzymatic roles of the kinase in mediating signals from certain GPCRs such as the sphingosine 1-phosphate receptor (261) or the LPA receptor (534). In contrast, PI3Kβ
was not a major contributor to growth factor-induced signaling (334) but made some contribution to insulin-mediated signals (261). Similar conclusions were reached when using PI3Kβ-specific inhibitors (534, 804). These studies were consistent with early observations that PI3Kβ can be activated by both RTKs and Gβγ subunits (835).

PI3Kβ was also found essential in male fertility (262), and in platelet functions, especially related to platelet integrin-mediated signaling (209, 1003). Recent studies reported that a majority of PI3Kβ was nuclear where it contributed to the control of DNA replication (998) and also to double-stranded break DNA repair (831), but it is doubtful that the functions of the enzyme would be only nuclear. PI3Kβ-deficient MEF cells showed impaired autophagy, and expression of the enzyme enhanced autophagy. Surprisingly, PI3Kβ was not acting via the Akt-mTOR pathway but was associated with the Vps34-Vps15-Beclin-1-Arg14L complex and promoted PtdIns3P synthesis (374). Reactive oxygen species production by neutrophil cells in response to fungal antigens mediated by integrins was also found to require either the PI3Kβ or the PI3Kδ isoforms (175). These studies suggest a clear specialization of PI3Kβ for certain tissues and processes, but more studies are needed to define the exact role(s) of this isoform under more acute inhibitory conditions and not only by genetic manipulations.

Deletion of the p85β regulatory subunit enhanced T-cell proliferation and decreased cell death (342). On the other hand, p85β showed redundant functions with p85α in early embryonic development and either one was necessary and sufficient to support membrane-ruffling responses to PDGF (176). The two p85 regulatory subunits were also found to be required but redundant in B-cell development (1156).

Curiously, PI3Kβ mutations are not commonly found in human cancers even though this isoform also has oncogenic potential (346, 758, 1813). There are, however, conditions under which PI3Kβ can also be a factor in tumorigenesis especially under PTEN loss (731, 1687). The transforming ability of PI3Kβ was dependent on Gβγ interaction (331). PI3Kβ appears to be more “constitutively active” than the wild-type PI3Kα behaving similarly to one of the oncogenic mutants of PI3Kα (N345K) (330). Comparison of the solved structures of the PI3Kα (660, 978, 1733) and PI3Kβ (1807) complexed with their respective regulatory subunits (or pieces of it) may explain some of these differences, although the conclusions of the different studies differ as to whether the catalytic domain of PI3Kβ is restrained more or less by interactions from p85β (nSH2, iSH2, and cSH2). Also interesting is the question of why the discrepancy between these highly homologous enzymes in their responses to tyrosine-phosphorylated peptides or Gβγ subunits liberated during stimulation of a restricted group of GPCRs. Recent identification of the Gβγ subunit interaction region in PI3Kβ is a major step toward understanding the unique regulation of this PI3K isoform (331).

3. PI3Kγ/PI3KCG

The earliest indications that there was an alternative way of PI3K activation via GPCRs and heterotrimeric G proteins were the PtdIns(3,4,5)P3 increases found in neutrophils after stimulation with GPCR ligands (1461, 1572). It was also observed that PI3K inhibition blocked chemotaxis and oxidative burst in neutrophils, and the GPCR-stimulated degranulation in basophilic leukemia cells (52, 1484, 1761). This was followed by the detection of a Gβγ-regulated distinct PI3K activity in myeloid cells (1468) and the subsequent cloning of the 110-kDa catalytic (1465, 1476) and 101-kDa regulatory subunits (1465) of what was named PI3Kγ. More recently, another regulatory subunit of PI3Kγ, called p84 or p87PIKAP, has been identified (1496, 1641). The tissue distribution of PI3Kγ and its adaptors is more restricted than those of other PI3K isoforms, showing highest expression in cells of myeloid origin, such as macrophages, neutrophils, eosinophils, and mast cells (128). However, these enzymes are also found in lower levels in other tissues such as the heart or the exocrine pancreas (128, 1476). The p84/87 regulatory subunit is especially enriched in mast cells, macrophages, and heart (157, 1641), whereas the p101 subunit is more abundant in neutrophils (1497).

PI3Kγ can be activated by both Ras and Gβγ subunits, and both pathways are important for the full repertoire of neutrophil responses, such as chemotaxis and respiratory burst in response to chemotactic stimuli (1497). The relative roles of the two regulatory adaptors p101 and p84 in mediating Gβγ and Ras stimulation were uncovered relatively recently. Early studies showed that the isolated p110γ catalytic domain of PI3Kγ can be directly stimulated by Gβγ subunits (887, 1476) as well as by Ras (1184, 1309). The direct stimulation of p110γ lipid kinase activity by Ras was found only modest in one study (1309), but oncogenic Ras mutants potently stimulated p110γ both in vitro and in transfected cells in another (1184). Importantly, the Gβγ sensitivity of PI3Kγ stimulation is greatly enhanced by the p101 regulatory subunit in vitro or in cells (833, 1465), yet p101 does not change the Ras sensitivity of the kinase. Accordingly, a mutant p110γ with impaired Ras binding still responds to Gβγ, and this response is still enhanced by p101 (1184, 1497). In contrast, p84/p110γ complexes are not sensitized to Gβγ stimulation. Instead, the p84/p110γ complex requires Ras binding for membrane recruitment and activation by GPCRs (833). Therefore, it appears that p110γ complexed to the two distinct adaptors is regulated in a clearly distinct manner and participates in distinct cellular responses. In support of this idea, recent studies showed that mast cell degranulation that depends on simultaneous engagement of FcεRI and the GPCR adenosine receptors relied on the p84/p110γ, while chemotaxis or Act...
activation were also supported by the p101/p110γ complex. Moreover, the fate of the PtdIns(3,4,5)P₃ made by the distinct complexes also differed; the one made by p101/p110γ quickly internalized while those made by p84/p110γ remained in the PM (157).

In addition to its lipid kinase function, PI3Kγ can also phosphorylate proteins such as MEK and activate the MAP kinase pathway through its protein kinase activity (1477). In elegant studies using activation loop swapping, PI3Kγ enzymes defective in either lipid or protein kinase activities were created to test the relative importance of lipid vs. protein kinase activities of the enzymes in initiating downstream responses (163).

The phenotypes observed after PI3Kγ deletion in mouse also supported the pivotal role of this enzyme in host defense and inflammation, consistent with the roles of this enzyme in chemotaxis of neutrophils and macrophages (624, 906, 1346). Analysis of PI3Kγ-deficient animals also revealed that the enzyme was critical in amplifying degranulation responses in mast cell and, accordingly, the knockout animals were protected from systemic anaphylaxis (847). Several other pathological conditions in which recruitment of neutrophils and macrophages by inflammatory chemokines is an aggravating factor have been alleviated by genetic or pharmacological inactivation of PI3Kγ. For example, atherosclerotic lesions are attenuated in apoE-deficient mice by inhibitors of PI3Kγ, or by replacing the macrophages of the apoE mice with ones originated from PI3Kγ-deficient animals (231, 456). Sepsis-induced multiorgan damage was also significantly reduced in mice with genetic or pharmacological inactivation of PI3Kγ (1002), and PI3Kγ deleted mice were largely protected in mouse models of rheumatoid arthritis (208). PI3Kγ inhibition also alleviated the severity of glomerulonephritis in a mouse model of systemic lupus (104). These studies and many others reviewed in more details (996, 1294) have identified PI3Kγ as a very desirable drug target for the control of a variety of inflammatory conditions, and several companies are developing subtype-specific PI3Kγ inhibitors (1294, 1310).

Even if the role of PI3Kγ in myeloid cell functions, chemokine responses, and inflammation are dominating themes, PI3Kγ has also been identified in other tissues and biological processes. These include a specific role of the kinase in platelet aggregation in response to Gt-coupled receptors, such as the ADP-receptor P2Y12 (623, 907), or in T-cell signaling (1346) and T-cell development resulting in a decreased CD4+/CD8+ T-cell differentiation ratio in PI3Kγ-deficient mice (1279, 1346). Moreover, PI3Kγ function is also important in the heart, especially under pathological conditions, as PI3Kγ deficiency protected mice from hypertrophy, fibrosis, and related dysfunctions caused by prolonged β-adrenergic exposure (1182). Strangely, PI3Kγ-deficient and “knock-in” mice expressing the kinase-dead version showed opposite responses to pressure overload following aortic constriction: knockout mice developed myocardial damage and heart failure while the knock-in mice were protected from all of these effects and even showed protection from fibrosis (1199). This striking difference revealed a kinase-independent function of PI3Kγ in the heart through its association with the phosphodiesterase PDE3B and stimulation of its PDE activity (1199). A PI3Kγ-mediated effect of angiotensin II (ANG II) on Ca²⁺ channels in vascular smooth muscle cells has also been described (1244), and PI3Kγ-deficient mice were protected from the vascular damage and hypertensive effects of ANG II administration (1623). An initial report found an increased incidence of colorectal cancer in PI3Kγ-deficient mice (1345), but this phenotype was not reproduced in other studies using independently developed PI3Kγ-deficient mice and has been attributed to some unrelated and yet unclear genetic constellations (105).
generation response under certain conditions (175, 285). These studies identified the PI3Kγ and -6 isoforms as potential targets to treat allergic diseases and also some autoimmune diseases including rheumatoid arthritis (1294, 1295, 1549). Moreover, PI3Kδ-specific inhibitors have shown some promise in the treatment of chronic lymphocytic leukemia (606) and multiple myeloma (681), and they were found potentially beneficial in reversing the glucocorticoid resistance in chronic obstructive pulmonary disease (1005, 1558). The recently solved structure of PI3Kδ (125) will certainly aid the development of PI3Kδ-specific inhibitors (41) that can be tested in a variety of immunological disease models.

5. Class II PI 3-kinases/PI3KC2s

Class II PI 3-kinases were discovered based on their sequence homology with class I PI 3-kinases (192, 369, 965, 1638). These enzymes also exist in three forms in mammalian cells: PI3KC2α, PI3KC2β, and PI3KC2γ (1347, 1610). Class II PI 3-kinases are larger proteins (170–200 kDa) that have highly homologous domains with other PI 3-kinases, such as the Ras binding domain, the helical and catalytic domains, but also have some distinctive features, such as a PX and C2 domains at their COOH termini. They are most dissimilar at their NH2 termini that differ in lengths but all contain proline-rich segments (FIGURE 5). Class II PI 3-kinases can phosphorylate PtdIns as well as PtdIns4P but not PtdIns(4,5)P2 in vitro, and based on this feature, it was initially believed that these enzymes produce primarily PtdIns(3,4)P2 from PtdIns4P in cells (369). However, increasing evidence suggests that class II PI 3-kinases also make PtdIns3P for specific processes (420). Since relatively little is known about the physiology of these enzymes, the three isoforms will be discussed together.

PI3KC2α and PI3KC2β are ubiquitously expressed, whereas PI3KC2γ shows more restricted expression mainly in liver and some other tissues depending on the species (627, 1061, 1171). PI3KC2α is found primarily in the trans-Golgi and in clathrin-coated vesicles (367) but was also localized to nuclear speckles (356). PI3KC2β, on the other hand, was found in the cytoplasm and in perinuclear puncta (1706), while PI3KC2γ was described in the Golgi (1171). Both PI3KC2α (484) and PI3KC2β (1706) were shown to bind clathrin, and clathrin stimulated the kinase activity of PI3KC2α (484, 485, 1583). These studies suggested a role of these kinases in clathrin-mediated endocytosis and trafficking. Although class II PI 3-kinases do not have any known adaptor proteins, several studies have indicated that PI3KC2α can respond to EGF (53), insulin (191), chemokine (1583), or TNF and leptin (829) receptor stimulation. Similarly, PI3KC2β associates with the activated PDGF and EGF receptors (53) and is stimulated by stem cell factor (51) and integrin engagement (1796).

As to the roles of these enzymes in cellular processes, several studies suggested that PI3KC2α is involved at multiple levels in the secretion and actions of insulin (418). PI3KC2α was found to regulate exocytosis of insulin in pancreatic beta cells (370), but was also activated by insulin via the small GTP binding protein TC10 (970) and played a role in insulin-induced Glut4 translocation (370). A role of PI3KC2α was also suggested in neurosecretion (1034), and in this context, it is notable that TC10 was recently found to regulate axon cone specification by inducing translocation of the exo70 exocyst complex to membranes (391). PI3KC2β, on the other hand, was found to regulate cell migration (368, 764, 971) via a Rac-dependent signaling pathway also involving PtdIns3P production. PI3KC2γ was claimed to play a role in regenerating liver (1171), but there is little knowledge concerning the involvement of this enzyme in any biological process. As pointed out above, it is increasingly evident that class II PI 3-kinases make PtdIns3P as their signaling molecule (370, 970, 1700). If the enzyme indeed makes primarily PtdIns3P, it is likely that it does so in very specific molecular contexts that differ from those regulated by the class III Vps34 enzymes. This has initiated a discussion as to what extent class II enzymes contribute to the bulk amount of PtdIns3P found in cells (418, 1034). It is worth pointing out that classII PI3Ks are less sensitive to the PI3K inhibitor Wm (369), and some of the PtdIns3P in cells is more resistant to inhibition by Wm (1464).

Genetic ablation of PI3KC2β in mice has been reported, but the mice are viable and fertile with no obvious specific phenotype reported (569). Inactivation of the enzyme’s ortholog in C. elegans causes fat accumulation mimicking the effects of inactivation of the insulin receptor homolog (57). Ectopic expression of the PI3KC2 ortholog or its kinase-dead version in fly epithelium caused pattern formation defects (966).

VI. PHOSPHOINOSITIDE PHOSPHATASES

Initial interest toward the inositol lipid phosphatases in the early 1980s paled compared with the excitement for lipid kinases and PLC enzymes, and only a few groups pursued research in this direction. PI phosphatase research was motivated primarily by the interest in the metabolism of the second messenger Ins(1,4,5)P3 (286, 1474) by 5-dephosphorylation and by the prominent effect of lithium on inositol phosphate metabolism causing an accumulation of Ins monophosphates and depletion of myo-inositol (34), with a possible link to the effect of Lith in manic-depressive disease (134) (see sect. III). Therefore, identification of the Ins(1,4,5)P3 5-phosphatase (that showed no lithium sensitivity) and the lithium sensitive inositol monophosphatase(s) were top priorities in the mid 1980s. However, Phil Majerus’ group had already focused on every form of inositol phosphate or -lipid phosphatases from early on and identified several enzymes that acted upon various inositol
phosphate isomers and some on the lipids themselves (see Ref. 972 for a review on these early studies). The interest level was significantly raised when the gene causing the X-linked human disease Oculo-Cerebro-Renal Syndrome of Lowe (OCRL) was mapped and found to have significant homology to an already known 75-kDa inositol polyphosphate 5-phosphatase (61). Subsequent research showed that OCRL was a PtdIns(4,5)P2 rather than an inositol polyphosphate 5-phosphatase (1166, 1805). Further momentum was gained when a PtdIns(4,5)P2 5-phosphatase associated with presynaptic membranes and named synaptojanin (Snj) was described and cloned (1022). In parallel studies, the same enzyme was purified and cloned as a PLD inhibitor (259), a feature that was due to its PtdIns(4,5)P2 phosphatase activity since PLD requires PtdIns(4,5)P2 for optimal activity (918). These studies helped disseminate the notion that inositol lipid dephosphorylation was an important regulator of inositol lipid signaling and that it was essential to determine whether a phosphatase acted on lipids or on soluble inositol phosphates in its natural cellular location. The next huge development of the phosphatase field was marked by the realization that the tumor suppressor gene PTEN (phosphatase and tensin homolog located on chromosome 10) was actually a PtdIns(3,4,5)P3 3-phosphatase (1123). Two of the SYNJ2 isoforms (SYNJ2B1 and -2) are highly expressed in nerve terminals and in the testes (1126). The hallmark of all SYNJs is a Schaedler domain of SYNJs can also dephosphorylate PtdIns3P and PtdIns(3,5)P2 in vitro (538, 1126). On the basis of these findings, it is believed that the Sac1-domain takes the 5-phosphatase product (mainly PtdIns4P) and further dephosphorylates it to PtdIns. Indeed, it has been shown that the activities of both the 5-phosphatase domain and the Sac1 domain of SYNJ1 were required to support synaptic vesicle recycling (981).

Both SYNJs were found to be critical in determining the fate of the endocytosed clathrin-coated vesicles (CCVs). SYNJ1 knockout mice develop and are born seemingly normally but die shortly after birth. These newborns show accumulation of CCVs in their nerve termini due to a defect in “uncoating” of the vesicles (301). This also causes poor recycling of vesicles into a fusion competent synaptic vesicle pool (794). SYNJ1 associates via its COOH-terminal domain with several proteins enriched in the nerve terminal such as amphyphysin (335) and with proteins that play important roles in the endocytic pathways, such as endophilin, intersectin, syndapin, and Eps15 (1418). SYNJ1 is constitutively phosphorylated and undergoes activity-dependent dephosphorylation by calcineurin, which, in turn, affects both its catalytic activity and interactions with other proteins (876, 1023, 1419). Both SYNJs interact with the SH3-domain containing adaptor protein, Grb2 and SYNJ2, but not SYNJ1, participates in clathrin-mediated EGF receptor and transferrin receptor endocytosis (974, 1314). These results suggest nonredundant functions of the SYNJ phosphatases in controlling PtdIns(4,5)P2 levels in the membranes of vesicles along the endocytic pathway. Interestingly, one isoform of SYNJ2, SYNJ2A, is targeted to the mitochondria via binding to the outer mitochondrial membrane protein OMP25 (1124). It is an intriguing possibility

1. Type II 5-phosphatases

A) SYNAPTOJANINS. As described above, synaptojanin-1 (Synj1) was discovered by the De Camilli group as a 5-phosphatase that functions in synaptic vesicle exocytosis and recycling (1022). SYNJ1 has multiple splice forms that differ in their COOH termini. SYNJ1–145 shows very high expression in the brain while SYNJ1–170 shows wider tissue distribution. A highly homologous sister enzyme, synaptojanin-2 (SYNJ2), is more widely expressed and also exists in three splice forms differing in their COOH termini (1123). Two of the SYNJ2 isoforms (SYNJ2B1 and -2) are highly expressed in nerve terminals and in the testes (1126). The hallmark of all SYNJs is a Schaedler domain of SYNJs located in the NH2 termini of the proteins. The isolated Sac1 domain of SYNJs can also dephosphorylate PtdIns3P and PtdIns(3,5)P2 in vitro (538, 1126). On the basis of these findings, it is believed that the Sac1-domain takes the 5-phosphatase product (mainly PtdIns4P) and further dephosphorylates it to PtdIns. Indeed, it has been shown that the activities of both the 5-phosphatase domain and the Sac1 domain of SYNJ1 were required to support synaptic vesicle recycling (981).

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A. 5-Phosphatases

This group of enzymes can remove the phosphate from the 5-position from PtdIns(3,4,5)P3, PtdIns(4,5)P2, and PtdIns(3,5)P2. They are divided into four types (I-IV), but the 43-kDa type I enzyme (INPP5A), which was the first 5-phosphatase isolated and cloned, does not act on lipids but dephosphorylates Ins(1,4,5)P3 and Ins(1,3,4,5)P4 (340, 862, 863). The type II 5-phosphatases include the synaptojanins, OCRL, INPP5B, INPP5J, and SKIP, while the two type III enzymes are SHIP-1 and -2. The only type IV enzyme is INPP5E. All of these enzymes belong to the AP family of endonucleases and have two signature motifs (F/Y)WXGDXN(F/Y)R and P(A/S)(W/Y)(C/T)DR(I/V)L(W/Y)separated by ~60–75 resi-
FIGURE 6. The inositol lipid 5-phosphatase family. These enzymes dephosphorylate PtdIns(4,5)P2 and/or PtdIns(3,4,5)P3 in the 5-position. There are three major subgroups called type II, III, and IV. [The type I enzyme is a smaller, 43-kDa protein that hydrolyzes the water-soluble Ins(1,4,5)P3 molecule but not the membrane-bound lipids.] The type II 5-phosphatase enzymes are encoded by six genes. Two of these, synaptojanin-1 and synaptojanin-2, exist in multiple splice forms differing in their respective COOH termini (only the two main forms are shown). The characteristic feature of these enzymes is the presence of a Sac1 homology domain upstream of their conserved 5-phosphatase domains. The NPF repeats of the longer form of SYNJ1 binds to the endocytic protein Eps15. OCRL and INPP5B are also very similar to one another, with a whole set of domains providing multiple interactions in addition to the catalytic 5-phosphatase domain. These include an NH2-terminal PH domain and COOH-terminal ASH and Rho-GAP domains. The ASH domain binds Rab5 and the adaptor protein APPL1, while Rho-GAP domain binds the small GTP binding proteins, Rac1 and Cdc42. OCRL1a contains two clathrin binding (CB) domains, the second of which is not present in OCRL1b. INPP5B has very similar structure, but it lacks the clathrin-binding domains and has a COOH-terminal CAAX domain. INPP5J and INPP5K are smaller proteins that have a SKICH domain downstream of their 5-phosphatase domain. This core structure is surrounded by proline-rich sequences in INPP5J. The type III 5-phosphatases are represented by the SH2-domain-containing enzymes SHIP1 and SHIP2. They have an NH2-terminal SH2 domain and C2 and proline-rich sequences downstream of their 5-phosphatase domain. SHIP2 also has a sterile alpha motif (SAM) at its very COOH terminus. The single type IV enzyme, INPP5E has a proline-rich sequence and a COOH-terminal CAAX box in addition to the 5-phosphatase domain.
that membrane deformations during mitochondrial fission and fusion are regulated by this enzyme in a manner similar to what occurs during endocytosis. An extra copy of SYNJ1 due to a trisomic arrangement found in the Ts65Dn mice, a model for Down’s syndrome, is associated with neurological defects and PtdIns(4,5)P2 dysregulation, and these aberrations are corrected by restoring SYNJ1 to disomy (1647). Since SYNJ1 is located on human Chr21 that is trisomic in Down’s syndrome patients, it is an attractive possibility that SYNJ1 is a major factor in the neurological dysfunctions found in these patients (1647). Recent studies also linked SYNJ2 to hearing and cochlear function. In a mouse strain, called Mozart, which exhibits progressive hearing loss, the causative mutation was mapped to a critical region within the phosphatase domain of the Synj2 gene (N539K) (983). Whether this gene is affected in human hereditary hearing disorders has yet to be investigated. SYNJ2 deleted mice have not been reported yet in the literature.

*S. cerevisiae* cells also express three phosphatases that show homology to mammalian SYNJs and contain both a Sac1-like and 5-phosphatase domains (1440, 1473). These enzymes, named Inp51, Inp52, and Inp53 (or “synaptotanin-like,” Sj1, -2, and -3, respectively) can be individually deleted, suggesting their significant functional redundancy, but triple deleted cells are nonviable (1440, 1473). Interestingly, a mammalian type II 5-phosphatase (75-kDa phosphatase, or Inpp5B) that does not contain a Sac1 domain still can restore viability in triple Sjl inactivated cells or alleviate the phenotypic changes observed in double-deleted strains (1155). The significance of this finding is that a functional Sac1 domain may not be necessary for the functions of the yeast Sjl proteins. This conclusion was supported by further reports that Inp52 (1453) or Inp53 (545) enzymes with mutated, inactive Sac1 but not inactive 5-phosphatase domains were able to restore the viability of triple Sjl inactivated strains. Nevertheless, the isolated Sac1 domains of Inp52 and Inp53 are able to hydrolyze PtdIns4P and PtdIns3P (538) and rescue defects caused by inactivation of the yeast Sac1 protein (667). Mutations in key residues within the Sac1 domain already render this domain of the Inp51 protein inactive. Individual or double mutant Sjl strains display functional defects that still suggest some specialization among the three Sjl proteins. The general conclusion reached based on a number of studies analyzing these mutants is that Inp51/Sj1 and Inp52/Sj2 have partially overlapping functions and that Sj1, in particular, is involved in the cell-wall integrity pathways and actin organization both of which requires PM PtdIns(4,5)P2 produced by the Stt4 PhK and the Mss4 PIP 5-kinase (see Ref. 1479 for a detailed review). In contrast, Inp53/Sj3 deleted strains display defects that suggest unique functions linked to clathrin-mediated trafficking both at the PM, such as endocytosis, or at the Golgi and TGN (544, 545, 1473) (and see Ref. 1479 for more details). The complicated phenotypes of the inactivation of the three Inp51–53 proteins in various combinations are due to several factors. First, the 5-phosphatase domains of the three enzymes control specific but perhaps overlapping pools of PtdIns(4,5)P2 and because of this the severity of the functional defects do not always correlate with overall PtdIns(4,5)P2 elevations. Second, the Sac1 phosphatase activity of Inp52 and Inp53 also contributes to the control of specific PtdIns4P and PtdIns3P pools and in most of these locations the Sac1 phosphatase has a redundant function with the Sjl enzymes (1479).

**b)** OCRL. As mentioned before, mutations in the OCRL gene were found responsible for the X-linked human disease OCRL (61). OCRL patients present with congenital cataract, renal tubular acidosis, aminoaciduria, and mental retardation (944). However, some patients with mutations of the same gene can also present with Dent’s disease with milder symptoms that are mostly confined to the kidney (647). The similarity of the OCRL protein to the 75-kDa inositol lipid 5-phosphatase (INPP5B) gave a clue that this protein was a 5-phosphatase, and subsequent studies showed that PtdIns(4,5)P2 was the preferred substrate for OCRL over the soluble Ins(1,4,5)P3 or Ins(1,3,4,5)P4, establishing OCRL as an inositol lipid 5-phosphatase (1805). OCRL prefers PtdIns(4,5)P2 over PtdIns(3,4,5)P3 and was also shown to hydrolyze PtdIns(3,5)P2 (1361), but it remains to be seen whether this lipid is indeed regulated by OCRL within the cells. The OCRL protein shows wide tissue expression, and its localization to the Golgi and TGN originally suggested that the OCRL disease is a result of a Golgi-originated trafficking defect (388, 1166, 1483). An attempt to make a mouse model of OCRL was complicated by the fact that OCRL deletion in mice does not reproduce the phenotypes associated with the human disease, apparently because of the overlapping function(s) of the homologous 75-kDa 5-phosphatase Inpp5b (722). Combined inactivation of both genes, on the other hand, manifests in early embryonic lethality (722). The difference between humans and mice in the ability of the 75 kDa phosphatase to compensate for OCRL functions must lie within the 75 kDa phosphatase itself, which differs between the two species in expression level and splicing. Indeed, replacement of the mouse Inpp5b with the human INPP5B version in an Ocrl (-/-) background created mice that show some of the symptoms found in the human disease (173). This mouse model should greatly facilitate the progress in understanding the molecular pathology of OCRL and Dent’s disease.

Since OCRL is only one of numerous 5-phosphatases within the cell, it is likely that it affects localized pool(s) of PtdIns(4,5)P2. Not surprisingly, it has been difficult to show increased levels of total PtdIns(4,5)P2 in cells obtained from OCRL patients, although in some cases such increases have been reported (1804). Other studies showed only indirect signs of aberrant PtdIns(4,5)P2 levels, such as altered F-actin distribution and dynamics in fibroblasts obtained from OCRL patients (1482). Although the bulk of the OCRL...
protein is associated with the Golgi/TGN (388, 1166, 1483), an increasing body of evidence suggests that its functions are more widespread. Localized functions of OCRL rely on the recruitment and regulation of the protein to different membranes and, therefore, the domain structure and multiple interactions of the protein with various regulatory proteins have attracted significant attention. In addition to its conserved 5-phosphatase domain, OCRL contains an NH2-terminal PH domain (988), an ASH (ASPM-SPD2-Hydim) domain (1232), and a COOH-terminal Rho-GAP-like domain that is catalytically inactive (407). OCRL has two clathrin binding domains: one located within the NH2-terminal PH domain (251, 988), while the other is found within the COOH-terminal Rho-GAP domain (250, 1594). None of the clathrin binding sites is present in the otherwise similarly built INPP5B protein (988). OCRL has two spliceoforms that only differ in a small eight-amino acid stretch within the Rho-GAP-like domain close to the clathrin binding site; the longer form binds clathrin with higher affinity (251) (FIGURE 6).

The OCRL protein associates with clathrin-coated transport intermediates and regulates trafficking between endosomes and the TGN (250). However, somewhat surprisingly, a prominent role of OCRL in the early steps of the endocytic pathway has been also suggested (407, 1634). Membrane recruitment and activation of the OCRL protein occurs via interaction with multiple Rab proteins (674) and the Rab5 effector APPL1 (adaptor protein containing PH domain, PTB domain and leucine zipper motif 1) protein (407). Interaction of OCRL with another Rab protein, Rab35, connects the phosphatase with abscission, the last step in cytokinesis (318). The structural details of the various domains of OCRL and their possible roles in activating and orienting of the phosphatase domain have begun to emerge based on the crystal structures of these domains with possible associated proteins (407, 652, 988, 1223, 1224). Taking all these data into consideration, the OCRL protein plays multiple roles in endocytic trafficking by its interaction with clathrin and Rab proteins (1223). It has been more difficult to connect the molecular details and cellular functions of OCRL to the defects underlying the pathophysiology of the disease. The similarity of the kidney symptoms of OCRL patients to those of Fanconi syndrome (236) led to the suggestion that the large LDL receptor family protein megalin, which recycles between the apical membrane and the TGN in proximal tubule cells, displays aberrant trafficking in OCRL patients (945, 1146). However, one study found no signs of aberrant megalin trafficking in human and canine renal epithelial cells after siRNA-mediated knockdown of OCRL, even though there was increased secretion of lysosomal hydrolyses (303). Recent studies also suggested that OCRL is important for the assembly and functions of the primary cilium, and this may be an important clue to understand the renal pathology of the disease (288, 956, 1263).

**C) INPP5B.** This enzyme is highly similar to the OCRL protein (45% identical at the amino acid level) and has a similar domain organization. In fact, INPP5B was identified before OCRL (1065). The enzyme, also called 75-kDa type II 5-phosphatase, is widely expressed, especially enriched in the kidney, lung, and testes (1438). Although initial studies claimed mitochondrial localization (1438), subsequent studies found the enzyme to be associated with the Golgi and ER exit sites (1718), and Rab proteins also affect the localization of the enzyme (1401, 1718). While INPP5B shows a similar domain structure to the OCRL 5-phosphatase, there are notable differences between the two enzymes. Unlike OCRL, INPP5B does not contain clathrin binding sites, and it has a COOH-terminal CAAX prenylation sequence (724) (FIGURE 6). The mouse has three splice variants of Inpp5B as 87-, 104-, and 115-kDa proteins, the expression of which is tissue and developmental stage specific (1013). INPP5B hydrolyzes equally PtdIns(4,5)P2 and PtdIns(3,4,5)P2 [and Ins(1,4,5)P3 and Ins(1,3,4,5)P4], but not PtdIns(3,5)P2 (724, 1361).

Homozygous mice deficient in Inpp5B are viable and appear normal, but do have testicular degeneration and defective sperm functions that manifest after sexual maturation (722). The sperm of these mice show reduced motility and reduced ability to fertilize eggs (722, 992). These defects can be the consequence of the impaired proteolytic processing of a sperm cell surface protein, fertilin-β, which is important for egg binding (596). In addition, there is a defect in the apical endocytosis in Sertoli cells causing swollen vacuoles (595).

**D) INPP5J.** This 5-phosphatase, also called proline-rich inositol polyphosphate 5-phosphatase (PIPP), was identified due to the presence of consensus 5-phosphatase sequences (1076). The enzyme is widely expressed and contains proline-rich regions both at the NH2 and COOH terminus and several 14-3-3 protein-binding motifs (1076). The enzyme also contains a SKICH (SKIP COOH-terminal homology) domain (540) that mediates PM association (1172) (FIGURE 6). In vitro, the 108-kDa enzyme can hydrolyze PtdIns(4,5)P2 and PtdIns(3,4,5)P3 as well as Ins(1,4,5)P3 and Ins(1,3,4,5)P4 (1076), but it is believed to regulate PtdIns(3,4,5)P3 levels in cells (1172). Overexpression of the enzyme in PC12 cells decreased PtdIns(3,4,5)P3 levels, Akt activation, and neurite outgrowth (1172). Recent studies identified collapsing response mediator protein 2 (CRMP2) as an INPP5J interacting partner and showed that the two proteins have opposing roles in regulation of nerve cell polarization, axon selection, and neurite elongation (59). Importantly, higher INPP5J expression levels have been correlated with better prognosis and disease outcome in breast cancer (1515, 1600).

**D) SKIP/INPP5K.** This enzyme is enriched in skeletal muscle and kidney (hence the name skeletal muscle and kidney...
enriched inositol polyphosphate phosphatase) and was identified also because of the presence of the two conserved regions found in 5-phosphatases (540). SKIP also contains a COOH-terminal SKICH domain that mediates its translocation from the Golgi/ER region to the PM after insulin stimulation (540) (Figure 6). It was shown that SKIP limits the insulin-mediated PtdIns(3,4,5)P₃ accumulation at the PM, decreasing Akt activation, GLUT4 translocation, and glucose uptake (679). The notion that SKIP might control the insulin sensitivity of skeletal muscles is supported by studies on SKIP +/− mice that show increased insulin sensitivity associated with increased Akt activation and glucose uptake in skeletal muscles of the mice (680). Homozygous SKIP −/− mice exhibit embryonic lethality (680).

2. Type III 5-phosphatases

A) SHIP1 and -2. These 5-phosphatases were named because of their NH₂-terminal SH2-domains. SHIP1 (also called INPP5D) was initially identified as an inositol lipid phosphatase associated with a number of adaptor proteins, such as Shc, Grb, and DOK (924), as well as with immunoreceptor tyrosine-based inhibitory (ITIM) or activation (ITAM) motifs of immune receptors (798, 1175). SHIP1 has several splice variants, a full-length (SHIP1/H9251) and s-SHIP1 (not shown in Figure 6) (1290). Structurally, SHIP1 and -2 contain a central 5-phosphatase domain followed by a C2 domain and two NPXY sequences in which the Tyr can be phosphorylated to interact with PTB domains. All SHIP variants except SHIP1β contain a COOH-terminal proline-rich sequence, and SHIP2 also has a COOH-terminal SAM (sterile alpha motif) sequence, whereas s-SHIP1 lacks the NH₂-terminal SH2 domain. The mechanism of SHIP1/2 activation is based on recruitment of the phosphatase from the cytosol to the membrane via the various protein-protein interaction domains present in the molecule. These proteins function as part of multi-protein complexes, and their cell- and tissue-specific functions are probably dictated by their binding partners. Initially, the SHIP proteins were believed to have a restricted substrate recognition only able to dephosphorylate the 5-position in PtdIns(3,4,5)P₃ and perhaps other soluble higher inositol phosphates (248, 1722). Recent studies, however, suggested that they can also hydrolyze PtdIns(4,5)P₂ and control clathrin-mediated endocytosis (1115). Nevertheless, SHIP proteins are still considered as key enzymes in the control of the PI 3-kinase products PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃ (112). It is important to note that the reaction product PtdIns(3,4)P₂ can also activate certain downstream targets, such as Akt (460); hence, SHIP1/2 can selectively inactivate signaling pathways initiated by PI 3-kinases (1290). The enzymatic activity of SHIP1 does not appear to be regulated by Tyr phosphorylation or protein-protein interaction (1213), but it is enhanced by binding of the product PtdIns(3,4)P₂ to the C2 domain (1170). Recent studies suggested that PLCβ3 and Lyn controls SHIP1 activity via phosphorylation of Tyr536 and Tyr564 (1742). Other studies identified a PKA phosphorylation site (Ser440) in SHIP1, which, when phosphorylated, significantly enhances the activity of the enzyme. This could contribute to the well-documented negative effects of cAMP-mobilizing signals on PI 3-kinase signaling in immune cells (1798). Some meroterpenoid compounds are also able to activate SHIP1 and exert strong anti-inflammatory effects that could represent a means of controlling PI 3-kinase downstream effectors in hematopoietic cells (1170, 1459, 1460).

SHIP1 and its splice variants are expressed exclusively in hematopoietic and spermatogenic cells (928), while SHIP2 (also called INPP5L1) shows ubiquitous expression (1095). Recent studies showed that SHIP1 expression is repressed by the human MIR155 microRNA (1154). Consistent with its expression pattern and enzymatic functions, SHIP1 deficiency results in enhanced PI 3-kinase signaling affecting cells and organs with hematopoietic cell involvement (B and T cells, macrophages, osteoclasts, mast cells, neutrophil cells, and platelets). Ship1 knockout mice develop a chronic myelogenic leukemia (CML)-like disease with myeloid proliferation, splenomegaly, and vast myeloid infiltration of the lungs causing premature death (593, 927). The significantly elevated PtdIns(3,4,5)P₃ levels induce a hyperproliferative response of hematopoietic progenitors to a variety of growth factors (593) and increased Akt activation in mature neutrophils and mast cells (927). Since PtdIns(3,4,5)P₃ has a pivotal role in amplifying polarization during chemotaxis both in phagocytic cells (1385, 1463) and lower organisms, such as Dictyostelium (976), there is a massive dysregulation of neutrophil polarization in Ship1 null mice (1140). The severe osteoporosis reported in Ship1 −/− mice is due to the hyperactivation of osteoclasts that are more numerous, enlarged, and hypernucleated (1523), resembling those in patients with Paget disease. Ship1 −/− mice also show deficient platelet aggregation (1386), an increased number of splenic dendritic cells that are hyperresponsive to GMCSF in vitro but have an immature phenotype (47).

The more ubiquitously expressed SHIP2 enzyme serves as a negative regulator of insulin signaling. Overexpression of SHIP2 antagonizes the PI3K angle of insulin signaling in 3T3-L1 adipocytes (1650) and cerebellar granule cells (1429). Additionally, transgenic mice, overexpressing SHIP2, showed signs of mild insulin resistance (744). Higher levels of SHIP2 expression were found in the cerebral cortex of the db/db obese mice (1429), and SHIP2 gene polymorphisms have been linked to metabolic syndrome (746, 991). A mouse strain lacking Ship2 (and the neighboring Phox2a gene) shows insulin hypersensitivity and severe hypoglycemia and has early neonatal mortality (271). Curiously, another study reported no such problems with a SHIP2 knockout strain and, to the
contrary, reported a resistance of these mice to weight gain in a high-fat diet (1417). A recent study identified Ser132 phosphorylation of SHIP2 that regulates its nuclear localization in nuclear speckles in a cell cycle-dependent manner. This study also found SHIP2 interaction with nuclear lamin A/C and that Ser132-phosphorylated SHIP2 also hydrolyzed PtdIns(4,5)P2 (400). Understanding the relationship between these novel features of SHIP2 and the phenotypic changes listed above will require further investigations. More details about SHIP2 can be found in Reference 401.

3. Type IV 5-phosphatase

The only type IV 5-phosphatase, also called INPP5E, 72-kDa polyphosphate 5-phosphatase, and Pharbin (801, 818), contains an NH2-terminal proline-rich segment, a central 5-phosphatase domain, and a COOH-terminal farnesylation CAAX motif (FIGURE 6). This enzyme can remove the 5-phosphate from PtdIns(4,5)P2, PtdIns(3,4,5)P3, and PtdIns(3,5)P2 and has about a 10 times higher affinity against PtdIns(3,4,5)P3 than SHIP1 (801, 818). INPP5E is located mostly in the Golgi and partially in the PM (818), but its distribution could be more specialized in specific cell types. Notably, INPP5E does not seem to be a general antagonist of PtdIns(3,4,5)P3 signaling. In macrophages, INPP5E is recruited to the phagocytic cup where it controls the conversion of PtdIns(3,4,5)P3 to PtdIns(3,4)P2, but unlike SHIP1 (294, 750), it only affects the FcyR- and not the complement receptor 3 (CR3)-mediated phagocytosis (649). Interestingly, overexpression of INPP5E in adipocytes does not antagonize insulin signaling but instead hydrolyzes PtdIns(3,5)P2 to PtdIns3P, which in turn increases GLUT4 translocation (817). Two recent studies linked INPP5E to the primary cilium. In one of these studies, mutations clustering within the 5-phosphatase domain of INPP5E were identified in patients with Joubert syndrome (145). These patients present with specific midbrain-hindbrain malformation, and variably with retinodystrophy, nephronophthisis, liver cirrhosis, and polydactyly and are considered as an emerging group of ciliopathies (1599). In this study, INPP5E was found to localize to the primary cilium in cells of the affected organs and the mutations caused destabilization of the cilia during stimulation (145). The other study found that disruption of the Inpp5e gene in mice caused multiorgan failure associated with defects in primary cilia (714). This latter study (714) also found destabilization of cilia in response to growth factor stimulation and identified a mutation in the human INPP5E gene that affected cilia localization in a family with MORM syndrome. The physical targeting of INPP5E to the cilia was recently shown to be dependent on a protein complex that included phosphodiesterase 6D, the Arf-like protein, ARL13B, and the centrosomal protein, CEP164, all of which had been linked to ciliopathies (668). These interesting discoveries should facilitate further studies to better understand the role of phosphoinositides in ciliary function and how INPP5E controls this process.

B. 3-Phosphatases

The phosphoinositide 3-phosphatases are comprised of two major groups. The first group contains PTEN (phosphatase and tensin homolog located on chromosome TEN), the voltage-sensitive phosphatases (VSPs) first isolated from the ascidia, Ciona intestinalis (1096) but also found in zebrafish (650) and Xenopus (1259), and the mammalian VSP homologs, the TPIP proteins (α, β and γ) (1163). These enzymes all share similar catalytic phosphatase domains (FIGURE 7), but differ in their substrate preference and what phosphates they remove. PTEN is one of the key enzymes that antagonizes PI 3-kinase action by removing the 3-phosphate from PtdIns(3,4,5)P3. In contrast, the nonmammalian VSPs are 5-phosphatases that act on PtdIns(4,5)P2 and PtdIns(3,4,5)P3 (550, 713). The mammalian TPIP proteins can dephosphorylate 3-phosphorylated inositides (1662), but it is not clear whether they can also act on PtdIns(4,5)P2. It is also questionable whether any of the mammalian VSP homologs are also voltage regulated (1163). In spite of the differences in their catalytic targets, these enzymes will be discussed in this section because of their close structural relationship to PTEN, an enzyme that has risen to prominence as a PtdIns(3,4,5)P3 3-phosphatase and a tumor suppressor gene. The second group of the 3-phosphatases are made up by the myotubulins (MTMs) with 15 structurally related members, 9 of which have phosphatase activity removing the 3-phosphate from PtdIns(3,5)P2 and PtdIns3P. The other six members are catalytically inactive proteins (1273) (FIGURE 7).

1. PTEN

This enzyme (also known as MMAC1 and TEP1) was first identified as a tumor suppressor gene after mapping its homozygous deletions in several human advanced cancers on chromosome 10 (897, 898, 1452). Due to its similarity to phosphatases and weak homology to tensin (there lies the origin of its name), PTEN was initially believed to be a dual-specificity protein phosphatase (1104). However, the enzyme shows low activity against protein and peptide substrates, and subsequent studies revealed that it was a PtdIns(3,4,5)P2 3-phosphatase (968, 969). Although able to dephosphorylate PtdIns(3,4)P2, PTEN is a lot more active against PtdIns(3,4,5)P3 (1015) and, therefore, is a functional antagonist of the class I PI 3-kinases. The protein and lipid phosphatase activities of PTEN can be dissociated, and a G129E mutation identified in a family with Cowden disease eliminates lipid but spares the protein phosphatase activity of the enzyme (481, 1103). Since Cowden disease is one of the cancer predisposition syndromes (641), these studies showed that the tumor suppressive function of PTEN is primarily associated with its lipid phosphatase activity. A mutant form of PTEN impairing protein but not
lipid phosphatase activity was recently identified showing that together with the lipid phosphatase activity, the protein phosphatase activity of PTEN was also necessary to maintain proper migration of glioblastoma cells (325).

PTEN has a catalytic phosphatase domain, with a conserved C-(X)₅-R signature motif, an NH₂-terminal phosphoinositide binding segment (PBM), a C2 domain toward the COOH terminus followed by two PEST (proline, glutamic acid, serine, threonine) sequences, and a COOH-terminal PDZ binding motif (FIGURE 7). The structure of the catalytic domain of PTEN has been solved showing similarities to those of phosphotyrosine and dual-specificity phosphatases (870). However, PTEN has a deeper substrate binding pocket that allows the bulkier PtdIns(3,4,5)P₃ headgroup to be used as a substrate. The C2 domain is important for membrane targeting and regulation of the enzyme. Unlike many other C2 domains, the C2 domain of

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**FIGURE 7.** The inositol lipid 3-phosphatases. A: the PTEN family of enzymes dephosphorylate PtdIns(3,4,5)P₃ at the 3-position and hence “antagonize” the class I PI3Ks. The conserved phosphatase domain is followed by a C2 domain and a PDZ binding sequence in PTEN. The PBM is a phospholipid binding domain present in PTEN and also in the voltage-dependent phosphatases (these latter enzymes are actually 5-phosphatases). The TPIPα and -γ enzymes have an NH₂-terminal putative transmembrane domain (TM) that is lacking in the β form. The TPTE enzyme is catalytically inactive. B: the myotubularin family of 3-phosphatases dephosphorylate PtdIns3P and PtdIns(3,5)P₂. They fall into three subgroups. All of these enzymes (except for MTMR14) have a PHG (PH and GRAM) domain and coil-coil (CC) regions in addition to their phosphatase domain. The phosphatase domain also contains a SET interaction domain (SID). MTMR3 and -4 also has a FYVE domain in their COOH termini. No specific domains beyond the phosphatase domain have been described in MTMR14. There are additional MTM-related proteins that lack catalytic activity. They are not shown in this figure.
PTEN does not bind Ca$^{2+}$, but it contributes to membrane binding through electrostatic interactions (323). However, membrane binding is not the sole function of the PTEN C2 domain (507), which also makes several contacts with the catalytic domain via hydrogen bonds (870). Truncation or mutations within the C2 domain of PTEN make the protein unstable (323, 506, 507). The NH$_2$-terminal phosphoinositide binding domain (PBM) of PTEN also contributes to membrane binding and localization. This domain in isolation can bind PtdIns(4,5)P$_2$, and mutation of its key basic residues (such as K13E) abrogates its lipid binding (1266). Basic residue mutations within the PBM in the full PTEN molecule or PtdIns(4,5)P$_2$ depletion prevent PTEN from efficiently localizing to the membrane (1249). Interestingly, PtdIns(4,5)P$_2$ binding to the PBM domain, is also present in the Ciona VSP, and seems to be important for the coupling of the voltage sensor to the phosphatase domain (846). A phosphorylation-dependent autoinhibitory role of the PBM has also been suggested for the mammalian PTEN enzyme (1249). The COOH-terminal tail contains a stretch of ~60 amino acids that can be phosphorylated by several protein kinases (22, 905) and phosphorylation renders PTEN inactive (1160, 1249) through interaction of the phosphorylated COOH terminus with the membrane interaction site of the C2 domain (1160). Since this COOH-terminal segment of PTEN is quite acidic and the protein phosphatase activity of PTEN is strongest against acidic peptides (1104), it was suggested that PTEN autocatalytically can activate itself through dephosphorylation of its COOH terminus (1247). The role of the PEST sequence within PTEN is not clear, but it may be related to the degradation of PTEN by proteosomes. Phosphorylation also affects the stability of PTEN (1562), and polyubiquitylation has been proposed to mark PTEN for proteosomal degradation (1561). The identity of the E3 ubiquitin ligase responsible for polyubiquitylation of PTEN has been controversial (458, 1674), but most recent studies suggest that WWP2 that belongs to the NEDD4-like family of E3 ligases is the elusive PTEN E3 ubiquitin ligase (967). Monoubiquitylation of PTEN, on the other hand, was found important for nuclear localization and tumor suppression (1579), at the same time mono- or poly-ubiquitylation was shown to inhibit PTEN phosphatase activity (964). A recent study found that PTEN gets sumoylated at residues K254 and K266 and that sumoylation is essential for the tumor suppressor function of the protein, presumably because of its contribution to membrane recruitment (521, 663).

The most important known function of PTEN is to serve as a negative regulator of PtdIns(3,4,5)P$_3$ and hence serving as a tumor suppressor. As detailed under class I PI 3-kinases, PtdIns(3,4,5)P$_3$ promotes carcinogenesis in a number of ways, both in a tumor cell autonomous manner, and also within the tumor cell environment. In the former, it stimulates proliferation, and protects cells from apoptosis, and it also increases the tumor cells’ ability to migrate and break down tissue barriers including those of capillaries, thereby promoting metastasis. In the tumor environment, PtdIns(3,4,5)P$_3$-dependent signaling has a crucial role in angiogenesis, stromal cell function, or immune surveillance. It has been very clear that germline mutations of PTEN cause increased incidence of multiple hamartomas and high risk of tumorigenesis observed in a number of human syndromes such as Cowden disease, Bannayan-Riley-Ruvalcaba syndrome, Lhermitte-Duclos disease, and Proteus- and Proteus-like syndrome (349, 1434). PTEN mutations have been detected in a variety of human cancers such as glioblastoma, breast, lung, colon, kidney, and uterine carcinomas or melanomas. Very often monoallelic mutations of PTEN are present in cancer tissues, but late-stage aggressive metastatic cancers show biallelic loss of PTEN (210, 349, 1434).

Most PTEN mutations cluster within the phosphatase domain making the enzyme catalytically inactive, but many mutations are found in other regions that affect the cellular localization or stability of the enzyme (999). It is also of great interest that PTEN is very sensitive to oxidative damage, and hydrogen peroxide rapidly inactivates its enzymatic activity (384, 842, 874, 888, 1297). One interesting and poorly understood aspect of PTEN tumor suppressor function is linked to its nuclear localization where a non-catalytic role has been recently proposed (1433). Clearly, there is more to PTEN function than PtdIns(3,4,5)P$_3$ signaling, and its link to nuclear proteins, chromosomal instability, DNA repair, and kinetochore assembly (1394) suggests a complexity that has yet to be fully understood.

Mouse models of PTEN inactivation have been providing more detailed information on the tumor suppressor as well as the developmental and tissue-specific roles of PTEN. Homozygous PTEN deficiency causes early embryonic lethality, and heterozygote mice also develop tumors (838, 1450, 1500). Remarkably, a dose-dependent reduction in PTEN even above monozygocity is associated with increased tumor formation (31, 1578). In addition, conditional and tissue specific PTEN knockout mice revealed a plethora of PTEN-associated functions in virtually all tissues and organ systems. This is not unexpected based on the multitude of processes regulated by PtdIns(3,4,5)P$_3$. More on the variety of the PTEN-related defects can be found in excellent reviews focusing on PTEN (805, 1434, 1501).

2. PTEN-related phosphatases

A) TPTE/TPIP PROTEINS. Humans express one TPTE (transmembrane phosphatase with tensin homology) (242, 1662) and several splice variants of TPIP (TPTE and PTEN homologous inositol lipid phosphatase) (α, β and γ) (1662) (FIGURE 7A). Of these, TPTE is specifically expressed in the testis and lacks phosphatase activity, while TPIPs are expressed in the testis, brain, and stomach and are enzymatically active against 3-phosphorylated inositides (1531, 1662). Mice also have a similar testis-specific protein called
PTEN2, but it does not seem to be the ortholog of the human TPTE or TPIP enzymes (535, 1738). TPIPα and -γ have NH2-terminal sequences suggestive of three to four transmembrane domains. Expressed TPIPα localizes to the ER, but TPIPβ, which lacks transmembrane segments, is cytosolic and has no significant phosphatase activity (1662). The transmembrane domains of TPIPγ show weak similarity to the voltage sensor of the ci-VSP (713, 1096). The functions of these proteins are presently unknown.

B) VOLTAGE-SENSITIVE PHOSPHATASES. Voltage-sensitive phosphatases (VSPs) were first identified in Ciona intestinalis based on their sequence similarity to PTEN and the presence of a voltage sensing domain within their transmembrane segments (1096). These enzymes are remarkable in that they hydrolyze phosphoinositides and increase their catalytic activity in response to depolarization. Similar enzymes have been found in other species, notably in Xenopus laevis (1259) and in zebrafish (650) with slightly different voltage characteristics, and the human TPIPs are likely to be their mammalian homologs (1163). The catalytic domains of VSPs are similar to that of PTEN, but they lack the COOH-terminal PEST sequences and the PDZ binding motif. However, in spite of their sequence homology to PTEN, these enzymes function as 5-phosphatases (as opposed to 3-phosphatases) acting on PtdIns(3,5)P3 and PtdIns(4,5)P2 (550, 713, 1097). The substrate specificity of VSP at least partially depends on a single Gly residue within the conserved C-(X)5-R sequence, and an Ala substitution at this position (G363A) abolishes the activity against PtdIns(4,5)P2 (713). Comparison of the structures of the catalytic domains of PTEN and ciVSP showed a reduced size of the catalytic pocket of ciVSP due to a protruding Glu411 residue (1009).

The voltage-sensing domain of VSPs shows significant homology to those of voltage-gated ion channels, and its voltage-sensing is manifested as a “sensing current” even in isolation (650, 1096). Mutations within the sensor module can shift the voltage dependence of the molecule (813). The exact mechanism of how the voltage-sensing unit couples to increased enzymatic activity is not yet understood. However, there is a close correlation between the conformational change of the voltage sensor and the activity of the enzyme (846, 1096, 1097). Also, the enzymatic activity of the phosphatase module has a retrograde effect on the voltage-sensing unit and phosphatase inhibitors and catalytically inactive Cys mutant catalytic modules alter the speed of the “sensing current” (650, 812). Recent studies found that the PBM domain of VSP has a critical role in coupling the voltage sensor to increased phosphatase activity (812, 846, 1637). This domain, which is homologous to the similar domain found at the NH2 terminus of PTEN, is a phosphoinositide binding module, and its deletion or mutations as well as PtdIns(4,5)P2 depletion affects the coupling between the two functional modules (812, 846, 1637).

The biological functions of VSP or its orthologs are not known, but their primary expression in the testes and its localization in the sperm tail in Ciona intestinalis (1096) suggests a role in fertilization. In spite of the limited understanding of its physiology, VSPs have become invaluable experimental tools. To study the multiple roles of PM PtdIns(4,5)P2, our ability to rapidly manipulate the level of this lipid in intact cells has been a highly desirable methodological invention. A major breakthrough in this area was the introduction of the rapamycin-induced translocation of 5-phosphatase enzymes to rapidly deplete PtdIns(4,5)P2 (1489, 1617). However, the VSP enzymes offer several further advantages over the rapamycin-inducible changes: they allow induction of extremely rapid, quantitatively precise, and reversible changes in PM PtdIns(4,5)P2. Therefore, VSP is becoming an important experimental tool in phosphoinositide research for the controlled manipulation of PM phosphoinositides (422, 609, 953).

C) PLIP/PTPTM1. A PTEN-like phosphatase (PLIP) has been described with unique preference to PtdIns5P as a substrate in vitro (1187). However, subsequent studies identified the same enzyme as a mitochondrion-localized protein tyrosine phosphatase (PTPTM1) that dephosphorylates phosphatidylglycerol-phosphate, an essential intermediate of cardiolipin synthesis (1797). Therefore, it is quite unlikely that this enzyme works on any phosphorylated inositide and its deletion does not affect PtdIns5P levels (1186).

3. Myotubularins

The myotubularin (MTM) family includes 15 members that share a distinctive phosphatase domain (FIGURE 7B) (776, 855, 856, 1536). Nine of these proteins possess 3-phosphatase activity against PtdIns3P and PtdIns(4,5)P2 and share a CXXGWDR sequence motif within their catalytic core. The other six homologs are catalytically inactive proteins often referred to as “pseudophosphatases” that associate mostly with and regulate the active MTM enzymes. The phosphatase active forms can be divided into four subgroups: the MTM1 group contains MTM1, MTMR1, and MTMR2; the MTMR3 group includes MTMR3 and MTMR4; and the MTMR6 subgroup has MTMR6, -7, and -8. MTMR14 stands alone in a separate group. The pseudophosphatases consist of MTMR5, -9, -10, -11, -12, and -13. In addition to their catalytic domains, these proteins contain several protein- and lipid-interacting modules, such as PH-GRAM domains (364), FYVE domains, coiled-coil domains, and COOH-terminal PDZ binding sequences (see FIGURE 7B for details). Most of the family members show expression in a wide range of tissues except MTMR5 and MTMR7 that are expressed exclusively in the testes and brain, respectively (440, 855, 856).

A) MTM1. This was the first MTM described as the gene mutated in X-linked myotubular myopathy (XLMTM) (858). MTM1 was originally believed to be a dual specific-
ity phosphotyrosine phosphatase (858), but its lipid phosphatase activity against PtdIns3P (152, 1536, 1814) was later demonstrated both as a GST-fusion protein and in cell expression studies. Many of the disease-causing mutations as well as mutation of the conserved Cys (C375S) residue render the enzyme catalytically inactive, and expression of such phosphatase inactive enzymes has dominant negative effects elevating PtdIns3P levels (1536). However, MTM1 can also dephosphorylate PtdIns(3,5)P2 and, therefore, increase PtdIns5P levels (1576). MTM1 knockout mice show perinatal mortality, and the pups that are born alive quickly succumb to muscle weakness and develop centronuclear myopathy with histological findings reminiscent of those seen in human XLMTM disease (196). Conditional and tissue-specific MTM1 knockout mice showed that MTM1 deficiency in skeletal muscle is the underlying cause of the disease, and it is not the development, but the maintenance of the skeletal muscle that requires this enzyme (196). MTM1 mutations were also identified in dogs suffering from X-linked myotubular myopathies (116). The intracellular localization of MTM1 has been controversial. Overexpressed MTM1 is mostly cytoplasmic with some perinuclear association, and it also shows up in membrane ruffles generated by overactive Rac (857). In other studies, endogenous MTM1 was found in early endosomes in association with the class III PI 3-kinase complex (212). It is likely that interaction with other proteins perhaps via phosphorylation-dephosphorylation cycles determine the location and therefore the localized regulatory function of the MTM1 protein. In support of this idea, MTMR12, a phosphatase-inactive MTM family protein, originally identified as an MTM1 adaptor protein (3-PAP), tightly associates with MTM1 (1117) and determines its localization (1118). MTM1 also associates with the intermediate filament desmin, and XLMTM-causing mutations in MTM1 disrupt this association leading to altered intermediate filaments, and abnormal mitochondrial positioning and functions (626). Recent data also suggest that MTM homologs in lower organisms control discrete pools of PtdIns3P specifically involved in endocytosis (1127, 1625).

B) MTM1 and -2. MTMR (R stands for -related) proteins are close homologs of MTM1. MTMR1 is in fact located next to MTM1 on the X-chromosome both in humans and mice as a result of gene duplication (799). Accordingly, the structure and enzyme activity of MTM1R is very close to those of MTM1 (1576). The function(s) of MTMR1 is not known, but it is likely that the restricted phenotype of MTM1 mutations are due to the fact that MTMR1 can substitute for MTM1 functions in all but a few tissues. MTM2, on the other hand, was discovered as the gene, whose mutations cause the hereditary recessive disorder Charcot-Marie-Tooth disease type 4B1 (CMT4B1), which is characterized by peripheral neuropathy due to demyelination (124, 159, 653, 792). Disease-causing mutations severely affect the ability of MTMR2 to dephosphorylate PtdIns3P and PtdIns(3,5)P2 (123, 792). The structure of MTMR2 has been solved and found to have a larger phosphatase domain than other PtdIns3P phosphatases (117, 118). This is because of presence of a “SID” [SET interaction domain (36)] domain within the phosphatase domain of MTMR2 (118). These studies also found that the NH2-terminal GRAM domain of MTMR2 has a similar architecture to PH domains, and it also binds phosphoinositides, primarily PtdIns(3,5)P2 (118). Expressed MTMR2 has been reported to be cytosolic or associated with perinuclear membranes or the nucleus (793, 1274). However, endogenous MTMR2 localizes to late endosomes (211). Recent studies showed that dephosphorylation of the NH2 terminus of MTMR2 facilitates its binding to early endosomes (462). MTMR2-deficient mice develop symptoms very reminiscent of those found in CMT4B1 patients with peripheral neuropathy and impaired spermatogenesis (158, 164). The myelin unfolding seen in these mice can be reproduced by selective deletion of the gene in Schwann cells (160, 161), confirming the primary defect in myelination. MTMR2 interacts with the catalytically inactive pseudophosphatases MTMR5 (also named Set binding factor 1 or SBF1) (793) and MTMR13 (also called SBF2), and these interactions greatly enhance the phosphatase activity of MTMR2, as well as determine its cellular localization (123, 1274). Mutations in MTMR13 cause Charcot-Marie-Tooth disease type 4B2 (CMT4B2) with similar defects to CMT4B1 patients with peripheral neuropathy and impaired spermatogenesis (158, 164), and these deficiencies can be replicated in mice with MTMR13 gene disruption (1275, 1545). In contrast, MTMR5 deletion in mice causes defective spermatogenesis without any peripheral neuropathy (440). Therefore, it appears that the MTMR2-MTMR5 complex functions in spermatogenesis, whereas the MTMR2-MTMR13 complex is essential for myelination in Schwann cells (1273).

C) MTMR3 and -4. These widely distributed phosphatases are characterized by the presence of a FYVE domain in their extended COOH termini (1659, 1814). Interestingly, however, the FYVE domain of MTMR3 does not bind PtdIns3P, nor is it responsible for the endosomal localization of MTMR3 (941). MTMR3, on the other hand, binds PtdIns5P at its GRAM domain (941), which may be important for allosteric activation of the enzyme by this rare lipid species (1355). Only few data are available on the functions of these phosphatases. Expression of human MTMR3 in yeast induces enlargement of vacuoles and increases yeast PtdIns5P levels (1659) consistent with its ability to dephosphorylate PtdIns3P and PtdIns(3,5)P2 (1659). Recent genome-wide association studies identified MTMR3 as a susceptibility gene in lung cancer in a large Chinese population (659) and also in early-onset inflammatory bowel disease in another study (598). It is not clear yet how the functions of these genes contribute to the respective pathologies. Nevertheless, evidence is accumulating that these phosphatases also regulate endocytic sorting (1122), routing, and inacti-
vation of receptors (1780) and also can be ubiquitylated (1227).

D) MTMR6, -7, and -8. MTMR6 and MTMR7 are both capable of hydrolyzing PtdIns3P and PtdIns(3,5)P \(_2\) (1355), but MTMR7, which is mainly expressed in the brain, also acts on water-soluble Ins3P (1075). MTMR6 has been shown to inhibit the Ca\(^{2+}\)-activated potassium channel K\(_{Ca}\)3.1 (1444), which requires PtdIns3P for its optimal activity (1442). Through this action, MTMR6 expression depolarizes T cells, thereby decreasing the driving force for the \(I_{\text{CRAC}}\) channels, and hence, inhibiting T-cell activation (1443). This action of MTMR6 is not mimicked by MTMR8 and, conversely, MTMR6 does not regulate other, highly related K\(_{Ca}\) channels (1442). Recent studies reported that the right balance between PI3K and MTMR8 is important for muscle (1024) and vascular development (1025) in zebrafish. Of the pseudophosphatases, MTMR9 interacts with both MTMR6 and MTMR7 and activates the phosphatase activity of the respective enzymes (942, 1075, 1832).

E) MTMR14. This member of the MTMR family was identified in silico (1566) based on its homology to MTMRs and its high sequence homology to a *Drosophila* protein named “egg-derived tyrosine phosphatase” (EDTP) (1756). Disruption of the gene in flies produced the “JUMPY” phenotype characterized by progressive loss of muscle control and shaggy movements (1382). Importantly, the human Jumpy/MTMR14, which displayed PtdIns3P and PtdIns(3,5)P \(_3\) 3-phosphatase activity, harbored catalytically compromising mutations in two patients suffering from centronuclear myopathy (1566). The same phosphatase was also identified as an inositol lipid phosphatase showing highest activity against PtdIns(3,5)P \(_2\) with prominent expression in skeletal muscle and heart and hence also named muscle-specific inositol phosphatase (MIP) (1393). MTMR14-deficient mice show muscle weakness and fatigue that develops with aging, due to a Ca\(^{2+}\) leakage from the SR-cisternae apparently because of increased PtdIns(3,5)P \(_2\) levels (1393). The progressive muscle weakness is attributed to an increased activity of ryanodine receptors by the PtdIns(3,5)P \(_2\) (1570), altered t-tubule morphology observed in MTMR14-deficient mouse (625), and impaired excitation-contraction coupling in MTMR14-depleted zebrafish embryos (382). MTMR14 levels are also decreasing with age in parallel to weakened muscle function, and deletion of the gene in mice significantly speeds up this process (1293). Since MTMR14 was also shown recently as a negative regulator of autophagy (1631, 1632), the rapid aging of muscles might be also related to an altered process of autophagy in MTMR14-deficient animals (625).

C. 4-Phosphatases

Phosphoinositide 4-phosphatases can remove the phosphate from the 4-position of PtdIns(3,4)P \(_2\) or PtdIns(4,5)P \(_2\). The enzymes that hydrolyze PtdIns(3,4)P \(_2\) are called INPP4A and INPP4B, while those that use PtdIns(4,5)P \(_2\) are TMEM55A and -B (the A and B forms of both groups are also called types I and II, respectively) (FIGURE 8A). The mammalian Rac guanine nucleotide exchange factors P-Rex1 and -2 also contain a domain homologous to the phosphatase domains of INPP4s, but these proteins do not have phosphatase activity (1317, 1698). There are two bacterial virulence toxins, IpGD from *Shigella flexneri* and SigD/SopB from *Salmonella*, that possess a wide range of phosphoinositide phosphatase activity in vitro but appear to act on PtdIns(4,5)P \(_2\) as a 4-phosphatase when expressed in cells, yielding the rare lipid PtdIns3P. Therefore, they are often used to study the functions and metabolism of PtdIns3P (1130, 1255, 1544). The Sac1 phosphatase dephosphorylates PtdIns4P, but it can also dephosphorylate PtdIns3P and will be discussed separately. No PtdIns(3,4,5)P \(_3\)-directed 4-phosphatase activity has been reported.

1. INPP4s

Two enzymes belong here, INPP4A and INPP4B. INPP4A, first isolated and cloned from rat brains, is ~100 kDa in size and dephosphorylates the 4-position in PtdIns(3,4)P \(_2\) and to a much lesser extent Ins(3,4)P \(_2\) and Ins(1,3,4)P \(_3\) (1149, 1150). Another form of the enzyme, INPP4B, was cloned subsequently showing highest expression in the heart and skeletal muscle (originally termed type II inositol polyphosphate 4-phosphatase) (1147). In addition to their conserved phosphatase domains, both enzymes have a C2 domain at their NH\(_3\) termini that show distinct lipid binding specificities. While the C2 domain of INPP4A prevents binding PtdIns(3,4)P \(_2\), PtdIns3P and PtdSer (712), that of INPP4B binds PtdOH and PtdIns(3,4,5)P \(_3\) (434). In addition, there is a PEST sequence in INPP4A (but not INPP4B) that is cleaved by calpain, thereby inactivating the enzyme, as shown in stimulated platelets (1148). Expressed INPP4A is localized to early and recycling endosomes and is activated by Rab5 (1401). Consistent with an endosomal function, knockdown of INPP4A decreases transferrin uptake (1401). Conversely, overexpression of the enzyme restores the morphology of enlarged endosomes caused by PtdIns3P deficiency (712), suggesting that INPP4A can produce PtdIns3P. INPP4A acts as a negative signal via decreasing the levels of PtdIns(3,4)P \(_2\) and attenuates Akt activation and also exerts an antiproliferative effect (712, 1649). Decreased levels of INPP4A and loss of heterozygocity of the enzyme are observed in a variety of cancers, suggesting that the enzyme acts as a tumor suppressor (428). No such functions have been attributed to INPP4B. A frameshift mutation that eliminates a functional Inpp4a in mice was found to cause the weeble phenotype (1153). These mice develop early onset ataxia due to the loss of Purkinje cells from the cerebellum. Interestingly, islands of Purkinje cells remain normal, and these express Eaat4, a glutamate transporter, suggesting that the lack of Inpp4a is associated with glutamate toxicity that is remedied by
glutamate removal by the Eaat4 transporter (1321). Recently, an Inpp4a knockout mouse was generated and showed rapid neurodegeneration in the striatum and basal ganglia, causing a phenotype similar to involuntary movement disorders such as Parkinson disease. The neurons of these mice undergo NMDA receptor-mediated cell death due to the increased levels of NMDA receptors at the postsynaptic density (1343). There is little known about the connection between Inpp4s and human disease. A polymorphism in INPP4A has been associated with severe asthma (1388), and a loss of INPP4A and -B has been reported in various human malignancies (107, 1317).

2. TMEM55

The two forms of these enzymes, A and B, originally called type I and type II PtdIns(4,5)P₂ 4-phosphatases, were cloned based on the presence of a conserved CX₅R motif also found in the Burkholderia pseudomallei virulence factor Bop, a putative phosphatase (1593). Both enzymes are expressed ubiquitously and act exclusively on PtdIns(4,5)P₂. The TMEM55 name refers to the two putative transmembrane domains found in the COOH termini of these proteins following their catalytic domains (1317). Overexpression of TMEM55A increases EGF receptor degradation (1593). TMEM55B, on the other hand, was shown to control nuclear PtdIns5P levels and respond to DNA damage by nuclear translocation, activating the ING2 protein (522) and promoting p53-mediated cell death (1833).

D. The SAC1 Family of Phosphatases

The prototypical Sac1 phosphatase was discovered independently in two laboratories as a mutant allele that rescued defects associated with temperature-sensitive actin mutants (1152) or secretion defects caused by sec14ts mutations in yeast (273). The connection between SAC1 being linked to actin dynamics on the one hand, and to the secretion process on the other, had not been fully realized for a long period, nor was it known what the Sac1p protein did in yeast cells. Eight years had passed from these original discoveries before it was realized that Sac1 is a phosphoinositide phosphatase (538, 667). On the basis of sequence similarities within their phosphatase domains, additional members of the SAC1 phosphatase family include the yeast Sac3 and yeast Fig4p work as PtdIns(3,5)P₂ 5-phosphatases. hSac2 is a 5-phosphatase acting on PtdIns(4,5)P₂ and PtdIns(3,4,5)P₃ yet, structurally is a homolog of the Sac1 family.
1. Sac1/SAC1M1L

The yeast Sac1p is capable of removing a phosphate from phosphoinositide monophosphates, such as PtdIns4P, PtdIns3P, and PtdIns5P, and also from bisphosphorylated PtdIns if the phosphates are not in vicinal positions such as in PtdIns(3,5)P$_2$, but not PtdIns(3,4)P$_2$ or PtdIns(4,5)P$_2$ in vitro (538, 667). Sac1-deleted yeast strains are viable, but show defects in actin dynamics, vacuolar sorting, and secretion and have cell wall abnormalities (273). They also show large (8- to 10-fold) accumulation of PtdIns4P with moderate increases in PtdIns3P and PtdIns(3,5)P$_2$ (455, 538, 667). Paradoxically, PtdIns4P increases are not associated with increased PtdIns(4,5)P$_2$; in fact, the latter shows moderate to substantial decreases depending on the study (455, 538, 667). The large increase in PtdIns4P in Sac1-deleted yeast requires a functional Stry but not Pik1, suggesting that Sac1 controls primarily the PtdIns4P pools generated by the Stry4P PI4K (455). This finding raised important questions since Sac1 is primarily found in the ER while Stry is in the PM, enriched in ER-PM junctional zones (79). Recent studies solved this apparent conundrum showing that ER-tethered Sac1 can act in trans to control PM PtdIns4P that is generated by Stry at ER-PM junctional zones (1454). However, it is still puzzling why the increased PM PtdIns4P is not reflected in increased PtdIns(4,5)P$_2$ levels. Sac1 is tethered to the ER by its COOH-terminal hydrophobic motif that binds to the ER dolicholphospho-mannose synthase enzyme Dpm1 (427). However, Sac1 cycles between the Golgi and the ER, and under nutrition deprivation, it is mostly localized to the Golgi and it is suggested that this relocalization limits PtdIns4P levels in the Golgi and, hence, inhibits secretion (427). Sac1 also associates with Orm proteins at the ER that are negative regulators of serine palmitoyltransferase, the rate-limiting enzyme in sphingolipid synthesis (178). Sac1 is also linked to the synthesis of complex sphingolipids in additional ways as it was found to be important for the synthesis of inositol phosphoceramide (IPC) (179). Accordingly, Sac1 deletions increase the sensitivity of yeast cells to Aerobasidin, an inhibitor of IPC synthesis (1529). All of these studies suggest that Sac1 performs multiple functions as part of numerous distinct protein complexes linked to different pathways.

The mammalian Sac1 enzyme is a ubiquitously expressed protein, first isolated from rat brain and showing the same substrate specificity profile as the yeast ortholog, capable of hydrolyzing all PtdIns monophosphates and PtdIns(3,5)P$_2$ but not PtdIns(4,5)P$_2$ (1125). Curiously, human Sac1 expressed as a GST fusion protein only acts on PtdIns3P and PtdIns4P in spite of 95% homology with the rat enzyme (1289). Mammalian Sac1 is mostly ER localized in steady state, but it cycles between the Golgi and the ER. A basic stretch of amino acids found at the Sac1 COOH terminus mediates its interaction with the coatamer COP II complex that mediates its retrieval to the ER (147). Under starvation, Sac1 interacts with COPII and undergoes oligomerization mediated by its NH$_2$-terminal leucine-zipper motif, and a significant fraction of Sac1 is then found in the Golgi (147). This process is reversed by growth factor stimulation via activation of p38 MAPK (147). Mammalian Sac1 is an essential protein, and deletion of the gene in mouse causes preimplantation lethality, and even in cells, knockdown of Sac1 leads to cell death, making it difficult to study the functions of the protein (929). Sac1-deficient cells show a major disorganization of their Golgi morphology without a gross defect in secretion. Importantly, using PtdIns4P-recognition PH domains, no sign of PtdIns4P accumulation in any cellular compartment could be detected, although an increased level of $[^3]$H]PtdIns4P was demonstrable in metabolically labeled cells (929). Sac1 knockdown also caused a high level of spindle defects, creating aberrant chromosomal segregation and a block in exit from mitosis (929). All of these defects can be rescued by overexpression of Sac1, and both its catalytic activity and ability to cycle between the ER and the Golgi were required for complementation (929). Sac1 mutant flies also die in embryonic stage and develop dorsal closure defects (1689). The structure of yeast Sac1 has been recently solved at 2 Å resolution. The enzyme consists of an NH$_2$-terminal lobe of yet unknown function and a COOH-terminal catalytic lobe showing both similarities and notable differences with other phosphoinositide phosphatases, such as PTEN and MTMR2 (979).

2. Fig4/SAC3

Yeast Fig4 was first identified in a screen looking for genes that are activated by pheromone-induced mating pathway (406). FIG4 was found as a nonessential gene that was required for cell and actin polarization and notably had a Sac1 homologous region (406). It took several years again before it was discovered that Fig4p is a phosphoinositide phosphatase that dephosphorylates PtdIns(3,5)P$_2$ at the 5-position (497, 1045). As already discussed in the Fab1p PtdIns3P 5-kinase section (sect. 5), Fig4p forms and stabilizes a trimeric complex with Fab1p and the scaffold protein, Vac14p located on the vacuolar membrane (390, 1042). This explains why deletion of Fig4 paradoxically decreases, rather than increases, PtdIns(3,5)P$_2$ levels (389, 390, 1311) as it is needed to keep Fab1p in a functional form. Therefore, Fig4 deletions cause vacuolar fission defects with enlarged vacuoles and defective retrograde transport (1311).

The mammalian homolog of Fig4 is called Sac3, and it should not be confused with the yeast Sac3 protein that is a member of the nuclear pore complex involved in mRNA export (878). The first mammalian Sac3 was identified as a human KIAA 00274 clone (1057) and characterized as the mammalian homolog of Fig4 (1353). Human Sac3 displayed features similar to its yeast counterpart: it hydrolyzed PtdIns(3,5)P$_2$, formed a complex with the mammalian
homologs of Fab1 (PIKfyve) and Vac14 (ArPIKfyve), and localized to endosomes (1353). Interestingly, in a parallel study, Sac3 was characterized in rats as a ubiquitously expressed protein that localized to the ER, Golgi, and recycling endosomes and played a role in neurite extension in response to NGF (1786). This latter report found Sac3 displaying a wider substrate profile of phosphatase activity acting on PtdIns4P, PtdIns3P, and PtdIns(3,5)P2. Genetic inactivation of Sac3 by an early transposon insertion into the mouse Fig4/Sac3 gene is responsible for the “paletremor mouse.” This Fig4−/− mouse is characterized with pigmentations defects and progressive spongiform neurodegeneration. Fibroblasts obtained from Fig4−/− mice show a decrease rather than an increase in PtdIns(3,5)P2 levels with prominent defects at the late-endosome to lysosome transition (and probably in many other trafficking steps). These are probably the underlying causes for the pigmentation defect and neurodegeneration (253). Pathogenic mutations in the human FIG4 gene have also been linked to a special form of autosomal recessive Charcot-Marie-Tooth disorder, CMT4J, characterized by motor and sensory neuropathy (253) and in a small percentage of patients with amyotrophic lateral sclerosis (ALS) (252). The most common mutation of Sac3 in CMT4J patients is 1411T, which decreases Sac3 association with the PIKfyve-ArPIKfyve (Fab1-Vac14) complex and enhances its proteosomal degradation (683, 884). However, the Fig41411T transgene over-expressed in Fig4−/− mice rescues the most severe phenotypes showing that this mutant protein still retains most of its functions (884). FIG4−/− mice also develop severe myelination defects that are especially pronounced in smaller caliber axons, and the number of myelinating oligodendrocytic progenitors are greatly reduced (1720). Remarkably, this defect can be corrected with neuron-targeted expression of Sac3/Fig4, suggesting that the defective oligodendrocytic progenitors are secondary to a neuronal defect. In this study, again the Fig41411T mutant enzyme was able to rescue the hypomyelination (1720).

3. hSac2/INPP5F

There is no yeast homolog of human Sac2. The human Sac2 enzyme was identified as a phosphatase showing significant homology to the yeast Sac1 and Fig4 proteins (1057). Strikingly, this enzyme is a PtdIns(4,5)P2 5-phosphatase capable of acting also on PtdIns(3,4,5)P3 but not on any of the other phosphoinositides or soluble inositol phosphates (1057). A genetrap mouse lacking a functional Sac2 shows no major abnormalities but is more susceptible to isoproterenol-induced cardiac hypertrophy, whereas overexpression of Sac2 in hearts protects animals from developing cardiac hypertrophy (1823). These studies suggested that Sac2 reduces PtdIns(3,4,5)P3 levels in mouse heart and hence antagonizes the activation of the fetal gene programs that underlie cardiac hypertrophy.

VII. PHOSPHOLIPASE C ENZYMES

PLC enzymes hydrolyze the phosphodiesters at the sn-3 position of phosphoinositides, yielding inositol phosphates and DAG. Other PLC enzymes (e.g., ones that hydrolyze PC) also exist justifying the term “phosphoinositide-specific” or PI-PLC to designate the substrate specificity of these proteins. Several bacteria also use PLC enzymes as virulence factors (528, 1646), and the bacterial PLCs do not hydrolyze phosphorylated PtdIns and act primarily on GPI linkages at the outer surface of cells. However, bacterial PLCs can also act on PtdIns itself. Because of this substrate restriction, the bacterial PLCs are also referred to as PtdIns (or PI) specific PLCs, which can cause some confusion. The eukaryotic PI-PLCs can use PtdIns, PtdIns4P, and PtdIns(4,5)P2 but not 3-phosphorylated inositol as substrates in vitro, but they are believed to act primarily on PtdIns(4,5)P2 in their native cellular environment. Only a few studies addressed this question rigorously, and a few reports suggested that PLCs act on PtdIns (1066) and that they are present in intracellular locations (149, 354), where they could act on PtdIns or PtdIns4P.

Initially, PLC enzymes were studied as activities responsible for phospholipid catabolism in the lysosome (703, 1272). After discovering that PLC was a key component of receptor-regulated signal transduction (1040) (see sect. III on the history of phosphoinositides), these enzymes were sought after as important receptor-regulated signal transducers. Having membrane-bound substrates, PLCs were studied in membranes, the red blood cell membrane being the purest case (383), but they were also detected and characterized in soluble fractions obtained from sheep seminal vesicle (630) or from rat and bovine brain (704, 1319, 1555). It was an important question to understand how PLCs were regulated by receptors. The Ca2+ requirement of PLC enzymatic activity and its strong stimulation by Ca2+ supported views that Ca2+ elevations might be the primary signals linking receptors to PLCs. These claims were later settled as PLC activation clearly precedes the cytoplasmic Ca2+ increases (see sect. III for more details). Also, several studies showed that the physicochemical state of the membrane determines the access of PLC to phosphoinositide substrates, raising the possibility that substrate presentation would be an effective way to regulate the enzyme (e.g., Refs. 631, 704). Soon it became clear that PLC enzymes are regulated by GPCRs via heterotrimeric G protein subunits (174, 571, 602, 1539). It was an important discovery in the late 1980s that GPCRs and RTKs generate Ins(1,4,5)P3 and evoke cytosolic Ca2+ increases with notably different kinetics (939, 1116), suggesting that the two kinds of receptors activate PLC enzymes with different mechanisms and possibly they act upon distinct PLC isoforms (67, 603, 1236). By that time two or three major forms of PLCs have been purified from various sources including the sheep seminal vesicle (630), liver (1521), and bovine brain (763, 1265, 1318, 1319). These activities differed in molecular size and immu-
noreactivity. These advances were rapidly followed by the molecular cloning of the first mammalian PLC enzymes (1493, 1494) that later became known as PLCβ, PLCγ, and PLCδ (1271). The protein initially designated as PLCα turned out to be an ER resident Cys-protease (1179, 1595), so there is no PLCα enzyme. The genomic era has led to the subsequent identification of several forms of each of the three classes and revealed additional families such as PLCε, PLCζ, and PLCη with a total of 13 isoforms belonging to six PLC families (572) (FIGURE 9).

Each PLC contains common basic building blocks, such as a PH domain, four EF-hands, a triose phosphate isomerase (TIM) barrel-like catalytic domain formed from X and Y conserved regions, and a C2 domain (found in this order from NH₂ to COOH terminus). In addition, most PLC families feature additional unique regulatory regions (FIGURE 9). PLC enzymes work mostly at the PM, and they are recruited to the membrane via multiple interactions with proteins and lipids mediated by the PH domain, the C2 domain, and a polybasic stretch within the CT-sequence found in PLCβ forms (419, 1214, 1673, 1760). The exact mechanism of membrane recruitment, however, is mostly obscure. The first mammalian PLC structure was that of PLCδ1 (408) followed by those of PLCβ2 (729), PLCβ3 (1657), and an invertebrate PLCβ (958). These structural studies revealed the molecular anatomy of the catalytic core and its relationship to the substrate PtdIns(4,5)P₂ (408). They also showed the mode of interaction of PLC enzymes with regulators such as Rac1 (729) and the Gαq subunit (958, 1657). These structural studies also helped identify an acidic autoinhibitory loop located between the X and Y conserved domains, which may act as a pseudosubstrate to occlude the catalytic active site of the enzymes (612).

**FIGURE 9.** The phospholipase C family. These enzymes hydrolyze PtdIns(4,5)P₂ to Ins(1,4,5)P₃ and diacylglycerol, hence are called phosphoinositide-specific (PI)-PLCs. They should not be mistaken for the bacterial PLCs that are specific to PtdIns, unable to hydrolyze phosphorylated PtdIns, and hence called PI-specific PLC (unfortunately similarly abbreviated to PI-PLC). Mammalian PLCs have a core structure consisting of a PH domain, followed by EF hands, a catalytic domain formed from X and Y conserved regions and a C2 domain. PLCδ enzymes show this minimal domain organization. The only exemption is PLCζ that lacks the PH domain. This basic core is then extended in the various PLCs: the β enzymes have a characteristic COOH-terminal extension (CTR) that lends regulation by heterotrimeric G protein alpha subunits. The X and Y domains are separated with a long insert in the PLCγ enzymes consisting of two SH2 domains, an SH3 domain, and this whole insert is sandwiched in between two half PH domains. The PLCγ enzymes have a long serine-proline-rich (S/P) segment in their COOH termini. The largest PLC enzyme is PLCζ, which has a cysteine-reach region (CR) and a RAS-GEF domain at the NH₂ terminus and two Ras association domains (RA) at the COOH terminus.
A thiol-reactive alkylation compound, U73122, has been widely used as an inhibitor of PLC (150). However, how this compound works is quite puzzling. It does behave as a PLC inhibitor in many cellular systems with a very narrow concentration range (2–5 μM) between effective and toxic doses (1423, 1552, 1732). However, it either has no effect on purified PLC enzymes or stimulates PLC activity in vitro at somewhat higher concentrations (~10 μM) (802). U73122 also inhibits a number of Ca2+-regulated processes independent of its putative effects on PLC (169, 1240, 1660, 1817). The fact that its nonreactive control compound U-73343 does not reproduce the effects of the active compound, by no means is a satisfying criterion to indicate specificity on PLC. Newly developed PLC inhibitors will be extremely helpful to better understand the role of PLC enzymes in various cellular settings and also to understand the mode of action of U73122 (664).

PLC enzymes have also been identified in lower eukaryotes such as the PLCα-like Plc1 in S. cerevisiae (447, 1771) and Dictostelium (387). A PLCβ-like protein called NorpA was found in Drosophila (153), and a homologous G protein-regulated PLC is present in the squid eye (1067). The latter two play a critical role in invertebrate photo-signal transduction. Deletion of Plc1 in yeast causes several abnormalities with severe growth defects, impaired cell wall integrity, decreased osmotic resistance, and an inability to use carbon sources other than glucose (447). Hyposmotic stress activates Plc1 in yeast, and the produced Ins(1,4,5)P3 is rapidly converted to InsP6 (1208). Intriguingly, PLC deletion in Dictostelium does not cause any major abnormalities, but PLC null cells fail to properly respond to chemorepellents while they do respond to chemotactants, suggesting a modulatory role of PLC in chemosensing (771). NorpA mutant flies are viable, but they have defective light response in the retina and develop retinal degeneration (153). NorpA also serves as a downstream effector of the fly rhodopsin (ninaE) in temperature discrimination (1395).

A. PLCβ

There are four members of the PLCβ family designated as PLCβ1–4. All PLCβ enzymes are activated by Gα subunits of the Gq/11 class (1425, 1538, 1656) and PLCβ2 and PLCβ3 also by βy subunits (174, 207). Therefore, PLCβ is a major downstream effector of GPCRs. It is now understood that GPCR activation of PLC that is resistant to pertussis toxin is mediated by the Gα subunits of Gq/11 (mostly PLCβ1, -β3, and -β4), while pertussis toxin-sensitive PLC activation by GPCRs occurs via βy subunits liberated from G and Gα proteins (mostly PLCβ2 and -β3) (183). PLCβ1 (and probably all other β isoforms) also acts as GTPase activating proteins (139). For a time it was believed that the characteristic COOH-terminal conserved coiled-coil (CT) domain found in all PLCβ is the site of activation by G protein α-subunits and is also the region that confers GAP activity (690, 1196, 1201, 1410). Structural studies allowed a more precise insight into the molecular details of Gq activation. They identified a unique, highly conserved helical extension of the C2 domain not found in any other PLC forms as the major site of Gq interaction (958, 1657). Most recent studies using expressed GFP-tagged enzymes in intact cells found that only PLCβ1 and PLCβ4 were enriched in the PM while PLCβ2 and PLCβ3 were mostly cytosolic and that the isolated CT domains of all but PLCβ2 were also localized to the PM (7). The importance of the CT domain was also highlighted in this study by the ability of the expressed isolated CT domains to inhibit Gq-mediated PLC activation and Ca2+ signaling apparently by interacting with the Gq alpha protein (7).

1. PLCβ1

This enzyme is broadly expressed but especially enriched in brain cortex and hippocampus. In fixed cells, the endogenous enzyme is found in the cytoplasm presumably associated with a variety of membranes (844), but an overexpressed PLCβ1 tagged with GFP associates with the PM (7, 8, 380). Two splice variants of PLCβ1 (PLCβ1a and -b), first described in heart, differ in their COOH termini and show different membrane localization (532). Both isoforms of PLCβ1 were also found in the nucleus (451, 987) and have a role in cell cycle control, lamin B1 phosphorylation, and G2/M progression (416, 443). PLCβ1 was also linked to myelodysplastic syndrome and its progression to acute myeloid leukemia (452). PLCβ1−/− mice show growth retardation, and although viable at birth, they start to die at the third week after severe generalized seizures without any sign of gross brain structural abnormality. The similarity of these seizures to those developing after GABAA receptor inhibition suggested that PLCβ1 deletion affects the function of inhibitory neurons (784). Interestingly, impairment of receptor-mediated increase in PLC activity showed remarkable selectivity in brain slices from PLCβ1−/− mice in that the response to muscarinic stimuli was greatly reduced, while that to mGluR agonist was enhanced and the response to serotonin receptor stimulation was unchanged (784). However, in another report, the cortical layering of neurons in the somatosensory cortex of PLCβ1−/− mice was found impaired and the InsP response to mGluR stimulation significantly reduced (565). Other studies found these mice still reaching normal age but showing memory deficits, hyperactivity, reduced acoustic startle response, attributed to an imbalance between muscarinic and dopaminergic or serotoninergic systems, reminiscent of animal models of schizophrenia (1020).

2. PLCβ2

This isoform is mostly expressed in hematopoetic cells (1194), but it is also found in other tissues such as in taste buds (787, 1070, 1299). PLCβ2 is primarily regulated by
βγ subunits released from pertussis toxin-sensitive G\(_{\alpha}\)/G\(_{\beta}\gamma\) proteins (174, 207, 1426). PLC\(_{3}\)2 is also activated by small GTP binding proteins of the Rho family (691, 692), and this regulation requires the PH domain (693, 1428). The structure of PLC\(_{3}\)2 with a bound Rac1 showed a direct interaction between the PH domain of PLC\(_{3}\)2 and the switch region of Rac1 (729), but no major conformational change in the catalytic site compared with the PLC\(_{3}\)2 structure alone. Rac1 was also shown to recruit the enzyme into more specialized membrane compartments relative to the more general membrane recruitment by G\(_{\beta}\gamma\) subunits (542, 693). PLC\(_{3}\)2 \(-/-\) mice are viable but, contrary to expectations (being enriched in hematopoietic cells), their immune responses to bacterial and viral pathogens are not impaired, if anything they are enhanced. At the same time, neutrophil cells of these animals showed a greatly reduced InsP\(_3\)\(_{2}\), Ca\(^{2+}\) and superoxide responses, but a variety of bone marrow-derived cells (including neutrophils) showed enhanced chemotaxis, suggesting that PLC\(_{3}\)2 may exert a negative rather than positive influence on chemotactic signaling (732, 906). This may be due to more PtdIns(4,5)P\(_2\) being available for PI 3-kinases. Interestingly and conversely, another study found that PLC\(_{3}\)2 and -β3 are critical for T-cell chemotaxis (72). PLC\(_{3}\)2 \(-/-\) mice are also impaired in sweet, amino acid, and bitter taste reception (1091, 1808).

3. PLC\(_{3}\)β3

This broadly expressed isoform can be activated by G\(_{\alpha}\)/G\(_{\beta}\gamma\) as well as G\(_{\beta}\gamma\) subunits (1215). The recent structure of PLC\(_{3}\)β3 in complex with activated G\(_{\alpha}\) defined the interface between the two proteins (1657). This enzyme has also been claimed to have an intracellular role to mediate the G\(_{\beta}\gamma\)/PKC\(_{\gamma}\)/PKD-mediated regulation of TGN to PM transport (354). PLC\(_{3}\)β3 \(-/-\) mice have a shorter life span and succumb to myeloproliferative diseases, lymphomas, and other forms of cancer (1742, 1743). These animals do not respond to stimuli that evoke an itching response apparently due to impaired signaling from H1 histamine receptors in C-fiber nociceptive neurons (563). Smooth muscle cells obtained from PLC\(_{3}\)β3 \(-/-\) mice failed to show Ca\(^{2+}\) elevations in response to opiate receptor stimulation. However, these mice showed high sensitivity to morphine and increased voltage-gated Ca\(^{2+}\) channel activity in response to opiates in their dorsal root ganglion neurons arguing against impaired opioid receptor signaling (1746). In zebrafish, PLC\(_{3}\)β3 was found critical for endothelin-1-driven regulation of pharyngeal arch patterning (1661) and a negative regulator of VEGF-mediated vascular permeability (629).

4. PLC\(_{3}\)β4

This enzyme is mostly expressed in specific areas in the brain, especially in the cerebellum (789, 868, 1526). A shorter splice variant of the enzyme has also been described showing high expression in the rat retina (5) but not responding to G\(_{\alpha}\)q stimulation (790). PLC\(_{3}\)β4 is regulated exclusively through G\(_{\alpha}\)q and not via G\(_{\beta}\gamma\) or Rac proteins (570, 867). Because of the closest homology of PLC\(_{3}\)β4 to the Drosophila NorpA protein that has key roles in photo signal transduction, and the presence of the enzyme in retina, it was assumed that this isoform had important roles in visual processing in mammals. PLC\(_{3}\)β4 \(-/-\) mice do, indeed, have impaired visual processing, but it is not related to defective photoreceptor light responses or structural aberrations of the retina but probably due to impaired signal processing (733). PLC\(_{3}\)β4 \(-/-\) mice develop normally but show ataxia with slower cerebellar development (784). The InsP\(_3\) responses to mGluR or muscarinic receptor stimulation were reduced in cerebellar slices obtained from PLC\(_{3}\)β4 \(-/-\) mice (784), and the process of climbing fiber synapse elimination in the developing cerebellum was also found impaired (759).

B. PLC\(_{3}\)δ

There are three different PLC\(_{3}\)δ enzymes, named PLC\(_{3}\)δ1, PLC\(_{3}\)δ3, and PLC\(_{3}\)δ4 (570) (PLC\(_{3}\)δ2 turned out to be the bovine version of human PLC\(_{3}\)δ4; Ref. 700). These enzymes all show broad tissue distribution but differ in cellular localization. PLC\(_{3}\)δ enzymes possess the minimal PLC building blocks: the NH\(_2\)-terminal PH domain, the EF hands, the conserved X and Y domains, and the C2 domain at the COOH terminus (FIGURE 9). The PLC enzymes of lower organisms (yeast and Dictyostelium) are orthologs of PLC\(_{3}\)δ. The regulation of this isoform is the least understood. The activity of the enzyme is increased with Ca\(^{2+}\) elevations in the physiological range (33) but not by the G proteins that activate other PLCs (neither the heterotrimeric, nor the small forms). However, it was suggested that PLC\(_{3}\)δ1 is activated by the atypical G protein, transglutaminase II, or GoH (429) or the small GTP binding protein RalA (515, 1406). According to current views, PLC\(_{3}\)δ1 is regulated via its PH domain that recruits the enzyme to the PM. The isolated PH domain of PLC\(_{3}\)δ1 was one of the first PH domains to be structurally characterized showing binding to both PtdIns(4,5)P\(_2\) and the soluble Ins(1,4,5)P\(_3\) (431, 882). Because of its binding to PtdIns(4,5)P\(_2\), the PH domain of PLC\(_{3}\)δ1 is widely used as a PtdIns(4,5)P\(_2\) reporter in live cells (1451, 1615). Therefore, the enzyme is recruited to the PM via binding to its own substrate, PtdIns(4,5)P\(_2\), and the production of Ins(1,4,5)P\(_3\) would displace the enzyme from the membrane providing with a self-limiting regulatory mechanism (936). However, PM recruitment may not be the sole regulation of the enzyme by PtdIns(4,5)P\(_2\), since the enzyme was strongly stimulated by the lipid even in cell free systems (936). Elimination of the PH domain, or single residue mutations within, strongly influence the activity of the enzyme but not its catalytic site suggesting an allosteric regulation (187, 1754). The C2 domain of PLC\(_{3}\)δ enzymes has also been implicated in Ca\(^{2+}\) regulation and membrane
association via nonspecific electrostatic interactions and PS binding (43, 409, 935).

1. PLCδ1

In addition to its role in hydrolyzing PtdIns(4,5)P₂ at the PM, PLCδ1 shuttles between the cytosol and the nucleus (1755). The enzyme accumulates in the nucleus during the G₁/S₁ boundary (1448, 1753) following an increase in nuclear PtdIns(4,5)P₂ levels (1448), and it also regulates cell cycle progression (1449). It is not clear how these nuclear changes contribute to the overall functions of the enzyme. PLCδ1 −/− mice are viable but lose hair within 10 days of birth and are susceptible to skin inflammation and also develop skin tumors (1109). These mice have a defect in keratinocyte differentiation in hair follicles and show an increased number and highly proliferative interfollicular epidermal cells and sebaceous glands. Keratinocytes from PLCδ1-deficient mice showed an impaired Ca²⁺ signal, PKC activation, and NFAT transcriptional activity (1109). PLCδ1 is also a downstream intermediate regulated by the keratinocyte-specific transcription factor Foxn1, the gene that is mutated in nude mice (1111). It is interesting to note that Orai1-deficient mice also develop hair loss and skin abnormalities and show defective keratinocyte differentiation (543). Orai1 is the channel component of the STIM1-regulated Ca²⁺ entry pathway that is activated upon ER Ca²⁺ depletion (435) and is highly expressed in epidermal keratinocytes (543). Extracellular Ca²⁺ is important for triggering keratinocyte differentiation, and it increases the expression of PLCγ1 and PLCδ1 (1241). PLCδ1 was also shown to be regulated by store-operated Ca²⁺ entry (795). Therefore, it is reasonable to assume that PLCδ1 and Orai1-mediated Ca²⁺ entry work in synergism to control keratinocyte differentiation. PLCδ1 −/− mice also develop skin inflammation due to the production of proinflammatory cytokines by the epidermis (676). Most recent studies reported that PLCδ1 −/− mice are protected from obesity when put on a high-fat diet apparently due to a higher metabolic rate and altered adipogenesis (622). The mechanism of how this is achieved is still under investigation.

PLCδ1 has been linked to several human diseases. Its levels were shown to increase and accumulate in neurofibrillary tangles in the brains of Alzheimer’s patients (1399, 1400). Reduced PLCδ1 levels due to hypermethylation of its gene have been reported in esophageal, gastric, and colorectal cancers (320, 471, 658), and the metastatic potency of breast cancer cells showed correlation with the expression levels of PLCδ1 and PLCδ3 (1264).

2. PLCδ3

This isoform showed a few notable differences from its sister, PLCδ1, in cellular distribution and sensitivity to lipids and detergents and in the importance of its PH domain in regulation (1202, 1203). Recent data indicated that PLCδ3 downregulates RhoA and promotes neurite outgrowth in primary cortical neurons (823). PLCδ3 was found to interact with myosin VI (Myo6) and contribute to inner-ear hair cell stereocilia stabilization and possibly to microvilli maintenance in the colon epithelium (1328). A uniquely tight coupling between Ca²⁺ entering via arachidonate-activated ARC channels and PLCδ3-mediated PtdIns(4,5)P₂ hydrolysis was also reported (1553). Knockout studies suggest that PLCδ1 and PLCδ3 have overlapping functions. PLCδ3 −/− mice show no obvious abnormalities, whereas a combined PLCδ1/PLCδ3 −/− knockout results in midgestational lethality caused primarily by a placental defect (1110).

3. PLCδ4

This enzyme has several isoforms in the rat, a smaller, 90-kDa form that is expressed in various tissues and a larger 93-kDa splice form that is specific to the testis (871). A third splice variant was also reported as a negative regulator of PLC activity (1105). There are four alternatively spliced forms in the mouse showing differential tissue expression (475). PLCδ4 is located primarily in the nucleus and shows changes with the cell cycle (925) and was shown to be increased in fast-growing human hepatoma cells (1338). The PH domain of PLCδ4 was reported not to bind Ins(1,4,5)P₃ (1105), and it was tested whether it would be a better PtdIns(4,5)P₂ reporter (872). However, this PH domain also has a lower PtdIns(4,5)P₂ affinity, and it does not contribute to the localization of the enzyme that this study found primarily in the ER (872). Overexpression of the enzyme upregulates the ErB1/2-Erk signaling pathway and promotes cell proliferation in breast cancer cells (889). PLCδ4 −/− mice develop normally, but the males are sterile due to the inability of their sperm to induce normal acrosome reaction and fertilization (476, 477).

C. PLCγ

PLCγ exists in two forms: PLCγ1, which is widely expressed, and PLCγ2 that is mostly expressed in hematopoietic cells (645). These enzymes have the most complex structure among PLCs in that they contain a split PH domain, two SH2 and one SH3 domains sandwiched between their catalytic X and Y domains (FIGURE 9). These enzymes are regulated by tyrosine phosphorylation by RTKs that occurs following interaction of the SH2 domains of the PLCγ enzymes with specific tyrosine-phosphorylated motifs of a variety of activated RTKs including receptors for PDGF, EGF, and FGF (and many others) (749, 994, 1008, 1027, 1077, 1086, 1654). It is particularly important for T- and B-cell activation that PLCγ associates with large cytoplasmic molecular complexes that are recruited to the PM and which contain non-receptor tyrosine kinases that activate the enzyme (165, 1101, 1197, 1291, 1374, 1694). In
some cases, PLCγ uses intermediary adaptor proteins to associate with the macromolecular complex, such as in T cells where the LAT (439, 1803) and SLP-76 (1751, 1752) proteins bring the PLCγ1 enzyme to the activated TCR complex. Recruitment of the PLCγ enzymes to the membrane via these protein-protein interactions is essential for the subsequent tyrosine phosphorylation and activation of the enzymes (786, 1008, 1139, 1653). Five Tyr residues in PLCγ1 were reported to be phosphorylated by RTKs: Tyr472, -771, -775, -783, and -1254 (786, 788, 797, 1383, 1652). Although conflicting reports have been published about the importance of some of these Tyr phosphorylations (1380, 1383), there is a clear consensus that phosphorylation of Tyr783 and perhaps Tyr775 are critical for the stimulated activity of PLCγ1 (526, 797, 1380). Phosphorylation of the equivalent residues in PLCγ2 (Tyr753 and Tyr759) are also critical in B-cell activation (797, 1278). How tyrosine phosphorylation affects enzymatic activity is a question that is just beginning to unfold (526). An intramolecular interaction between the COOH-terminal SH2 domain and the phosphorylated Tyr783 evokes a conformational change that relieves autoinhibition of the enzyme (526, 653). In addition to Tyr-phosphorylation, PLCγ1 was shown to be directly regulated by PtdIns(3,4,5)P3 either via interaction with the PH domain (419) or the SH2 domain (77, 1253). In addition, PtdIns(3,4,5)P3 also regulates PLCγ activity via activation of Tec-family tyrosine kinases, an effect that is most pronounced in immune cells and controlled by SHIP1 (448, 1356, 1357). Recent studies suggest that the sprouty proteins, negative regulators of RTK signaling (220), can associate with both PLCγ isoforms and inhibit their activation by RTKs (19).

1. PLCγ1

Homozygous PLCγ1 deletion in mice causes embryonic lethality at day 9 (730), apparently due to a loss of vasculogenesis and erythropoiesis (909). This is consistent with zebrafish studies that showed impaired vasculogenesis and ventricular contractility upon loss of PLCγ function (861, 1301). These phenotypes suggest that the effects of VEGF on vascular endothelium are mediated mostly by PLCγ-mediated pathways (1035, 1301, 1516). Since PLCγ1 activation is a major signaling route initiated by RTKs, it has been assumed, and in many cases shown, to be critical for the mitogenic, pro-proliferative effects during stimulation of these receptors. Indeed, elevated levels of PLCγ1 were found in aggressive breast cancers (56), and the enzyme was found critical for the metastatic properties of breast cancer cells (1329). However, even early studies suggested that RTKs, such as the FGFR, can induce mitogenesis without activation of PLCγ (1212), and this is consistent with the relatively normal development of mice up to day 8 without PLCγ1 (730). In Drosophila, PLCγ defects cause impaired wing and eye development (984).

2. PLCγ2

An additional and unique regulation of PLCγ2 is via interaction with Rac proteins (1221). Rac interacts with the split PH domain of PLCγ2 (199, 1663) and regulates the membrane recruitment and activity of the enzyme (413, 1221). It is assumed that Rac interaction with the split PH domain relieves an autoinhibition, independently of Tyr phosphorylation (413). PLCγ2 −/− mice are viable but are immunocompromised because of defective B-cell responses to immunoglobulins (1666). In addition to B cells, PLCγ2 deficiency also affects neutrophils (717), platelets (698), and dendritic cells (300). Recently, activating mutations of PLCγ2 were found in patients with spontaneous inflammations and autoimmunity (414), and activating deletions in PLCγ2 were found in families with cold urticaria, immunodeficiency, and autoimmunity (1169).

D. PLCε

This enzyme was a late-comer as it was discovered as a novel, Ras-regulated PLC enzyme identified in C. elegans and named PLC210 (1398). The mammalian homologs were then cloned independently in three laboratories (773, 938, 1431) and named PLCε. Two splice variants differing in their NH2 termini, called PLCε1a and PLCε1b, exist, but no functional difference between them has been reported (1436). It is generally accepted that the enzyme is widely expressed, although only at low levels making its detection difficult (1668). PLCε has several regulatory domains in addition to the classical building blocks, namely, an NH2-terminal cysteine-rich region followed by a Ras GEF domain (called here CDC25 GEF), and two Ras-association (RA) domains at the COOH terminus (FIGURE 9). The Ras GEF domain can activate Rap1 (736), while the two RA domains bind H-Ras and Rap1, with the second one (R2) being more important in this regard (1431). Therefore, PLCε is both an activator and a downstream target of Ras/Rap1 possibly representing a feed-forward activation loop. Receptor-induced activation of PLCε can be achieved from RTKs, such as the EGF receptor that requires H-Ras (1431) and probably involves translocation of the lipase from the cytosol to the PM (736, 1431). PLCε also translocates to the perinuclear area after Rap1 activation (736). It was proposed that Ras is responsible for the rapid phase of PLCε activation, while Rap1 is for a prolonged activation of the enzyme (1432). In addition, GPCR stimulation with LPA, or thrombin also activates PLCε, and this activation involves the G12/13 family of heterotrimeric G proteins (549, 774). The G12/13-mediated activation of PLCε is not direct; it goes through the Rho GEF proteins p115RhoGEF or LARG yielding GTP-bound Rho (549). Interestingly, while the EGF/Ras-mediated activation requires the COOH-terminal RA domains, the Rho-mediated activation does not require this segment but one within the conserved Y catalytic domain (1378). Rho-mediated activation of...
PLCe can also be achieved via Gq-coupled receptors, but in this case it is the p63RhoGEF that mediates Rho activation (18). The Ras and Rho-mediated activation of PLCe via the two different regions of the protein can work in a synergistic manner (1379). PLCe can also be activated via increases in cAMP levels mediated by the guanine exchange factor Epac, which acts on Rap2B (412, 1161, 1364).

Since a whole set of other PLCs can respond to stimulation of a variety of cell surface receptors, it is of particular interest to what extent PLCe contributes to the overall PLC activity during activation of specific receptors. This question was examined after RNAi-mediated knockdown of PLCe or probably more reliably in cells derived from PLCe−/− mice (1511, 1668). RNAi studies in Rat1 fibroblasts suggested that PLCβ3 was more important in the rapid phase, while PLCe in the prolonged phase of InsP3/DAG formation during endothelin, ET-1 receptor, LPA, or thrombin stimulation (772). Studies in astrocytes from PLCe−/− mice showed that PLCe is a major contributor to InsP3/DAG generation after LPA, thrombin, and S1P receptor stimulation and that LPA and thrombin use pertussis toxin-sensitive Gi/o proteins, after LPA, thrombin, and S1P receptor stimulation and that entire PLCe have generated PLCe-deficient mice, one eliminating the enzyme in cardiac development and notypes (detailed in Ref. 1424), but they both highlighted a truncated protein that is catalytically inactive missing of a variety of cell surface receptors, it is of particular interest to what extent PLCe contributes to the overall PLC activity during activation of specific receptors. This question was examined after RNAi-mediated knockdown of PLCe or probably more reliably in cells derived from PLCe−/− mice (1511, 1668). RNAi studies in Rat1 fibroblasts suggested that PLCβ3 was more important in the rapid phase, while PLCe in the prolonged phase of InsP3/DAG formation during endothelin, ET-1 receptor, LPA, or thrombin stimulation (772). Studies in astrocytes from PLCe−/− mice showed that PLCe is a major contributor to InsP3/DAG generation after LPA, thrombin, and S1P receptor stimulation and that LPA and thrombin use pertussis toxin-sensitive Gi/o proteins, while thrombin uses RhoA via G12/13 (264). Two groups have generated PLCe-deficient mice, one eliminating the entire PLCe protein (PLCe−/−; Ref. 1668), the other making a truncated protein that is catalytically inactive missing the EF-hands and part of the catalytic domain (PLCeΔνΔα; Ref. 1511). These mice show some differences in their phenotypes (detailed in Ref. 1424), but they both highlighted critical roles of the enzyme in cardiac development and functions. PLCeΔνΔα mice have developmental defects in the aortic and pulmonary seminal valves with sequential ventricular dilation and hypertrophy (1511). This pathology is not seen in PLCe−/− mice (1668). PLCe−/− mice, on the other hand, are more susceptible to cardiac hypertrophy, possibly because of upregulation of the Gq-mediated PLC pathways, since downregulation of PLCe in vitro in ventricular cardiomyocytes greatly blunts their “hyper trophy” response, which is the opposite of what the knockout mice would predict (1424). PLCe−/− mice also have impaired cardiac contractility that may be related to scaffolding functions with muscle-specific AKAP proteins as well as the ryanodine receptors (1424, 1799). In addition, PLCe has been linked to inflammation and tumorigenesis in skin (687) and intestine (900) in mouse models. As for human diseases, PLCe mutations have been found in patients presenting with childhood nephrotic syndrome probably linked to altered podocyte functions (621). Interestingly, no kidney pathology was found in PLCe mice, but PLCe deletion in zebrafish causes developmental problems with the filtration barrier of the fish “kidney” (621). PLCe was found significantly downregulated in patients with sporadic colorectal cancers (1675), and the PLCE1 gene was found as a susceptibility locus for gastric and esophageal squamous cell carcinoma (3).

E. PLCeζ

This is the smallest PLC enzyme that even lacks a PH domain (Figure 9). It was first isolated from mouse testis and is exclusively expressed in the spermatids (1349). PLC activation during the acrosome reaction has been described earlier (575), and injection of PLCeζ cRNA into mouse eggs initiates Ca2+ oscillations and triggers egg activation, suggesting that it is the “sperm factor” that initiates egg activation upon fertilization (822, 1349). Human sperm devoid of PLCeζ fails to trigger Ca2+ oscillations and egg activation (1774). The enzyme is localized at the equatorial region of human sperm (1774) and is found in the pronucleus during mouse egg activation (1770). PLCeζ is the most Ca2+-sensitive PLC already being activated at basal levels (100 nM) of Ca2+ concentrations (822). Regulation of PLCeζ appears to be different from the other PLCs. First, expression of the enzyme in CHO cells failed to induce any Ca2+ oscillations in spite of good expression and high level of PLC activity measured from the cytosol in vitro (1218). Most recent studies showed that PLCeζ acts on a distinct vesicular pool of PtdIns(4,5)P2 within the egg cortex as opposed to the PM, showing clear distinction from PLCe1 which affected the PM pool of the same lipid (1784). Also, unlike in other PLCs in which the linker region between the X-Y domains contains several acidic residues and exerts autoinhibition (612), in PLCeζ this region contains a stretch of basic residues that mediate binding to membrane PtdIns(4,5)P2 (1144), and removal of this region inhibits rather than activates the enzyme (1145). A basic stretch found within the X-domain has also been implicated in nuclear localization and nucleo-cytoplasmic shuttling of the enzyme during Ca2+ oscillations (834).

F. PLCeη

These enzymes have two isoforms, PLCe1 and PLCe2. They were identified independently in four laboratories in 2005 (673, 1108, 1471, 1820). They show highest expression in the brain and they are structurally related to PLCe1 (Figure 9). In addition to the usual domains present in PLCs, they have a serine- and proline-rich region and a PDZ-domain interacting sequence in their COOH termini (1470). Relatively little is known about the regulation of these enzymes. PLCe2 can be activated by G protein βγ subunits shown both in expression studies in COS-7 cells (1820) and in reconstitution assays (1819). Neither the PH domain nor the COOH-terminal unique sequence is required for Gβγ activation of PLCe2 (1819), but the PH domain was shown to be needed for membrane localization (1108). PLCe2 also shows high Ca2+-sensitivity even more so than PLCe1 (1108). A PLCe2 knockout mouse with a LacZ knockin has revealed high expression of the enzyme in the habenula and retina and suggested a possible role in retina development or maturation, but the mice showed no major abnormalities (753).
G. PLC-Related Proteins

In 1992 the Hirata laboratory isolated a protein from rat brain cytosol that showed high-affinity binding to Ins(1,4,5)P₃ (756). This protein, initially named p130, turned out to be identical to a PLC-like protein called PLC-L that was deleted in the cancer tissue of a patient suffering from small cell lung carcinoma (755, 811). Another homolog named PLC-L(2) was also described and showed that these enzymes have high sequence homology to PLC81 but are inactive because of a mutation of a conserved His within their catalytic domains (755, 1181). These proteins were later named PRIP-1 and -2 (for PLC-related but catalytically inactive protein), the former being highly expressed in brain, while the latter showing wider tissue distribution (1007, 1181). PRIP-2 localizes to the ER and perinuclear region (1181). The isolated PH domains of both proteins showed high-affinity binding to Ins(1,4,5)P₃ and PtdIns(4,5)P₂ and in the case of PLC-L(2)/PRIP-2 it also localized to the perinuclear ER (1181), while in the case of PLC-L/p130/PRIP-1 it was found cytosolic (1616). Contrary to these findings, the PH domains of PRIP-1 and -2 both showed PM localization (490). PRIP-1 has a role in GABA receptor function indirectly by affecting the trafficking and phosphoregulation of GABA receptors (757, 1546). PRIP-1 interacts with the β-subunit of GABAA receptors and the associated protein GABARAP (754), and it augments the transport of y2-containing GABAA receptors to the cell surface (1071). PRIP-1 knockout mice show anxiety-related behavior and reduced benzodiazepine sensitivity (754) and also exhibit seizure-like EEG activities (1822) indicating dysfunction of the GABAA receptors. PRIP-2 knockout mice develop normally and show no obvious abnormalities, but their B cells are hyperresponsive and display increased Ca²⁺ influx and NFAT activity after receptor crosslinking (1519). This may be related to the inhibitory effect of PRIP-1 on endogenous PLC activation and quenching some of the Ins(1,4,5)P₃ generated (1524).

VIII. PHOSPHATIDYLINOSITOL TRANSFER PROTEINS

The first functional studies on a phosphatidylinositol transfer protein (PITP) were described in yeast, when the Sec14p protein was found to be important for the transport of secretory proteins from the Golgi (98, 102). Sec14p is a cytosolic protein that associates with the Golgi and is capable of either PtdIns or PtdCho transfer (98). Rescue studies have indicated that expression of Sec14 mutants defective in PtdIns but not PtdCho transfer are able to rescue the secretory defect of Sec14-deleted strains (1217), questioning whether PtdIns transfer is the primary role of the protein. Remarkably, genetic interference with PtdCho synthesis rescues Sec14p secretory defects (272), and Sec14p itself is an inhibitor of the CDP-Cho pathway (1018, 1414). Deletion of Kes1p, one of the oxysterol binding proteins of yeast also “bypasses” Sec14 defects initially raising the possibility that Kes1p mediates the PtdCho “toxicity” seen in Sec14-deleted strains (424). More recent studies indicate that Sec14p is essential for the maintenance of a Golgi pool of DG, which is spared when PtdCho synthesis is limited. Also, deletion of Kes1p, which can “extract” PtdIns4P and exchanges it for ER-derived sterols, spares the Golgi PtdIns4P pool (339). Importantly, “Sec14 bypass” conditions require a functional yeast PLD enzyme, Spo14p, presumably to supply DG from PtdCho (1748). More on Sec14 and its possible modes of action can be found in several recent reviews (100, 103).

The first mammalian PITP was isolated from bovine brain as two proteins of ~30 kDa size capable of transferring PtdIns from labeled liver microsomes to PtdCho vesicles (597) and subsequently cloned from rat (355). PITPs were also isolated independently by two separate efforts purifying cytosolic factors that were required for G protein-mediated PLC activation (767, 1551) and ATP-dependent priming of exocytic vesicles (583). The mammalian PITPs [PITPs and its sister, PITP3 (1527), now called class I PITPs] show little sequence homology to the yeast Sec14p protein, yet mammalian PITPs can partially rescue yeast Sec14–1⁴ defects (but not SEC14 deletions) (1413), and conversely, Sec14p can restore PLC activity in mammalian cells (309). Curiously, unlike in the case of the yeast Sec14p (1217), the PtdIns transfer activity of mammalian PITPs was found to be important for complementation of the Sec14 defect is yeast (24, 1413). Another group of PITPs were initially identified in Drosophila, as one of the genes responsible for light-induced retinal degeneration phenotype, called RdgB (1636). Mammalian homologs of RdgB (variably named PITPα or RdgBα) have been subsequently found (15, 232, 537), and it was recognized that these larger proteins possess at their NH₂ termini a PITP-domain that is highly homologous to the class I mammalian PITPα and PITPβ. The same group of proteins was also isolated as interactors with the Ca²⁺-regulated tyrosine kinase, PYK2 and called Nir1–3 (891). Nir1, however, lacks the NH₂-terminal PITP domain but shows homology to the other Nir proteins in the rest of their sequences (891). Finally, a PITP that is most homologous to the PITP domain of RdgBs but lacks all the other domains of the RdgB homologs was cloned and named RdgBβ (480). Nir2 and Nir3 are now called class IIA, while RdgBβ is named class IIB PITP (1575) (see FIGURE 10). Given the functional similarity between mammalian PITPs and Sec14p, it is noteworthy that both yeast and mammalian cells have Sec14 homologs that are lipid transfer proteins with cargoes other than PtdIns (1088, 1136). This raises the question of why the Sec14 scaffold was replaced by that of PITPs in metazoans for PtdIns/PtdCho transfer while still kept for other lipid transport purposes.
A. Class I PITPs

PITPα and PITPβ belong to this group, the latter having two splice variants that differ in their very COOH termini (277). Several details on the structure and PI-transfer characteristics of these PITPs have been uncovered, yet we still do not fully understand what these proteins do and how they work inside the cells. The structure of PITPα has been solved both with and without a PtdIns cargo molecule within the binding cavity (1366, 1557), and the headgroup of the lipid faces the interior of the cavity. Notably, this is the opposite orientation from what is found in the structure of the Sac14p protein (1387). The COOH-terminal last 10 amino acids are important to close onto the bound lipid, and PITPα mutants lacking the 5 or 10 last residues at the COOH terminus exert dominant negative effects when expressed in cells, while they still retain PI transfer activity (568). PITPα is mostly cytosolic, but PITPβ is found Golgi-associated (1216). Membrane association of PITPβ depends on specific COOH-terminal sequences unique to the PITPβ form (1216) but also require two conserved hydrophobic residues (Trp202, Trp203) found in all PITPs (1557). Since PITPs can transfer PtdIns (and to a smaller extent PtdCho) between membranes, it was initially believed that they transfer the ER-synthesized PtdIns to the PM (and perhaps to other membranes) for phosphorylation by PI4Ks and PIP5Ks, and hence supply PLC with its substrate PtdIns(4,5)P2. However, it appears that the mode of actions of PITPs is more complex (279, 1136), and recent models suggest that they can also present PtdIns for enzymes that use it as substrate (PI kinases or PLC enzymes) (830, 1354). Recent models posit that the cycling of the lipid through PITPs is an integral part of the ability of lipid modifying enzymes to act upon PtdIns. The term nanoreactor has been introduced to emphasize this catalytic/chaperone rather than transport function (689, 1136, 1354).
The vibrator mouse that shows early-onset action tremor, progressive spinocerebellar neurodegeneration, and juvenile death expresses very low level of PITPa (557, 1692). PITPa deleted mice are born normal, but develop spinocerebellar degeneration and soon succumb into hypoglycemia as well as intestinal and hepatic steatosis and die by day 12, apparently due to their inability to handle the milk fat during suckling (23). Cells obtained from these mice showed no significant change in phosphoinositide content, or problems with ER to PM secretion, exocytosis, endocytosis, or signaling, and therefore, the defects seemed to be confined to the defects in neurons, enterocytes, and liver cells, probably all related to problems with lipid handling (25, 99). The PtdIns binding of PITPa is critical for its function, as a PtdIns binding-deficient mutant protein cannot rescue the null phenotype (26). A detailed analysis of the pathology of mice expressing various levels of PITPa suggested that the lifespans of these animals were proportionally shortened with decreased level of the protein. These studies also showed a close relationship between the steatotic defect, hypoglycemia, and cerbellar inflammation, but the neuronal defects and early lethality still are maintained even when the lipid defects are corrected in the intestine (26).

At the cellular level, PITPa was shown to associate with the netrin receptor and to play a role in neurite outgrowth (1747). Morpholino-mediated knockdown of PITPa in zebrafish causes early developmental arrest (688). In Drosophila, mutation of the single class I PITP Giotto causes defects in male germline cytokenesis (500, 508), with a phenotype, similar to the defect in the PI4KB homolog fud (182).

PITPB deletion is incompatible with life resulting in an early implantation defect in mice (25). The zebrafish homolog PITPB is required for proper development of double cone cell outer segments in the retina (688). Knockdown of PITPB in HeLa cells causes altered Golgi morphology and distorted nuclei and a retrograde transport defect between the Golgi and the ER (219). A few studies suggested that the sp1 splice variant of PITPB is capable of sphingomyelin binding and transport in vitro and inside cells (341, 1605). However, other studies concluded that this feature of the protein might not be critical for its cellular functions (219, 1377). Given its essential role in mammalian organisms, it is generally believed that PITPB delivers PtdIns for PI kinases and hence is necessary to maintain the PtdIns(4,5)P2 pools available for PLC enzymes (279, 310). However, a direct proof of this concept is still to be obtained.

B. Class II PITPs

The RdgB proteins contain several domains other than the PITP domain: their NH2-terminal PITP domain is followed by an acidic stretch and several hydrophobic domains initially believed to be membrane-spanning segments. The acidic region also includes an FFAT domain that anchors the protein to the ER via binding of VAP proteins. The RdgB proteins also contain a DDHD domain with homology to some of the PLA1 enzymes. Another conserved section, called LNS2, is homologous to that found in lipins, the phosphatidic acid phosphohydrolases. It is located close to the COOH terminus which also was identified as the site of PYK2 interaction (890). (FIGURE 10). The founding member of this family, the RdgB protein of Drosophila, has a uniquely important role in invertebrate photosignal transduction. Flies mutated in this gene develop light-induced retinal degeneration and show impaired electric response to light evident in electroretinograms (1636). These defects can be fully remedied by expression of the full-length RdgB protein or its isolated PITP domain, but not by rat PITPa or a chimeric protein with a rat PITPa fused to the rest of Drosophila RdgB (1055). The two mammalian homologs of RdgB (called RdgBa1, Nir2 or PITPnm1 and RdgBa2, Nir3 or PITPnm2) are also able to rescue the visual defects of the RdgB fly, although Nir2 works better than Nir3 (232). The Drosophila protein is localized to submicrovillar cisternae that are special regions of the ER at the base of the rhabdomeres (1502). It is currently assumed that RdgB is essential for the transfer of PtdIns from the ER to the PM (rhabdomeres) in the fly retina, and its defects interrupt the supply of this lipid to the PM and hence rapidly deplete the PtdIns(4,5)P2 pools that are important for proper PLC-generated signals during light exposure (1575).

Less is known about the mammalian RdgB homologs. In the mouse, Nir2 is ubiquitously expressed, while Nir3 is primarily found in the retina and some regions in the brain (948). Nir3 knockout mice show no obvious phenotype while Nir2 knockout is embryonic lethal (947). At the cellular level, Nir2 is Golgi localized (16, 921) where it helps maintain DG levels (919). However, Nir2 also gets associated with lipid droplets after oleic acid treatment (920), and it changes localization with the cell cycle, being found in the midbody and the cleavage furrow in telophase and anaphase, respectively (921). Functionally speaking, Nir2 was shown to regulate protein export from the ER and contributes to the structural integrity of the ER (39). The interaction of the Nir2 and Nir3 proteins with the ER is mediated via the acidic FFAT domain that is responsible for interaction with the ER-resident VAP-B proteins (39). It is very likely that Nir2 proteins work at membrane contact zones between the ER and the Golgi, and the interaction of Nir2 with PI4KA suggests that it may help deliver PtdIns for this PI 4-kinase to regulate exit of proteins from the Golgi (16). The small RdgB protein also having two splice forms is mostly found in the cytosol (480). Although the function of this protein is still unknown, it was shown to interact with the
angiotensin II AT₁ receptor interacting protein ATRAP with its lipid-binding domain, whereas it interacts with 14-3-3 proteins at its COOH terminus, and this latter interaction affects the rapid degradation of the protein (278, 495). Most recent studies found that Rdgββ is capable of PtdOH binding and hence may function as a PtdOH transfer protein (494).

IX. PHOSPHOINOSITIDES AT THE PLASMA MEMBRANE

It is hard not to notice the hierarchical organization of phosphoinositide distribution between the various membrane compartments. As one moves outward from the nucleus toward the PM, the extent of inositol lipid phosphorylation is increased in the membranes: the ER and nuclear envelope mainly contain PtdIns and the Sac1 phosphatase that localizes to these membranes ensures that any phosphorylation of PtdIns would be properly counteracted. The endosomal compartments, including the Golgi, contain monophosphorylated PtdIns, such as PtdIns4P or PtdIns3P, and have phosphatase enzymes that act both on mono- and bis-phosphorylated PtdIns, such as the MTMs and the 5- or 4-phosphatases. The PM has the bulk of PtdIns(4,5)P₂ and PtdIns(3,4,5)P₃ and the phosphatases that dephosphorylate them. The one exception to this rule is PtdIns(3,5)P₂ that is formed at the multivesicular body, which is an endomembrane compartment. Yet, one can argue that MVB invaginating membranes [where PtdIns(3,5)P₂ function is critical] are destined to the lysosome, the content of which can be viewed as “external” to the cell (FIGURE 11). The existence of the same process supporting the externalization of membranes at the PM (as in the case for virus budding) lends some support to these views (1016). Based on this distribution, PtdIns4P, PtdIns(4,5)P₂, and PtdIns(3,4,5)P₃ are the major inositol lipids in the PM. PtdIns4P in the PM is used to be considered only as an intermediate of PtdIns(4,5)P₂ synthesis, but this simplistic view is increasingly challenged by solid data that suggest that PtdIns4P can support the functions of ion channels (see Ref. 559 and discussions below) and also contributes to the anchoring of lipid-modified targeting sequences that contain polybasic domains (559). We still need to learn more about PM PtdIns4P, but currently PtdIns(4,5)P₂ is recognized as the most important inositide at the PM with diverse processes being controlled by changes in its levels (FIGURE 12). PtdIns(4,5)P₂ is the precursor of Ins(1,4,5)P₃ and DAG after PLC activation and hence is pivotal for early signaling from cell surface receptors. Second, PtdIns(4,5)P₂ is a substrate of class I PI3Ks and the precursor of PtdIns(3,4,5)P₃, the latter being critical for growth factor signaling, and also for PM polarization and membrane dynamics. Third, PtdIns(4,5)P₂ itself is probably important for the proper operation of ion channels and transproters and plays roles in exo- and endocytosis. These processes will be briefly discussed below.

A. Canonical PI Signaling Triggered From Cell Surface Receptors

This topic has been in the frontiers of inositide research for some time, receiving enormous coverage and could fill a book easily by itself. This will be summarized only briefly just for the sake of completeness. Studies unraveling the connection between receptor-mediated stimulation of “PI-turnover” and Ca²⁺ signaling placed phosphoinositides in the center of attention in the early 1980s and generated knowledge that has become part of every textbook on cell physiology. The basic principle of receptor-initiated PLC activation with generation of Ins(1,4,5)P₃ and DAG, two very different messenger molecules, has been firmly consolidated (130, 135). Yet, the variations of how many different PLC enzymes can be activated in how many different ways could not have been foreseen even with the wildest imagination (see sect. VII on PLCs). There are a large number of molecules regulated by DAG, starting with numerous PKC isoforms (1270, 1298) and extending beyond them (216, 1306). Similarly, the details of how Ins(1,4,5)P₃ releases Ca²⁺ from the ER via three different types of InsP₃ receptors (1050) and the complex regulation of the gating of these receptor/channels by Ca²⁺ and Ins(1,4,5)P₃ (1335) now represents a whole new research field. The same features of the InsP₃ receptors underlie Ca²⁺ waves and oscillations (136). A new chapter in PI signaling from the PM has begun with the identification of PI 3-kinases. Their close association with growth factor and oncogenic signaling has propelled this field and the literature on various isoforms of PI3Ks, and the variety of their activation mechanisms have been overwhelming (see sect. VC). This diversity is only paralleled by the number of effector proteins that bind and are regulated by the 3-phosphorylated PIs. The list includes guanine exchange factors and GTPase activating proteins for a variety of small GTP binding proteins, serine/threonine and soluble tyrosine kinases, and scaffolding proteins that organize signaling complexes. Through these pathways, inositides play pivotal roles in transmembrane signaling of every cell in an organism. These processes have been touched upon in the respective sections describing the enzymes and will not be further discussed here. However, there are several processes regulated by PIs in various membranes that historically are not covered under the canonical PI signaling umbrella but rather belong to membrane trafficking or to the ion channel fields. These are the areas that will be reviewed in some detail in the subsequent sections.

B. Ion Channels and Other Transporters

Early observations already hinted that phosphoinositides can directly influence ion transport pathways. A stimulatory effect of membrane PtdIns(4,5)P₂ on PM Ca²⁺ pump activity was found as early as 1981 (1132), and the sarcoplasmic reticulum Ca²⁺-ATPase was shown to tightly associate with phosphoinositides (1619). However, the
phosphoinositide regulation of ion channels/transporters has been first clearly postulated in the case of Na\(^+\)/Ca\(^{2+}\) exchanger (NCX1) and K\(_{ATP}\) potassium channels studied in giant excised patches of the heart (616). In this and many subsequent studies, the lipid regulation was noted in excised patch recordings as a “run-down” of the current with time, which then was reversed by the inclusion of ATP (to resynthesize PPIs) or inositol lipids themselves to the cytosolic face of the membrane. The explanation for this phenomenon was that inositol lipids slowly disappear via dephosphorylation in the excised membrane patches. Most of our knowledge on the direct regulation of ion channels by inositides has originated from studies on excised patches taken from *Xenopus* oocytes expressing the channels in question (1282). Only recently had it become possible to test the regulation of these channels in intact cells with the aid of drug-induced inositol lipid manipulations (1489, 1617). There is ample literature on the regulation of ion channels and transporters by phosphoinositides (488, 617, 932, 1138, 1285, 1487). Here we only provide a summary of these studies in **TABLE 2**, which might be more useful for a quick overview. Inter-

**FIGURE 11.** Distribution of phosphoinositides in various membrane compartments in a generic cell. The majority of PtdIns(4,5)P\(_2\) is located in the plasma membrane (PM), and that is where it is converted to PtdIns(3,4,5)P\(_3\). PtdIns4P is also found in the PM, but it is also highly enriched in the Golgi and in some endosomes that are part of the TGN. PtdIns3P is formed on endosomes such as the early endosomes (EE) and their precursors and is converted to PtdIns(3,5)P\(_2\) at the level of sorting endosomes (SE) at regions that form the invaginating membranes destined to become luminal in multivesicular bodies (MVB). PtdIns is generated in ER-derived compartments that can reach every other membrane, but the mechanism of lipid transfer between these compartments is still unknown. It is important to note that this distribution does not rule out that each of these lipids can be present in small, undetected amounts in other membrane compartments.
Calcium signaling has always been very closely linked to PLC-mediated phosphoinositide breakdown, namely through InsP₃-induced Ca²⁺ release from the ER. The enormous progress concerning the details of InsP₃-induced Ca²⁺ signals will not be discussed here but have been the subject of numerous great reviews (129, 1050, 1051, 1534). However, less is known about how phos-
Phosphoinositides control Ca\(^{2+}\) influx, a topic I will discuss in some detail.

1. Store-operated Ca\(^{2+}\) entry (SOCE)

It was recognized in the late 1970s and early 1980s that Ca\(^{2+}\) influx increases after stimulation of so-called “Ca\(^{2+}\)-mobilizing receptors,” which led James Putney to postulate a Ca\(^{2+}\) entry pathway that is activated by emptying the ER Ca\(^{2+}\) stores (capacitative Ca\(^{2+}\) entry) and thus currently named store-operated Ca\(^{2+}\) entry (SOCE) (1242). The molecular entities that constitute this pathway had been enigmatic for a long period, with the nonspecific cation Trp (transient receptor potential) channels taking center stage (1193). The breakthrough in this field came when large-scale small interfering RNA (RNAi) studies identified the STIM1 protein as the ER Ca\(^{2+}\) sensor necessary for SOCE (916, 1296). This was followed within a year by the discovery of the Orai1 protein as the channel component of \(I_{\text{CRAC}}\), the highly Ca\(^{2+}\)-selective current representing the major form of SOCE (435, 1635, 1802). A lot of progress has been made in understanding the mechanism of STIM1-Orai1 activation during ER Ca\(^{2+}\)-store depletion covered by several recent reviews (345, 896, 1427). For this summary it is enough to say that the ER-resident single transmembrane STIM1 protein has a luminal Ca\(^{2+}\)-sensing EF hand domain that triggers the oligomerization of the protein upon Ca\(^{2+}\) unbinding. This, in turn, induces a conformational change in the cytoplasmic aspect of STIM1 allowing it to interact and activate the Orai1 channels at junctions where the ER and the PM are in close proximity. The connection of this form of Ca\(^{2+}\) entry to inositol lipids was first noted when Wm was shown to inhibit the \(I_{\text{CRAC}}\) channels, and curiously the inhibition seemed to be related to changes in PtdIns4P rather than PtdIns(4,5)P\(_2\) (184). Since STIM1 has a polybasic tail at its very COOH terminus, it has been suggested that it interacts with the acidic phospholipids possibly with PtdIns(4,5)P\(_2\) in the PM, a view now generally accepted (217, 1664). In contrasting findings, PtdIns(4,5)P\(_2\) was found not to play a significant role in STIM1 movements or activation of Orai1, and instead, PtdIns4P seemed to be implicated in the regulation of Orai1 itself (820, 1618). More studies will clarify which, if any, of these inositides and by what mechanism regulate this important Ca\(^{2+}\) entry pathway.

2. Voltage-regulated Ca\(^{2+}\) entry

The old classification that “excitable” cells contain voltage-gated Ca\(^{2+}\) channels and “nonexcitable” cells use SOCE has now become outdated, since most excitable cells do contain channels formerly thought to operate in nonexcitable cells (e.g., CRAC and Trp channels) and, conversely, many cells formerly considered nonexcitable do have Ca\(^{2+}\) entry channels that are regulated by voltage changes (e.g., most endocrine cells). As detailed in the next paragraph, a great number of ion channels and transporters require phosphoinositides for proper function or are even regulated by physiological changes in the PM levels of these lipids, most notably PtdIns(4,5)P\(_2\) or PtdIns4P. These can have direct effects on Ca\(^{2+}\) entry when the affected channels conduct Ca\(^{2+}\), but they can also indirectly influence Ca\(^{2+}\) influx if they change the membrane potential through modification of potassium channels or chloride channels. Membrane potential changes not only influence the gating of channels (such as the voltage-gated Ca\(^{2+}\) or Na\(^{+}\) channels), they also change the electrical driving force for Ca\(^{2+}\) (as in the case for CRAC channels).

3. Phosphoinositides: regulators or permissive factors for channels?

An important question often debated about inositol lipids and channel/transporter function is whether the lipids are permissive for optimal function of the proteins, or indeed are regulatory within the range of inositol lipid changes that occur under physiological conditions. One can easily envision that highly acidic inositol lipids in the inner leaflet of the PM engage positive residues at the cytoplasmic aspect of the TM helices of channels, thereby serving as structural reference points keeping the protein in the right conformation. This would ensure that channels are not active during their transport in the internal membranes and become functionally competent only in the PM. On the other hand, it has also been shown convincingly that PtdIns(4,5)P\(_2\) changes evoked by agonist stimulation are faithfully followed by changes in the activity of several potassium channels to the extent that these channels are used as “PtdIns(4,5)P\(_2\) reporters” (422). It is worth pointing out, however, that we often use very high supraphysiological concentrations of agonists in our cellular studies and at normal level of receptor occupancy the overall lipid changes in the PM may not be very significant. Whether a channel responds to small changes in phosphoinositides also depends on the binding affinity of the channel to the lipid; in fact, the activity of a channel with very high affinity to an inositol may depend on this lipid, yet even large lipid changes will not cause a change in channel activity.

The molecular mechanisms by which inositol lipids can control ion channels mostly remain elusive. Almost all ion channels and transporters contain clusters of basic residues in their membrane-adjacent regions facing the cytosol or within their COOH-terminal tails. For example, inositol lipid regulation was mapped to the TRP domains of the cytoplasmic tails of TrpM8 channels (1138, 1284). An interesting and common feature of the PtdIns(4,5)P\(_2\) regulation of many potassium and TRP channels is that the lipid alters the interaction of the channels with other specific regulators such as calmodulin (843), \(\beta\)y-subunits (661), or ligands such as ATP (113). In addition, indirect regulation of channels via inositol-binding and channel-interacting proteins is also possible as suggested for Pirt, a molecule
<table>
<thead>
<tr>
<th>Channel</th>
<th>Ins Lipid Regulator</th>
<th>Reference Nos.</th>
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<td>883, but (−) 27, 742</td>
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<td>883, but (−) 742</td>
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<td>Hyperpolarization-activated HCN2 channels</td>
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<td>ROMK1 (Kir1.1)</td>
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<td>K(_{ATP}) (Kir6. x)</td>
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<tr>
<td>Two pore</td>
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<td>937, but see 245, 911</td>
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<td>422, 1488, 1794</td>
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<td>BK (Ca(^{2+}) activated)</td>
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<td>1598</td>
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<td><strong>Ion exchangers and transporters</strong></td>
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<tr>
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<td><strong>Ion pumps</strong></td>
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<td>PM Ca(^{2+})-ATPase</td>
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<td><strong>Ligand-gated channels</strong></td>
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*Continued*
that interacts with TRPV1 channels and also binds phosphoinositides (780), or AKAP150, an A-kinase anchor protein that associates with TrpV1 (728). Two recent reports on the structures of two closely related Kir channels bound to PtdIns(4,5)P2 began to shed light on the structural basis of lipid regulation of these channels: in the case of the Kir2.2 K+ channel, PtdIns(4,5)P2 binds at an interface between the transmembrane domain (TMD) and the cytoplasmic domain of the channel such that the fatty acid chains interact with the TMD and the phosphorylated headgroup with the cytoplasmic domain bringing the latter closer to the membrane and helping to open the inner gate (566). In the case of the GIRK2 channel, PtdIns(4,5)P2 occupies a similar position at the interface between the TMD and the cytoplasmic domain. However, here the position of the cytoplasmic domain already being closer to the inner membrane interface is not affected dramatically with lipid binding. Instead, PtdIns(4,5)P2 has a more profound effect on the opening of the inner gates in a mutant channel that mimics the conformation of the Gβγ-activated state (1715). In other words, the lipid had an important effect on G protein-induced activation, consistent with previous conclusions based on functional analysis (661, 1795).

Another form of channel regulation by inositol lipids is the stimulated insertion of the channel from intracellular inactive compartments to the PM. This process is usually regulated by PtdIns(3,4,5)P3, made by PI 3-kinases, such as reported for L-type Ca2+ channels (1633) or for the maintenance of AMPA receptors at the postsynaptic density (54). Since this form of regulation is essentially a trafficking process, it will not be further discussed here.

### C. Exocytosis

The earliest indications that phosphoinositides may be important for membrane fusion came from studies on dense core vesicle (DCV) exocytosis, where it was recognized that phosphoinositides were important for priming exocytic vesicles (395, 582, 583). In the process of regulated secretion, DCVs undergo maturation that increases their competence to dock and fuse with the PM when a rapid rise in Ca2+ concentration triggers the fusion process. Some of the mature granules under the membrane will dock to the PM, but these prelocked vesicles still have to undergo “priming” to become the “readily releasable pool” that is first fused upon stimulation (312, 715, 1191). During isolation of cytosolic factors needed for “priming” of DCVs, the mammalian PTP and PIP 5-kinase proteins have been identified (582, 583). Moreover, it was shown that PLC-mediated PtdIns(4,5)P2 hydrolysis was necessary for exocytosis to take place (558, 1707, 1708). These observations then led to the notion that PtdIns(4,5)P2 is an obligatory factor required for generating competent exocytic vesicles, and increased PtdIns(4,5)P2 turnover promotes Ca2+-triggered fusion of vesicles with the PM (643). However, for a long period, the question of whether PtdIns(4,5)P2 was necessary for priming at the PM membrane or at the surface of the exocytic vesicle has not been clarified. In the last 10 years or so, several proteins relevant to exocytosis have been found to bind and to be possibly regulated by PtdIns(4,5)P2 (see Table 3). There are other proteins important for exocytosis that bind phospholipids, such as the DOC2 protein that supports slow asynchronous exocytosis (1762). However, DOC2 binds PtdSer via its C2 domain but does not bind phosphoinositides.

It is not clear at present how PtdIns(4,5)P2 binding to any of these proteins regulate the exocytic process. The best studied of these is the Ca2+-sensitive phosphoinositide-binding regulator protein of exocytosis CAPS (46, 1313). This protein acts at a Ca2+-dependent prefusion step that is rate limiting and which requires the ATP-dependent priming of the DCVs (530) to promote the assembly of SNARE complexes (718, 719). CAPS requires PtdIns(4,5)P2 at the PM.
been some ambiguities whether PtdIns(4,5)P2 binding of the
ability to facilitate exocytosis (529). Although there have
effect that requires PI4KB (531). In another study, re-
increases glucose-induced insulin secretion by increasing
yeast frequenin, which interacts with PI4KB (see sect. V),
during exocytosis. PIP5K
calcium sensor 1 (NCS-1), a homolog of the
/H9002
centrations, which would be consistent with inhibition of
particularly PAO sensitive (83). In a number of studies,
would be of interest to know which PI and PIP kinase(s)
ing yet another mechanism to promote the fusion process. It
was the relevant lipid that determined the size of the readily
releasable pool (1056). With the use of reporters for
PtdIns(4,5)P2, such as PLC81PH-GFP or fluorescent neo-
mycin analogs, it was possible to show that there is an
increased concentration of PtdIns(4,5)P2 at the sites of exo-
cytosis (258, 551, 643, 718, 1056). A recent study found
that PtdIns(4,5)P2 binding to synaptotagmin-I increases the
Ca2+
binding affinity of the protein 40-fold (1602), provid-
ing yet another mechanism to promote the fusion process. It
would be of interest to know which PI and PIP kinase(s)
contribute to the generation of PtdIns(4,5)P2 at the PM
during exocytosis. PIP5K is the enzyme that is important
for synaptic vesicle exocytosis in the brain (1704) and is
also critical in DCV exocytosis in neuroendocrine cells
(520). Less clear is the identity of the PI4K enzyme. Isolated
chromaffin granules were found to contain a PAO-sensitive
PI4K (1716), and PAO-sensitive, type III PI4Ks were found
to be crucial to determine the size of the readily releasable
pool of exocytic vesicles in pancreatic beta cells (1168).
However, the PI4K purified from chromaffin granules
turned out to be the type IIa PI4K enzyme (108) that is not
particularly PAO sensitive (83). In a number of studies,
secretion was inhibited by Wm or LY294002 at higher con-
centrations, which would be consistent with inhibition of
type III PI4Ks (492, 1256, 1480, 1682). Several data suggest
that PI4K is important for regulated exocytosis: neuronal
calcium sensor 1 (NCS-1), a homolog of the Drosophila
and yeast frequenin, which interacts with PI4KB (see sect. V),
increases glucose-induced insulin secretion by increasing
the readily releasable vesicular pool in pancreatic beta cells,
an effect that requires PI4KB (531). In another study, re-
ducing the level of PI4KB with RNA interference inhibited
nutrient-induced insulin secretion (1683). Since NCS-1 was
discovered as a protein regulating synaptic development
and plasticity (reviewed in Ref. 615), it is a reasonable
assumption that PI4KB also functions in the genesis, trans-
port, and/or exocytosis of synaptic vesicles (1702).

The role of PI3Ks in exocytosis and secretion has also been
explored. PI 3-kinase inhibition was found to increase exo-
cytosis in adrenal chromaffin cells (1056, 1701) or insulin-
secreting beta cells (411, 1790) apparently due to a tran-
sient increase in PtdIns(4,5)P2 level. In contrast, a signifi-
cant amount of literature documents the need for PI3K in
secretion from immune cells, most notably for histamine
release from mast cells following FcR stimulation (997,
1164, 1207, 1761). However, in this case, PI3K, specifically
the PI3Kε (30) and PI3Kγ isoforms (157, 847), act as im-
portant amplifiers to control PLCγ activation, and hence,
Ca2+
signaling and PtdIns(3,4,5)P3 may not be directly in-
volved in the control of the exocytic machinery (30, 157,
847). Recent studies implicated PI3K-C2ε in the control of
exocytosis of insulin-containing vesicles in pancreatic
β-cells (370, 879) and neurosecretory cells (1034, 1700).
It should be remembered that PI 3-kinase inhibitors can also
indirectly affect secretion by altering the various Ca2+
in-
flux pathways (see above) or actin dynamics as shown in
pancreatic β-cells (282, 1222).

### D. Endocytosis

Most cell surface molecules undergo endocytosis and recy-
cling, a process that ensures delivery of nutrients to the cell
interior, and one that controls receptor number at the cell
surface and also serves as a quality control mechanism.
There are a number of forms of endocytosis, such as the
clathrin-mediated and clathrin-independent endocytosis
and endocytosis via caveolae as described in many detailed
reviews (366, 655, 1019). The most studied and hence un-
derstood form of endocytosis is mediated by clathrin using
a process that requires the participation of a great number
of proteins that form the clathrin lattice and bring cargo
molecules to this structure (1019, 1243, 1537). Endocytosis
is also regulated by inositol lipids at several biochemical
steps along the formation, maturation, and fission of the
endocytic particle. One of the first indications that inosi-
tides have to do with endocytosis was the finding that the
tetrameric clathrin adaptor protein AP-2, a key component
of the clathrin-mediated endocytic machinery, was a “re-
ceptor” for InsP6 (1639). Subsequently, it was shown that
the natural binding partner of this protein is PtdIns(4,5)P2
(482, 1288). Studies on synaptic vesicles revealed that
the PtdIns(4,5)P2 5-phosphatase synaptojanin (981, 1022) and
the PtdIns4P 5-kinase PIP5Kγ (352, 1704) are crucial reg-
ulators of synaptic vesicle cycling and neurotransmission
(1702). Recent studies using rapid depletion of PM
PtdIns(4,5)P2 in intact cells have unequivocally proven that

### Table 3. PtdIns(4,5)P2-interacting proteins related
to exocytosis

<table>
<thead>
<tr>
<th>Protein</th>
<th>Interacting Domain</th>
<th>Reference Nos.</th>
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<td>Syntaxin-1A</td>
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<td>CAPS</td>
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<td>MUNC13</td>
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<td>Granuphilin</td>
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<td>Rabphilin</td>
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</table>
clathrin-mediated endocytosis (CME) of nutrient receptors and GPCRs require PtdIns(4,5)P2 (2, 1569, 1617, 1831). Moreover, Arf6-regulated non-clathrin-mediated endocytic pathways also depend on phosphoinositides, although in this case the molecular identities of the lipid-dependent steps are less clear (17, 189, 646).

It has been shown that endocytic vesicles normally lose PtdIns(4,5)P2 by 5-dephosphorylation during separation from the PM, and if they do not, they will be coated with actin and accumulate and aggregate in the cell interior (e.g., Ref. 189). This observation fits with the notion that PtdIns(4,5)P2 is an identity code for PM. However, as more and more fine details are emerging on the hierarchy of the molecular events that control CME, it appears that several rounds of dephosphorylation and repolyphosphorylation cycles of PtdIns(4,5)P2 are necessary to maintain the flow of sequential steps in the initiation, maturation, invagination, and fission of internalizing vesicles (48). Moreover, in these different steps, distinct PtdIns4P 5-kinases and PtdIns(4,5)P2 5-phosphatases may have specific roles. Overexpression or knockdown of PIP5Kα or -β were shown to alter internalization of transferrin or EGF receptors (106, 1185). At the same time, several 5-phosphatases, such as SHIP1 (1115), OCRL (251, 988), and Sjn1 (1209), were all found to associate with components of the clathrin-coated pits (CCPs). A detailed TIRF analysis of different steps in CME during manipulation of PM PtdIns(4,5)P2 found no recruitment of PIP5Kα to CCP, yet their overexpression increased the number and size of CCPs (433). In the same study, several 5-phosphatases did show association with CCPs (although to significantly different extent and not to all CCPs), and the most important of these, Sjn1 inhibited the initiation and stabilization of early CCPs, but promoted the maturation of CCVs at later stages (433).

So how does PtdIns(4,5)P2 regulate endocytosis? Several proteins of the endocytic pathways bind phosphoinositides, primarily PtdIns(4,5)P2 with low affinity (see Table 4). Lipid binding contributes to the recruitment of such proteins to the PM. Most of these molecules are adaptor proteins that link cargo to clathrin, but they also include proteins that contain BAR (Bin-Amphiphysin-Rvs), F-BAR, or I-BAR domains (466, 1520, 1606). The latter three domains consist of dimers of long helical segments that generate a rigid banana shape with radiiuses that differ between the three families. They have positive residues on the concave (BAR and F-BAR) or convex (I-BAR) surface that interact with acidic phospholipids in curved membranes (1211) and also have membrane-bending and curvature-generating activities (465). Some BAR domain-containing proteins also possess other phosphoinositide binding modules, such as PX domains that are found in a group of sortin nexin (SNX-BAR) proteins (304). Most of these proteins play important roles in tubulation and scission at different endomembranes (see below), but SNX9, in particular, is important during the formation of endocytic tubes (1437, 1537, 1765) and also interacts with dynamin 1 and N-WASP, a protein that controls actin polymerization (1402). Dynamin is the GTPase that is essential for the scission of the endocytic vesicle, and its PtdIns(4,5)P2 binding PH domain is critical for its function (1362). Although the PH domain of dynamin has low affinity to PtdIns(4,5)P2, the assembling dynamin molecules on the neck of endocytic vesicles form a ring of multiple lipid binding modules (1475, 1506, 1816). How PtdIns(4,5)P2 affects dynamin is not fully understood, but it can recruit the molecule to CCPs, and it also increases dynamin’s GTPase activity (1816). It is clear, however, that dynamin interacts with a host of other proteins in the forming of endocytic vesicles via its other domains, and it controls not only the last step of CCPs life, i.e., its scission from the PM, but also regulates early steps during the formation and maturation of CCPs (see Refs. 432, 1362 for recent reviews on dynamin).

E. Plasma Membrane Polarization

Most cells in an organism develop a lateral organization of their PM with significant differences in membrane compositions and functions as they make contacts with other cells and form tissues and organs. The best examples are epithelial cells that have apical and basolateral membranes or neurons with axons and dendrites with distinct features. Even solitary cells of the innate and adaptive immune system show significant polarization as cells chemotax and phagocytose or communicate with one another. Cell migration and the invasive properties of cancer cells also depend on cell polarization and make all the difference between curable and deadly forms or stages of the disease. In all of these processes phosphoinositides play fundamental roles in multiple ways. There are three areas that will be discussed in some detail: chemokaxis, phagocytosis, and the organization of polarized cells as they best show the principles that apply to many other polarization processes.

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**Table 4.** PtdIns(4,5)P2-interacting proteins related to endocytosis

<table>
<thead>
<tr>
<th>Protein</th>
<th>Interacting Domain</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynamin</td>
<td>PH</td>
<td>4, 1332, 1816</td>
</tr>
<tr>
<td>AP-2</td>
<td>α and µ</td>
<td>482, 1288</td>
</tr>
<tr>
<td>AP180/CALM</td>
<td>ANTH</td>
<td>453, 819</td>
</tr>
<tr>
<td>Epsin</td>
<td>ENTH</td>
<td>711</td>
</tr>
<tr>
<td>Dab2/1</td>
<td>PTB</td>
<td>654, 665, 1062, 1788</td>
</tr>
<tr>
<td>Hip1/Hip1R</td>
<td>PTB</td>
<td>711</td>
</tr>
<tr>
<td>Arh/Arh1</td>
<td>PTB</td>
<td>1063</td>
</tr>
<tr>
<td>Snx9</td>
<td>PX and BAR</td>
<td>1764, 1765</td>
</tr>
<tr>
<td>Arrestin</td>
<td></td>
<td>483</td>
</tr>
<tr>
<td>Myosin VI</td>
<td></td>
<td>1439</td>
</tr>
</tbody>
</table>
1. Chemotaxis

A significant portion of our knowledge came from studies using the model species Dictyostelium discoideum, a unicellular organism that responds with chemotaxis to cAMP signals originating from neighboring cells (reviewed at great detail in Refs. 206, 815, 1505, 1691). How cells sense and respond to a chemotactic gradient are exciting problems since the concentration difference between the cells leading and trailing edges is extremely low. This makes a tiny difference in signaling strength when considering the dose-response relationship of the chemotactic receptors (usually GPCRs) and their G protein activation. These small differences have to be amplified for the cell to be able to respond with robust polarization. Accordingly, it has been found that distribution of GPCRs or their G protein activation do not show prominent polarization (735, 1744). However, it was recognized for the first time in Dictyostelium that the PI3K product PtdIns(3,4,5)P3 localizes prominently to the leading edge of the cell (373, 666, 1026). Deletion of the three PI3Ks in Dictyostelium indeed compromises although does not eliminate the ability of cells to respond to chemotactic stimuli (1518). Importantly, the PtdIns(3,4,5)P3 phosphatase PTEN was found to localize to the trailing end of the cell (677), and its deletion increases overall PtdIns(3,4,5)P3 levels and dissipates the PtdIns(3,4,5)P3 gradient interfering with efficient chemotaxis (677). Since PtdIns(4,5)P2 binding of PTEN is important for PTEN localization (678), a decreased PtdIns(4,5)P2 level at the leading edge because of the active PI3Ks favors PTEN accumulation at the trailing end. PLC activity can also modify the distribution of PtdIns(4,5)P2 and PTEN, affecting the PtdIns(3,4,5)P3 gradient (771). These observations together led to the suggested that localized stimulation (by PI3Ks) together with a global inhibition (by PTEN) form the basis of the amplification of gradient sensing in which the lipid PtdIns(3,4,5)P3 has a central role (815, 1505, 1626). Several other molecules also show polarized distribution in Dictyostelium. These include activated RasC and RasG; the TorC2 complex; a number of downstream PtdIns(3,4,5)P3 effectors that contain PH domains, such as Crac, PKBA, and PhdA (1505); and several recently identified proteins with unknown function (1801). RasG activates PI3Ks, while RasC is linked to TorC2, and it is part of the regulatory loops that control chemotaxis (205, 1342).

Very similar principles were found to drive the chemotactic responses of mammalian cells, such as neutrophils and macrophages (1463, 1691). Accumulation of PtdIns(3,4,5)P3 in the leading edge of differentiated HL60 cells have been described parallel to the findings in Dictyostelium (1385) and confirmed in many subsequent studies (430, 1667) including mouse neutrophils (1140). In this case, however, there are some differences in the players that form the positive and negative feedback loops in building up the PtdIns(3,4,5)P3 gradient. In mouse neutrophils, it was shown that SHIP1 rather than PTEN is important to serve as the negative regulator that keeps overall PtdIns(3,4,5)P3 levels down (1140), and that Gβγ (1497) subunits liberated by the activated chemotactic receptors together with Ras (1342, 1497) strongly stimulate PI3Kγ at the leading edge of the cell. It has also been shown that a positive feedback loop between PtdIns(3,4,5)P3 and the small GTPases, Rho, Rac and Cdc42 and actin polymerization also contributes to the sharp polarization (1441, 1667, 1693). An additional positive feedback is provided by the secondary recruitment and activation of class IA PI3Ks by PI3Kγ-mediated increase in PtdIns(3,4,5)P3 (285, 1131). Although PTEN may not be the major phosphatase to influence the PtdIns(3,4,5)P3 gradient, it has been shown that PTEN −/− neutrophils are easily “distracted” and cannot properly “prioritize” their chemotactic cues (591). The complexity of the various pathways PtdIns(3,4,5)P3 influences chemotaxis, and migration is demonstrated by the enhanced transendothelial migratory ability of PTEN (−/−) neutrophils which is counterintuitive to the concept of too much PtdIns(3,4,5)P3 causing a problem with directional sensing (904, 1341). This is related to the fact that PtdIns(3,4,5)P3 not only controls the polarization and amplifies the directional sensing of the cells, but also affects their adhesion, rolling, and motility, via other effectors including integrins and the actin cytoskeleton (1463) (and see below). It is important to note, however, that PtdIns(3,4,5)P3 is not the molecule that sets up the direction, as chemotaxis in Dictyostelium cells still occurs without PI3Ks (45, 628) and neutrophil cells will still migrate toward gradients without PI3Kγ (430, 1140). Nevertheless, PtdIns(3,4,5)P3 is definitely a signal that amplifies and increases the precision of directional sensing, and engages the mechanisms that move cells along chemotactic gradients. There have been a number of proteins identified in neutrophils that are regulated by PtdIns(3,4,5)P3 (827). These included serine/threonine kinases, such as PDK-1, mTOR2, and Akt/PKB; the tyrosine kinase Btk; GAP and GEF proteins for Arf; Cdc42; Rho and Rac; and cytoskeletal proteins such as WAVE2 and ezrin (827, 1463). Moreover, motor proteins, such as myosin X that has three PH domains (one is a split one), can bind PtdIns(3,4,5)P3 and be recruited or conformationally regulated by this lipid species (293, 950, 1228, 1591). All these proteins mediate a different aspect of the migrational response, and it is very likely that they may have their own dedicated source of PtdIns(3,4,5)P3 produced at different phases of the cell movement.

2. Phagocytosis

Phagocytosis is a critically important process for innate immunity and for clearance of cell debris during inflammation and tissue remodeling (6, 1821). It is one of the most spectacular membrane polarization events that one can observe under the microscope. Particles or cells to be phagocytosed initially attach to the cell surface, mostly via FcγRs or CR3 complement receptors (1592). This binding initiates a whole set of signaling events that induce the raising of the
PM around the object (called pseudopods) until this membrane ring is high enough to close above the particle thereby engulfing it. The engulfed particle surrounded by a membrane then undergoes maturation upon which various endocytic compartments fuse with the phagosome lowering its pH, changing its membrane composition, and supplying degradative enzymes that eventually degrade the particle. This complex coordinated membrane deformation sequence is associated with characteristic changes in membrane phosphoinositides at the different stages of the phagocytic process (1766). After the particle is bound to the PM, increased synthesis of PtdIns(4,5)P$_2$ was found to occur during formation of the phagocytic cup due to the recruitment and activation of PIPK$_{ia}$ (172). However, PtdIns(4,5)P$_2$ suddenly disappears upon sealing of the phagosome caused partially by its PLC$_{\gamma}$-mediated hydrolysis (172), and this latter step is essential for proper particle ingestion (1370). The PtdIns(4,5)P$_2$ decrease is the result of several additional processes: a drop in the surface charge of the formed phagosome releases PIPK$_{ia}$ (1767), PtdIns(4,5)P$_2$ is consumed by PI3Ks that form PtdIns(3,4,5)P$_3$ on the bottom of the phagocytic cup as the pseudopod is raising (295, 1000, 1094), and several phosphatases that act both on PtdIns(4,5)P$_2$, such as OCRL and INPP5B (155), or on PtdIns(3,4,5)P$_3$, such as SHIP (14, 294) and Pharbin (649) are recruited to the phagosome. The sequential recruitment of the multiple inositol lipid metabolizing enzymes and the resulting localized and temporally organized spectacular changes in the various PI species during phagocytic membrane remodeling raises the question of what importance these lipid changes have and how they participate in the organization of this process.

As for PtdIns(4,5)P$_2$, the changes in this lipid correlate very closely with the formation of actin during the pseudopod extension and with actin disappearance after particle engulfment (1370). The different type I PIP kinases appear to have subtle and distinct roles in the process: deletion of PIPK$I_1$ from bone marrow-derived macrophages yields defective attachment of IgG-coated particles to the cells (989). These cells have increased amount of highly polymerized actin and fail to cluster their Fc$\gamma$R upon engagement (989). They also show enhanced RhoA and reduced Rac1 activation. In contrast, PIPK$I_2$ deletion inhibits the ingestion process and prevents activation of WASP and actin polymerization without altering attachment (989). The connection between PtdIns(4,5)P$_2$ and actin dynamics has long been appreciated not only in phagocytosis but in virtually any membrane deforming events, including chemotaxis (1505, 1691), endocytosis (1320, 1768, 1800), and exocytosis (1574, 1681). Several actin capping and severing proteins were shown to bind PtdIns(4,5)P$_2$, and therefore, the lipid can affect actin polymerization in a number of ways. It can dissociate the capping protein gelsolin from the (+) end of actin (720, 721), it can increase actin nucleation by the Arp2/3 complex via binding to the N-WASP protein (1048, 1287), and it can inhibit the actin-severing activities of coflin and the actin depolymerizing factor ADF (1162, 1772, 1812). Profilin and $\alpha$-actinin, two additional proteins promoting actin assembly and crosslinking, are also regulated by PtdIns(4,5)P$_2$ (237, 516, 1412). Moreover, several actin bindin proteins containing FERM (four-point one-erzina-radin-moesin) domains contain PtdIns(4,5)P$_2$ binding modules, and their PM association is determined by lipid binding to these domains (255, 556, 982). Hydrolysis of PtdIns(4,5)P$_2$ by PLC$_{\gamma}$ is necessary for completion of phagosome formation (172, 1370). This not only allows the drop in PtdIns(4,5)P$_2$ levels and hence facilitates actin disassembly, but also generates DAG to activate other downstream effectors, such as PKCa (238, 859) and Ras/Rap1 (171).

PI 3-kinase inhibition by Wm or LY294002 inhibits phagocytosis of large ($>3 \mu m$) particles but has a relatively small effect on the smaller ones (50, 295). Yet, localized prominent PtdIns(3,4,5)P$_3$ increases in the phagocytic cup have been observed (750, 1000). A recent study found a close connection between PI3K activation and Cdc42 cycling during development of the phagosome. Cdc42, which promotes actin polymerization, also activates PI3Ks in the phagocytic cup; the 3-phosphorylated lipids, in turn, inactivate Cdc42 allowing actin depolymerization and recycling, which is important for phagosome formation (115). Relatively little is known about which PI3K isoform(s) are responsible for the generation of PtdIns(3,4,5)P$_3$, but the class IA enzyme, via recruitment through p85 adaptors, is believed to be the most important for this process (750). In contrast, PI3K$\gamma$, which is abundant in phagocytic cells, plays little if any role in phagocytosis of opsonized particles (1740). However, the role of the PI3K subclasses can be more specialized, as recent studies found that phagocytosis of the parasite *Leishmania mexicana* is strongly impaired in PI3K$\gamma$-deficient cells or by specific inhibitors of this PI3K isoform (308). As for PtdIns(3,4,5)P$_3$ effectors, the small GTP binding proteins of the Rho/Rac family have well-established roles in phagocytosis (215, 648) and GEFS for these proteins, such as VAV or the Ras GEF protein SOS, are activated by PtdIns(3,4,5)P$_3$ (322, 696, 1588, 1624). PtdIns(3,4,5)P$_3$ also can recruit protein kinases, such as Akt and Btk, and while the former is not desirable and hence being limited by several mechanisms (155), the latter serves as a positive regulator of PLC$_{\gamma}$ activation and helps complete phagosome formation (42, 738).

Recruitment of PI 5-phosphatases that act upon PtdIns(4,5)P$_2$ and PtdIns(3,4,5)P$_3$ is necessary to terminate the processes described above, and prepare the completely ingested phagosome to its intracellular journey and maturation. It is not clear why phagocytic cells need four different 5-phosphatases (OCRL, INPP5B (155), SHIP (14, 294), and Pharbin (649)), but it is likely that each of these is specialized to disassemble specific protein-lipid signaling complexes. Ac-
cordingly, Pharbin (INPP5E) inhibited FcγR-mediated, but not CR3 complement receptor-mediated phagocytosis, while SHIP1 was found to be more important for CR3-mediated phagosome formation (649). OCRL and INPP5B, on the other hand, were important at a later stage to limit Akt activation in the already ingested phagosome (155). The internalized phagosome is then mostly stripped of PtdIns(4,5)P$_2$ and PtdIns(3,4,5)P$_3$, and the major inositol lipid associated with it is PtdIns3P generated by the class III PI3K and possibly PtdIns4P, the product of PtdIns(4,5)P$_2$ dephosphorylation but also produced by type II PI4ks (156). In this regard, the limiting membrane of the phagosome is similar to that of other endocytic vesicles. There is, however, an interesting recent observation that suggests that even the endocytosed phagocytic vacuole has a small amount of PtdIns(4,5)P$_2$ and PtdIns(3,4,5)P$_3$ associated with it, which helps generate actin comets that propel the vacuoles inside the cells. This study suggested the class III PI3K via generation of PtdIns3P helped dislodge Inpp5B from the nascent phagosome and allow class I PI3Ks to generate PtdIns(3,4,5)P$_3$ (156). The subsequent fate of the maturing phagosome is a complex process involving a number of Rab proteins and the PI lipids PtdIns3P and PtdIns(3,5)P$_2$. Although phagosome maturation has its unique features reviewed recently (446), from a PI lipid standpoint, it is not too dissimilar to other endocytic processes that will be discussed below.

An important trivial question rarely asked is what keeps these very local lipid gradients from spreading given the rapid rate of lateral diffusion of PIs within the PM. Such gradients can be maintained only if the lipid production is highly concentrated at these sites against a high background of degradative activity. Alternatively, and probably complementary to the former, the engagement of the lipid by protein binding partners could shield the lipids from degradation. To answer these questions, one would need to know the diffusion speed of lipids when they are free and when proteins are bound to them, or the average time a specific protein module spends bound to a PI species. A few studies have addressed these issues using fluorescent tools in living cells (518, 561, 840). They demonstrated that lipids indeed diffuse rapidly, so there is a need for localized synthesis/degradation or physical diffusion barriers, to maintain gradients. Second, protein effectors interact only transiently and can only act over micrometer distances. Recent studies using similar techniques found that significant diffusion barriers exist around the phagocytic cup keeping PtdIns(4,5)P$_2$ in place (517).

3. Epithelial cell polarity

The classical example of membrane polarization is found in epithelial cells that form a barrier separating the inner milieu from the external world or line cavities in multicellular organisms. In the classical case, these cells have apical and basolateral membranes separated by tight junctions that link the cells together and the protein and lipid compositions of the apical and basolateral membranes are significantly different. How cells maintain this polarity has been the subject of intense research as newly synthesized molecules traffic to their proper destination, and to do so, they must use molecular codes for apical and basolateral targeting (194, 1030, 1696). Since different membrane compartments within the cells have characteristic PPI compositions, it is an important question whether the apical and basolateral membranes have unique PPIs associated with them. Indeed, it has been reported that when MDCK cells from an epithelial layer around a cyst in a three-dimensional culture, their apical membranes are enriched in PtdIns(4,5)P$_2$, while their basolateral membranes are enriched in PtdIns(3,4,5)P$_3$, and this lipid is excluded from the apical membrane (499, 1004). These studies also found that this lipid distribution is important for proper targeting of proteins to the respective membranes, and this process can be disturbed by exogenous delivery of the lipids to the “inappropriate” membranes (499, 1004). In subsequent studies, also using human colon cancer cells, it was found that the PtdIns(3,4,5)P$_3$ 3-phosphatase PTEN localizes to the apical membrane explaining the lack of PtdIns(3,4,5)P$_3$ in that compartment. Indeed, PTEN downregulation disrupted intestinal epithelial polarity and increased the invasiveness of human colon cancer cells (851). A possible link between PtdIns(4,5)P$_2$ and the apical polarity was established when annexin2, which had been previously shown to bind PtdIns(4,5)P$_2$ (1268), was found to be enriched in the apical membranes and also to recruit Cdc42, a small GTP binding protein. Cdc42, in turn, is critical in defining the apical membrane as it targets and enriches the Par6/aPKC polarity complex at the apical membrane (1004). What is not clear is which PI3Ks maintain the high PtdIns(3,4,5)P$_3$ levels at the basolateral membranes during epithelial morphogenesis, but integrins interacting with matrix components are probably part of the PI3K activation mechanism.

Another role of PtdIns(4,5)P$_2$ in the formation of adherens junctions has been recently described. One study showed increased PtdIns(4,5)P$_2$ production by PIPK1y at the adherens junctions, which was required to recruit the Exo70 exocyst subunit to the newly formed E-cadherin junctions (1749). Another study described that OCRL1 5-phosphatase was part of junctional complexes and was critical for the maturation of polarized epithelial cells (527). Consistent with the role of PtdIns(4,5)P$_2$ in adherens junctions, an elegant EM study on PtdIns(4,5)P$_2$ distribution found the lipid highly enriched in gap junctions, but otherwise showed an even distribution between the apical and basolateral membranes (1183). PIs certainly play additional roles in intracellular trafficking decisions, driving the selective delivery of cargoes to the respective PMs, but those processes belong to the intracellular roles of PIs and will be touched upon later. The aforementioned studies just began to explore the PM phosphoinositide subcompartments in
epithelial cell polarity. Clearly, these lipids must have a role not only in targeting proteins to the right PM domains, but also in the regulation of channels and transporters that are so critical to the barrier and transport functions of epithelial layers. It is also important to note that the PtdIns(3,4,5)P3/PtdIns(4,5)P2 distribution described above may not be a general feature of all epithelial cells and could also change with the developmental stage (1397). It is expected and certainly hoped that research in this area will significantly increase and will be extended to planar cell polarity. This latter refers to the alignment of a collection of cells within a cell sheet, another exciting form of polarization that has not yet set an eye on phosphoinositides.

F. How Do Cells Maintain Their PM PtdIns(4,5)P2 Pools?

The key role of the PM pool of PtdIns(4,5)P2 to so many cellular functions makes it essential for the cells to control the level of this lipid in the membrane. Since PtdIns(4,5)P2 represents only a small fraction of the total cellular PtdIns, cells have to replenish this pool especially during prolonged and robust PLC activation. Early studies have already established that the entire PtdIns(45)P2 pool is turning over several times during even a 10-min stimulation (e.g., Ref. 299). This happens as the larger PM pool of PtdIns undergoes sequential phosphorylations by PI 4-kinases and type I PIP 5-kinases (see sects. II and III). It has been observed that Li+ inhibits several inositol phosphate phosphatases and ultimately the inositol monophosphatase that converts inositol monophosphates to inositol (34). Since recycling of inositol is essential for the maintenance of the “PI turnover,” Li+ ions are able to disrupt the cycle leading to the accumulation of inositol phosphates and CDPP-DG and an eventual rundown of the PtdIns pools (87, 90, 133, 727) (see sect. IV and Figure 2B). Although it takes a longer period for the smaller PtdIns(4,5)P2 pools to be depleted in this manner, it has been proposed that the therapeutic benefits of Li+ treatment in manic-depressive disease might be related to the preferential effects of Li+ on neurons that show the highest activity (134). Since Li+ also affects other enzymes, most prominently GSK-3 that acts on the Wnt signaling pathway (739), this attractive Li+ hypothesis has never been unequivocally proven or disproven.

Another important and unresolved issue is which PI4Ks are involved in this process and how PtdIns gets to the PM since it is synthesized in the ER. It has been shown that inhibition of type III PI4Ks by higher concentrations of pan-PI3K inhibitors, such as Wm, are able to block PtdIns 4-phosphorylation and PtdIns(4,5)P2 maintenance in highly stimulated cells (1112). Since type II PI4Ks are insensitive to Wm (386, 1112), and PI4KB-specific inhibitors do not have the same effects, by way of exclusion it was concluded that PI4KA is the enzyme responsible for this process (82, 84). This was corroborated by earlier findings that the yeast homolog of PI4KA, Str4p is responsible for PM PtdIns(4,5)P2 maintenance in yeast (63). However, the primarily ER localization of the mammalian PI4KA enzyme (1107, 1726) and the modest effects of its cellular knockdown on PtdIns(4,5)P2 maintenance in stimulated cells made these conclusions somewhat circumstantial (82, 84). A recent study shed new light on this question: this study found that the EFR3 and TTC7 proteins are capable of recruiting PI4KA to the PM and confirmed that this kinase is the primary source of PtdIns4P in the PM (1114). That still leaves us with the question of how PtdIns gets to the PM. Although PI transfer proteins (PITPs) are capable of PtdIns transfer between membranes (1551) and have been shown to present the lipid for PI4Ks (1354) and PI3Ks (1189), there are no solid evidence that these proteins are indeed mediate PtdIns transfer from the ER to the PM. In fact, PITPs were mostly implicated in the synthesis of PtdIns4P at the Golgi (1354) and not at the PM. In a recent study we found that the PI synthase enzyme that was formerly believed to reside in the ER, can associate with a highly mobile membrane compartment that originates from but is not identical to the ER intermediate compartment (ERGIC). This mobile platform might be able to deliver PtdIns to various membranes via transient contacts (796). However, how lipid transfer takes place during these membrane contacts, and how the system senses and adapts to the PtdIns(4,5)P2 needs of the PM still needs to be clarified.

Another related question is how the PtdIns(4,5)P2 5-phosphatase-PIP5-kinase cycle affects PM PtdIns(4,5)P2 levels. The rapid turnover of the 5-phosphate in PtdIns(4,5)P2 has long been described, and it was attributed to “futile cycles” of dephosphorylation and rephosphorylation believed to take place in the PM as it happens in erythrocyte membranes (580). However, it must be clear by now that the multiple 5-phosphatases and 5-kinases that are often part of the same signaling complex and regulate several steps at the endocytic cycle, must be, in big part, responsible for the high turnover of the 5-phosphate. This means that what appeared to be “futile cycle” might be the sum of several processes along the endocytic pathway in which PIP5Ks and 5-phosphatases generate a local turnover feeding specific signaling steps. This raises the question of whether a pathway exists by which the bulk of uncoated endocytic vesicles recycle back to the PM before reaching the early endosome/sorting endosome stage allowing their PtdIns4P content to be rephosphorylated by PIP5Ks? Another recent puzzling observation was that PtdIns(4,5)P2 levels can be maintained at apparently very different and greatly reduced PM PtdIns4P levels, a finding that is hard to reconcile with the high turnover rate of the phosphate in the 5-position (559). We do not have a clear answer to these observations, nor do we understand what happens to the PtdIns4P that is left behind on the early endocytic compartments, since another lipid, namely, PtdIns3P, is found to be important for the formation of early endosomes from the CCVs. Lastly, it is
completely unknown what mechanism delivers the PtdOH generated at the PM by the phosphorylation of DG to the ER-localized CDS enzyme(s) for the recycling of the DG for PtdIns synthesis (see Figure 2B).

X. PHOSPHOINOSITIDES AT INTERNAL MEMBRANES

The ultimate precursor of all phosphoinositides is PtdIns, a lipid synthesized in the ER and in an ER-derived subcompartment (796). PtdIns is first phosphorylated to PtdIns3P and PtdIns4P in endosomes and Golgi, respectively, and then further to PtdIns(3,5)P2 and PtdIns(4,5)P2 in late endosomes/MVB and the PM. Parallel to these “synthetic” intermediates exist the “degradative” intermediates that are derived from the dephosphorylation of PtdIns(4,5)P2 and PtdIns(3,4,5)P3 producing PtdIns(3,4)P2 and PtdIns4P in membranes that are moving inside from the PM. As a result, internal membranes contain a variety of PI lipids, most of which have been linked to the regulation of specific trafficking pathways. Some authors refer to PI lipids as identity codes marking specific membrane compartments. While these lipids, indeed, can be characteristic of specific intracellular membranes, it may be misleading to assign their functions to those membranes as much as it can be misleading to draw conclusions from the steady-state distribution of proteins concerning their functions when they cycle between various compartments (e.g., transferrin receptors are mostly found in ER, yet their function clearly requires visiting the PM). Also, using our imaging and antibody tools, we make conclusions about the lack of lipids in internal compartments just as much as we do about their presence. This can also be misleading. It is quite possible that what we see is a larger “precursor” pool that is phosphorylated and dephosphorylated with a very short-lived intermediate, and the invisible, short-lived lipid is in fact the important regulatory entity. There are probably cases for both, as we can draw parallels from the PM, where PtdIns(4,5)P2 is a regulatory lipid, a product of PI3Ks, but it also is a precursor of PtdIns(3,4,5)P3 made by the PI3Ks and PtdIns(3,4,5)P3 is rapidly removed by 3- or 5-phosphatases. Therefore, one should be careful making strong inferences just from the localization of inositol lipids or the lack thereof, and also consider the location of the enzymes both for the production and elimination. Even though it seems counterintuitive to have both the kinase and the phosphatase enzymes at the same place apparently working against one another, it makes perfect sense if one sees this arrangement as a rapid cycling between active and inactive forms of a regulator (a lipid, in this case) just as it happens with small GTP binding proteins with their GEFs and GAPs (see this analogy discussed in Ref. 85) (Figure 13).

A. ER

As described in earlier sections, PtdIns is produced in the ER and in a highly mobile organelle that still awaits further characterization (796). Further phosphorylated PtdIns are not detectable in the ER, probably due to the high enrichment of the PtdInsP monophosphatase enzyme Sac1 in the ER. In fact, Sac1 cycles between the ER and the cis-Golgi compartments (147, 426, 427) and according to recent studies it can also act in trans on PtdIns4P located in the PM at ER-PM contact zones (1454). There are, however, PI kinases found associated with the ER, namely, PI4KA (and possibly its yeast ortholog, Stt4p), which has been shown to regulate ER exit sites in mammalian cells (154, 425). In other studies, PtdIns4P made by the PI4KB (or Pik1p in yeast) was found necessary for fusion of COPII vesicles with the early Golgi compartment but not for budding from the ER (940). The inhibition of HCV replication by PI4KA knock-down or inhibitors revealed that this enzyme is important for the generation of the membranous webb, the membrane platform of HCV replication that is likely to be of ER origin (122, 1267, 1513, 1514, 1577, 1597). Our knowledge is still very limited on the question of what proteins and by what mechanism respond to PtdIns4P made in ER membranes or on the surface of vesicles that leave the ER. PtdIns4P is also targeted by the pathogen Legionella pneumophila, which uses its SidC (1248) and SidM (188, 1824) proteins, together with Rab1 to form a replication-permissive vacuole from ER-derived vesicles (1248).

B. Golgi

It has been the identification and cloning of the first yeast PI4K, Pik1p, that drew attention to the fact that the Golgi is an important site of PtdIns4P production and actions (444, 555, 1479, 1655). This was soon followed by studies showing the presence of mammalian PI4Ks in the Golgi (1036, 1106, 1107) and the role of Arf1 in their Golgi recruitment (514). Moreover, several PH domains with PtdIns4P binding were identified in proteins that showed Golgi localization (381, 893–895, 1783), strongly suggesting that PtdIns4P is an important regulator of Golgi functions (337). Functional studies showed that PI4KB (or Pik1p in yeast) is responsible for post-Golgi trafficking of several protein cargoes (64, 193, 514, 555, 1509, 1655). Since all four mammalian PI4Ks are found in the Golgi/TGN compartment (81), the question arises as to what extent can PI4Ks be linked to specific Golgi-related functions and how much functional overlap and redundancy exist among these enzymes. PI4KA is found in the ER and probably the cis-side of the Golgi (1107, 1726), PI4KB is localized along the whole Golgi compartments (337), while PI4K2A and PI4K2B are found in the trans-side of the Golgi and the TGN (83, 1680, 1690, 1697). This distribution, however, probably shows some changes depending on the status of the cell. More on the regulation of the PI4Ks in the Golgi can be found in section V.
The known effectors regulated by PtdIns4P at the Golgi can be grouped into three categories: the first includes clathrin adaptors, both the tetrameric forms, such as AP-1 and AP-3 (1330, 1680) and the monomeric GGA proteins (344, 1671). These proteins can bind PtdIns4P, and in mammalian cells their Golgi/TGN recruitment is closely associated with the functions of the type II PI4Ks (344, 1671). However, in yeast, the Golgi binding of the AP-1 and Gga2p proteins is functionally linked to Pik1p (316, 344). A significant complexity is layered on this process by the fact that the Golgi recruitment of either the clathrin adaptors or the Pik1p/PI4KB enzymes depends on the activation of Arf1 proteins (512, 514). This is clearly illustrated in a recent study showing in yeast that AP-1 and Gga2p are sequentially recruited to the TGN membrane and that PtdIns4P made by Pik1p determines how tightly these two events are coupled. Moreover, Pik1p itself is helped in its recruitment by direct binding to Gga2p (316) and also to the Arf1 GEF protein, Sec7p (512). It is clear from these yeast studies that Arf1- and PtdIns4P of clathrin-dependent sorting out of the Golgi compartment. The mutual reliance on a physical association between PI4K2A with AP-3 in the localization and functions of these two proteins has also been shown in mammalian cells. Here, PI4K2A is both a regulator of AP-3 and a “cargo” via its dileucine-based sorting motif, and these two proteins are mutually required for correct localization and for proper sorting of cargos to the lysosome (298). More details on the dual requirement for Arf1 and PtdIns4P of clathrin-dependent sorting out of the Golgi can be found in several recent reviews (314, 315, 523).

The second group of effectors is comprised of lipid binding proteins. Notably, most PH domains that recognize PtdIns4P are found in proteins that transfer lipids between membranes (381, 894, 1783). These include the mammalian oxysterol binding protein (OSBP) (894), some of its mammalian homolog ORPs (1167), and the yeast homolog OSH proteins (893, 1304, 1783). Further members of this group are the ceramide transfer protein CERT (564, 895) and the glycosyl-ceramide transfer protein FAPP2 (381, 513) and its truncated sister protein, FAPP2 (381). All of these proteins contain PtdIns4P binding PH domains that...
require both Arf1-GTP and PtdIns4P for Golgi association (895). The recently solved structure of the FAPP1 PH domain identified separate binding sites for binding of these two regulatory components (587, 885). An important common feature of all of these lipid-transfer proteins is the presence of a FFAT [two phenylalanines (FF) in an acidic tract] motif that provides an ER-association signal via interaction with ER-localized VAP (VAMP-associated) proteins (747, 931, 1049). Both the FFAT domain and the PH domain is essential for the lipid transfer (but not lipid cargo binding) functions of the proteins (564, 769, 1210), although the exact mechanism of how PtdIns4P regulates this process remains obscure (1262). The obvious question regarding the physiology of these proteins is which PI4K(s) are associated with their functions. Based on studies using the isolated PH domains of these proteins, their Golgi localization is supported by both PI4KB and PI4K2A (84), but the OSBP and FAPP1 PH domains show slightly different localization, and more importantly, their overexpression distorts Golgi structure differently (94). This probably reflects their PtdIns4P-dependent binding to additional and different proteins. When studying the lipid transfer functions of these proteins, it was found that ceramide transfer by CERT (that is needed for SM synthesis) relies on PI4KB more than PI4K2A (313, 1567), whereas FAPP2-mediated GlcCer transfer (required for synthesis of complex glycoinositolphospholipids) is linked to PI4K2A more than to PI4KB (313). However, this kinase preference may not be absolute as the upregulation of SM synthesis by oxysterols was shown to depend on PI4K2A (97). In yeast, where the type II PI4K homolog Lsb6p is not a major Golgi regulator, the Golgi recruitment of all of the OSH proteins studied were dependent on Pik1p (1783). Moreover, the unique OSBP homolog Kes1p, the deletion of which alleviates the need for the PI transfer protein Sec14p (902), has a complex sterol-regulated antagonistic relationship with TGN/endosomal PtdIns4P signaling (1089). Recent studies have shown that Kes1p (also called Osh4p) exchanges sterols picked up from the ER for PtdIns4P at the Golgi membrane and, hence, the Golgi PtdIns4P made by Pik1p drives Kes1p-mediated sterol transport between the ER and Golgi membranes (339). This suggests a reciprocal relationship between PtdIns4P controlling lipid transfer and lipid transfer proteins regulating PtdIns4P-mediated signaling. The existence of additional links between PI4Ks and lipid metabolism is revealed by yeast studies finding genetic interactions between Stt4p and sphingolipid synthesis (1510). Although this connection is more closely related to the role of Stt4p in producing PtdIns(4,5)P2 at the PM (415), an ER-component is indicated by the synthetic lethality (1510) and physical association of Stt4p (502) with the ER-localized Ssc2p protein, the yeast homolog of the mammalian VAP proteins. Moreover, Ssc2p was shown to bind phosphoinositides, and this binding was found important for Ssc2p function (745). There have been no similar studies described for PI4KA in mammalian cells. Finally, if PI4Ks are important for lipid transport and maintenance of lipid homeostasis, it is expected that the lipid composition of membranes have an impact on PtdIns4P levels. Indeed, sphingolipids were found to be important for the PM association of the yeast PIP 5-kinase Mss4p (806). Similarly, an association was found between the Sac1 PtdIns4P phosphatase and the Orm proteins that control the rate-limiting step in sphingolipid synthesis in yeast. This also suggests a link between sphingolipid status and PtdIns4P regulation (178). Although much less is known about lipid regulation of PI synthesis in mammalian cells, it is notable that the distribution, palmitoylation, and activity of PI4K2A is regulated by the cholesterol content of internal membranes (949, 1686).

A third and different kind of PtdIns4P effector was discovered by a study that identified novel PtdIns4P-binding domains (357). This study found that the mammalian Golgi protein GOLPH3 binds specifically to PtdIns4P and controls Golgi morphology by forming a bridge between the Golgi membranes and the actin cytoskeleton through its binding to unconventional myosin, MYO18A (357). This finding that linked Golgi morphology to the actin cytoskeleton was somewhat surprising in light of the well-documented importance of the microtubular rather than the actin network in the maintenance of Golgi structure. The yeast homolog of GOLPH3, Vps74p, also binds PtdIns4P, and its steady-state Golgi localization requires Pik1p (357, 1731). The Vps74p protein was found necessary to retain several glycosyl-transferases in the Golgi (1365) and was identified as an essential component of the retrograde trafficking of these enzymes (1731). These studies also suggested that in addition to regulating anterograde trafficking, Pik1p was also important for the retrieval of specific cargos, namely, Golgi glucosyltransferases. Curiously, the GOLPH3 locus is amplified in a number of human cancers and was linked to oncogenesis through enhanced mTOR1 signaling (1371). How these three aspects of GOLPH3/Vps74 biology are linked together is an exciting question that is surely being studied in several laboratories.

C. Endosomes and Lysosomes

Endosomal function is a term that covers a lot of processes. Newly synthesized molecules leaving either the ER or the Golgi (where PtdIns4P is the critical regulatory PI lipid) have to be sorted either toward the PM or the lysosome. At the same time, the PM undergoes constant internalization [a process where PtdIns(4,5)P2 is a major controlling PI lipid] and the internalized membranes and luminal contents have to be sorted either to the lysosome for degradation or back to the PM (FIGURE 11). During these processes membrane vesicles fuse, their membranes and luminal contents can mix, and then the membranes get separated segregating both luminal and membrane components to form new vesicular entities. At all of these steps, PI lipids, namely, PtdIns3P, PtdIns(3,5)P2, and PtdIns4P have fundamental...
roles. This is a huge research area that will be summarized here only with broad brush strokes.

1. PtdIns3P and PtdIns(3,5)P$_2$ in endomembranes

Identification of the yeast Vps34p protein as a PI 3-kinase was a major milestone in highlighting the importance of PtdIns3P in intracellular trafficking (1367). It was soon followed by the recognition that a unique cysteine-finger protein module present in several proteins, namely, the yeast Fab1, the C. elegans YOTB/ZK632, the yeast Vac1, and the mammalian EEA1 (hence comes the name FYVE domain) (1090), is necessary to target the EEA1 protein to endocytic membranes (1458), and it is a PtdIns3P recognition module (200, 501, 836, 1064). FYVE domains have since been found in a great number of proteins (1457), and it has been firmly established that PtdIns3P, together with Rab5 proteins, controls endocytic vesicle fusion (1100, 1409). The yeast PI3K Vps34p is essential for sorting proteins to the yeast vacuole (605, 1367), and among the FYVE domain containing proteins, the yeast Fab1p turned out to be a PtdIns3P 5-kinase that was necessary for maintaining vacuolar morphology and function (498, 1757). These studies have established PtdIns3P and PtdIns(3,5)P$_2$ as key lipid regulators of endocytic trafficking. Another PtdIns3P binding module called phox-homology (PX) domain was later found in several proteins such as the sorting nexins (SNX) (304, 1540). Although the first PX domains were found to bind PtdIns3P (752, 1750), and all yeast PX domains bind this lipid (1782), some metazoan PX domains bind PtdIns(4,5)P$_2$ (327, 810), and the lipid-binding specificity of many mammalian PX domains remains unknown. It is also important to note that PX domains also bind proteins (1540, 1643). Recent studies also suggest that class II PI3Ks produce some of the PtdIns3P in higher eukaryotes (370, 970, 1700), and the molecular cloning of the type II PI4Ks (108, 1058) revealing their presence in endosomes (83, 1060) also suggested the involvement of these latter enzymes in endocytic trafficking in mammalian cells (83, 1060). PtdIns3P controls several aspects of the biology of endosomes. It has a role in targeting newly synthesized lysosomal proteins to the lysosomes (vacuole in yeast), and it also controls retrieval of proteins from the lysosome to endosomes. It regulates the sorting of endocytosed cargoes for recycling or degradation and also plays a critical role in autophagy, a process by which starving cells degrade their own cytoplasm and organelles (1631). For all these different processes, a whole set of regulatory proteins are recruited to the endocytic membrane in specific sequences.

For example, soluble proteases have to reach their destination, the lysosomes. For these proteins, special receptors exist such as the yeast Vps10p and the mammalian mannose-6-phosphate receptor (M6PR). These transmembrane proteins cycle between the Golgi and prevacuolar compartments, with each round delivering a batch of enzymes for further sorting to the lysosomes. As pointed out in earlier sections, PtdIns4P is important for the exit of these transport carriers from the Golgi using clathrin-coated vesicles. However, retrieval of Vps10 from the endosomes relies on other inositol lipids and proteins that form a complex called the “retromer” (1373). In yeast, Vps29, Vps30, and Vps35 are important components of the retromer (1373), and one of these, Vps30, as well as another protein, Vps38, are both accessory proteins to the PI 3-kinase PtdIns3P (201). Moreover, two proteins, important for vesicle budding, Vps3 and Vps17 that are also part of the retromer (1372) possess PX domains that bind PtdIns3P (201). The mammalian Vps5 homologs SNX1 and SNX2 play similar roles in mammalian cells as part of the mammalian retromer complex. SNX1 shows PtdIns3P and PtdIns(3,5)P$_2$ binding via its PX domains (297) and was found responsible for retrograde trafficking of the M6PR (213, 307).

Another role of PtdIns3P is to promote homotypic fusion of endocytosed uncoated vesicles. For this, tethering by the EEA1 proteins and Rab5 are both essential (1409). However, not all endocytic vesicles are equal, and different cell surface receptors are already routed to different destinations even at the early steps of the endocytic process. FYVE domain containing proteins other than EEA1, such as WDFY2 (585), can define special endocytic compartments with unique signaling properties (1665). The recycling of several receptors back to the cell surface requires PI3Ks (2, 670, 886, 1601). In this process, proteins are retrieved at different stages of vesicle maturation as they progress from early endosomes to late endosomes. A whole set of PtdIns3P binding proteins participate in this process that seems to be different for specific groups of molecules. Various PX domain-containing SNX proteins were identified as uniquely important for these processes (305, 307). For example, SNX4 controls trafficking of TIRs between the early and recycling endosomes (1571), and SNX3 is central to a newly recognized unique retromer pathway by which the Wnt sorting receptor Wntless is retrieved from the endosomes to the TGN (307, 576). Yet another SNX, SNX17, which has a FERM domain that recognizes NPXY sequences on transmembrane proteins, is important for LDL-like receptor cycling (372, 1472) as well as the maintenance of integrins in the PM (1456).

Molecules that are not recycled back to the PM are taking a route toward degradation. This process uses another form of cargo selection that is based on poly-ubiquitylation, which can also serve as part of ER quality control of misfolded proteins. Many PM proteins such as RTKs that undergo endocytosis and are destined for degradation become multi- or polyubiquitylated and recognized by proteins that possess UbI-binding domains. Here the Vps27 protein and its mammalian homolog Hrs are master regulators, both having a FYVE domain and being regulated by PtdIns3P (73, 146, 766, 1250, 1447). The central compartment
where proteins are sorted for degradation is the MVB where the sequential assembly of a protein complex called the ESCRT machinery (ESCRT-0, -I, -II, and -III complexes) is essential for segregation of the ubiquitylated proteins into inwardly invaginating membranes (70, 71, 671, 763). PtdIns3P and Vps27/Hrs are necessary for the recruitment of the ESCRT-0 and hence the subsequent members of the complex to the MVB (73, 766, 1251, 1542). ESCRT-I does not show PtdIns3P binding on its own (821), but ESCRT-II does via the PH domain-like GLUE domain of Eap45 (the mammalian Vps36) (1416). ESCRT-III complexes are the ones that deform membranes of the MVB and generate the inwardly budding vesicles (567, 1327), and several proteins of this complex, such as CHMP3 (and its yeast homolog Vps24p) (1710) and CHMP2a and CHMP4b (225) can bind PtdIns3P, this complex, such as CHMP3 (and its yeast homolog Vps24p) (1710) and CHMP2a and CHMP4b (225) can bind PtdIns3P, PtdIns(3,5)P$_2$, or both. Recent studies reconstituted the assembly and lipid deforming effects of the ESCRT machinery in giant unilamellar vesicles in vitro and only found a moderate enhancement of intraluminal vesicle release by PtdIns3P or PtdIns(3,5)P$_2$ (1723). Although this finding suggests that these lipids promote but are not essential for this reaction, they probably play a significantly more important facilitating role within the cell where these proteins are not supplied in such abundance.

Another important endomembrane process that requires PtdIns3P is autophagy (more precisely, macroautophagy) (281, 1073). Starving cells use this process to degrade a fraction of their cytoplasmic proteins and organelles, such as mitochondria. The hallmark of this process is the formation of a double membrane from internal membranes (also called phagophore), the origin of which is somewhat debated but likely being the ER with mitochondrial and possibly other membrane participation (548, 1565). This membrane gradually engulfs a piece of the cytosol and organelles to be degraded and eventually fuses with the lysosome. The phagophore can be recognized because of its high enrichment of the protein LC3 (microtubule-associated protein light chain 3, Atg8p in yeast) that gets phosphatidylyethanolaminated, a unique lipid modification characteristic of this process (675). The initiation of the phagophore requires the class III PI3K, Vps34, its regulator, Vps15, and Atg6/Beclin1 forming a core complex. There are several additional proteins associated with this complex that play a role in the maturation and subsequent fusion of the autophagosome with the lysosome, and some are shared with the vacuolar sorting pathway. For example, the Vps34/Vps15/Atg6 complex related to autophagy (also called complex I) contains Atg14 (Barkor/Atg14L in mammalian cells), whereas complex II that functions in vacuolar sorting contains Vps38 (UVRAG in mammals) instead of Vps14/Barkor (234, 1157). It is not surprising that some of the steps in phagophore maturation and lysosomal fusion show the same PI3K involvement as the vacuolar sorting pathway. What was surprising, though, was the finding that autophagosome formation also requires PtdIns 3-phosphatases, such as Jumpy (MTMR14) (1631, 1632), which suggests that the turnover of PtdIns3P must be more important than the actual levels during this process. Although the literature of autophagy is increasing every day with intimidating complexity (281, 1073), the exact role(s) of PtdIns3P in this process remains to be understood.

An important point to be emphasized is the close connection between autophagy and nutrient sensing, both of which have 3-phosphorylated inositide regulatory components (Figure 14). All metabolic processes in the cell have to be harmonized with the supply of nutrients, and the central hubs for this information network are the TOR proteins identified in 1991 as the yeast targets of the drug rapamycin (592). The TOR protein (TOR1 and TOR2 in yeast, and mTOR in mammalian cells) is a Ser/Thr kinase that belongs to the PI kinase-related kinase family and is evolutionary highly conserved. The mTOR catalytic subunit exists in two functionally distinct protein complexes, mTORC1 and mTORC2 (454). Each of these complexes contains several proteins, some of which are shared, but mTORC1 contains Raptor, while mTORC2 contains Rictor as unique scaffolding protein components. Rapamycin binding to FKBP12 induces a complex formation with mTORC1 (but not mTORC2), which, in turn, allosterically inhibits mTORC1 activity, explaining why acute rapamycin treatment only inhibits mTORC1. mTORC1 collects information through various pathways on cellular stress, energy levels, amino acids, and growth factors and controls cell growth, metabolism, cell cycle progression, and macroautophagy (854). In contrast, mTORC2 is regulated by growth factors and acts on several downstream AGC protein kinases to control actin cytoskeleton, apoptosis, and cell proliferation (454). It is beyond the scope of this review to get a detailed summary of the mTOR literature (for reviews, see Refs. 454, 854, 1830), but there are important connections between mTOR and PI3Ks that are worth mentioning. Most, if not all, growth factors activate the PI 3-kinase signaling cascade at the cell surface, primarily the class I p85/p110α complex. The resulting PtdIns(3,4,5)P$_3$ engages a range of proteins that signal to mTORs. PtdIns(3,4,5)P$_3$ recruits to the membrane both the PDK1 protein kinase and its downstream target, the Akt/PKB kinase, via their PH domains that bind PtdIns(3,4,5)P$_3$ [and PtdIns(3,4)P$_2$]. PDK1 phosphorylates Akt/PKB at position Thr308, and another phosphorylation at Ser473 within the hydrophobic motif, carried out by mTORC2, is needed for full activation. PtdIns(3,4,5)P$_3$ also activates mTORC2 kinase activity by a poorly understood mechanism (489, 1430). Akt/PKB have several downstream targets that are part of the cell survival pathway (860, 1620, 1806). Akt/PKB inactivates the TSC1-TSC2 (hamartin-tuberin) complex, which is a critical inhibitory regulator of mTORC1 (491, 697, 1541). The TSC1/2 complex is a GTPase activating protein for the Rheb (Ras homologue enriched in brain) GTP binding protein that integrates signals from energy levels, O$_2$
supply, and growth factors (985). The amino acid sensing, however, is mediated by a different pathway that involves the Rag GTPases (785, 1336) and one that requires translocation of the mTORC1 complex to the surface of the lysosomes (1335, 1829). Curiously, the Class III PI3K hVPS34 was found to be important for the amino acid sensing pathway for mTORC1 activation (536, 1143). It has also been shown that this pathway works parallel to the

FIGURE 14. Integration of metabolic control, cell growth and proliferation, and their regulation by phosphoinositides. One of the main effectors of PI3K activation is the Akt/PKB protein kinase that is activated by translocation to the membrane by binding via its PH domain to PtdIns(3,4,5)P3 [and PtdIns(3,4)P2]. This recruitment is important for phosphorylation of Akt/PKB by two protein kinases, PDK1 (on Thr308) and the mTORC2 complex (on Ser473). These two kinases are also activated by PtdIns(3,4,5)P3. Although Akt signals in a number of other directions (not shown here for simplicity), one of its major functions is to control the activity of the mTORC1 complex. Active Akt stimulates mTORC1 by inhibiting the GAP activity of the TSC1/2 complex, the latter limiting the activity of the Rheb GTP binding proteins that are upstream activators of the mTORC1 complex. Active mTORC1 is a signal for cell growth and proliferation and inhibits apoptosis and autophagy. In case of starvation, energy depletion, stress, or hypoxia, mTORC1 is inhibited, and these signals activate the TSC1/2 complex. Under these conditions autophagy is stimulated. A different pathway is used during amino acid sensing. Amino acids activate the Rag GTPases and induce translocation of the mTORC1 complex to the surface of the lysosomes. The classIII PI3K hVPS34 is also important for the amino acid sensing pathway for mTORC1 activation using a pathway that works parallel to the Rag GTPases and which requires PLD1, and PtdIns3P binding to the PX domain of PLD. Recently, the class II PI3Ks and PtdIns(3,5)P2 production was also shown to stimulate mTORC1. Paradoxically, autophagy also requires PtdIns3P and VPS34, and it is most likely that the VPS34 that serves as a mediator of mTORC1 activation by amino acids works in a different complex and location than the one that promotes autophagy.
Rag GTPases and requires PLD1 and binding of PtdIns3P made by hVPS34 to the PX domain of PLD1 (1773). Since mTORC1 inhibits autophagy, and autophagy also needs VPS34, it is most likely that the VPS34 that serves as a mediator of mTORC1 activation by amino acids is found in a different complex and location than the one that promotes autophagy. Another study found that inositol phosphate multikinase (IPMK) also has a noncatalytic role keeping the mTORC1 complex together during its translocation to the lysosomal surface (791). More studies are needed to explore the complex PPI regulation of the mTOR-metabolic network with special emphasis on the compartmental organization and membrane association of these complexes.

Significantly less clear are the mechanistic details of how the PtdIns3P to PtdIns(3,5)P2 conversion controls the lysosomal sorting process and the relevant downstream targets of the lipid PtdIns(3,5)P2 (377). It is well established that Fab1p deleted yeast strains have huge vacuoles and impaired transport of CPY to the vacuole (498). These defects are also associated with weakened vacuole acidification (1159) and chromosomal segregation defects, the latter probably secondary to the enlarged vacuoles (1757). However, autophagy in yeast does not require PtdIns(3,5)P2 (379). The limited known PtdIns(3,5)P2 targets include the PROPPINs that are seven-β-propeller containing proteins, such as the yeast Atg18p (also known as Svp1p) (379) or its mammalian homolog WIPI1(725). Atg18p and WIPI1 and the other PROPPINs (Atg21p and WIPI1–4) are important in autophagy (397, 533, 825, 1238). Atg18p depletion leads to PtdIns(3,5)P2 accumulation and to enlarged vacuoles, a phenotype similar to that of Fab1p deletion, indicating a role in determining vacuolar size (397). However, as pointed out above, the sorting of hydrolyses and initiation of autophagy are two separable functions of Vps34p linked to complex II and complex I, respectively (779, 825). Since autophagy in yeast does not require PtdIns(3,5)P2 (379), Atg18p may have a dual role: it could be important for autophagy as a PtdIns3P effector and for vacuolar morphology as a PtdIns(3,5)P2-regulated protein. A recent study also found that PtdIns(3,5)P2 regulates the activation and localization of mTORC1 in mammalian cells and that the lipid interacts with the mTORC1 component raptor (180). Another group of effectors of PtdIns(3,5)P2 was recently identified as the mammalian TRPML proteins or mucolipins and their yeast homolog, Yvc1/TRPY1 (371). These are identified as the mammalian TRPML proteins or mucolipins and their yeast homolog, Yvc1/TRPY1 (371). These defects are needed to explore the complex PPI regulation of the mTOR-metabolic network with special emphasis on the compartmental organization and membrane association of these complexes.

2. The role of PtdIns4P in endomembranes

Although PtdIns4P has been primarily linked with Golgi and PM functions, the endosomal localization of type II PI4Ks suggests that this lipid may also be important for certain trafficking steps in endosomes. Knock-down of PI4KIIα abolished the Golgi recruitment of the heterotrimeric adaptor AP-1 (1680), and also impaired the recruitment of AP-3 in endosomes (1330). PI4KIIα was also shown to regulate the late-Golgi transport of VSV-G and influenza hemagglutinin (1680). The type II PI4Ks were implicated in transferrin receptor endocytosis and recycling (83), and PI4KIIα has a major role in EGF receptor degradation in the late endosomal pathway (1060). Recent studies showed that knock-down of type II PI4Kα causes enlargement of prelysosomal/lysosomal compartments (298, 741, 1128), and PI4Kα is required for lysosomal delivery of the Gaucher disease enzyme β-glucocerebrosidase and its receptor, LIMP-2 (741). It will be important to understand where and how the PtdIns4P to PtdIns3P switch happens in the various endocytic compartments. Transport vesicles coming from the ER and the Golgi contain PtdIns4P. Moreover, the endocytozed vesicles also should have PtdIns4P as a result of the action of numerous 5-phosphatases. All of these internal vesicular intermediates will have to lose their PtdIns4P and acquire PtdIns3P. How this process is regulated enzymatically and why this switching is important are questions still to be investigated.

D. Nucleus

The presence of several enzymatic activities related to the “PI cycle,” such as PLC, DG kinase, and PI kinase in nuclear matrix, raised the possibility that a separate nuclear phosphoinositide system exists (275, 1205). Since PPIPs are presumed to be membrane associated, the presence of the lipid as well as the enzymes in the nucleus initially raised suspicions that contamination with nuclear membranes might be responsible for these activities. However, phosphoinositide changes in the nuclei of Swiss 3T3 cells stimulated with IGF-I were found significantly different from those elicited at the PM, and the nuclear lipids were identified in the nuclear lamina rather than in the membranes (360, 1001). With the cloning of the various PI lipid metabolizing enzymes and the availability of antibodies for immunofluorescence, it has become clear that many enzymes of the PI cycle do localize to the nucleus. These include several isoforms of PLC enzymes [β1 (451, 952, 987), δ1 (302, 1448), and δ4 (925)], both forms of the type III PI4Ks [PI4KA (748) and PI4KB/Pik1 (332, 343, 1478)], the yeast PIP 5-kinase Mss4 (62) and the mammalian PIP 5-kinase 1α (168, 1029), and the type II IP5KB (263). In addition to the kinases, inositide phosphatases were also shown to move in and out of the nucleus. An increased nuclear retention of PTEN was shown after oxidative stress (229), and the Ser132-phosphorylated SHIP2 was found localized to the nucleus and...
also becoming active against PtdIns(4,5)P$_2$ (400). Moreover, the lipid PtdIns(4,5)P$_2$ itself could be localized by antibodies or by PtdIns(4,5)P$_2$ recognizing PH domains in the nuclear matrix, especially enriched in nuclear speckles (1087, 1176, 1684), the dynamic organelles that have high pre-mRNA splicing activity (849).

There are two major questions raised by these findings; one is related to the physical state of the lipids and another to the nuclear proteins that are regulated by the nuclear lipids. Although we have yet to find answers to the first question, our mind has to be open to the possibility that nuclear lipids may exist in association with proteins, or forming a unique lipid phase like a micelle or liposome. For example, phospholipids, including phosphoinositides, have been found tightly bound to nuclear receptors (151, 828, 903). This does not rule out that phosphoinositides are functionally important in the inner nuclear membrane, where several studies showed their presence (204, 496, 1421). This latter might be significant in light of the nuclear Ins(1,4,5)P$_3$ (519) and Ca$^{2+}$ signals (396) and the high transcriptional activity located in juxtamembrane regions of the nucleus (20, 1512). There have been more advances in the identification of nuclear proteins that respond to lipid changes. A noncanonical poly(A) polymerase, named Star-PAP, has been identified as a protein that interacts with PIPKIIα in nuclear speckles (1029). This protein adds poly(A) tails to a selected group of mRNAs, and its activity is highly stimulated by PtdIns(4,5)P$_2$ (1028, 1029). Curiously, a low activity form of a DNA polymerase was also shown to be activated by PtdIns4P or its hydrolytic product, Ins(1,4)P$_2$ (1507, 1508).

Although PtdIns(4,5)P$_2$ appears to be the most abundant and recognized nuclear phosphoinositide, studies on type II PIPKs that phosphorylate PtdIns5P to PtdIns(4,5)P$_2$ revealed an unexpected twist in their nuclear functions. The nucleus-localized PIPKIIβ (263), most likely in the form of a heterodimer with the catalytically more active PIPKIIα (1672), has an equally, if not more, important role in regulating PtdIns5P levels than controlling nuclear PtdIns(4,5)P$_2$ (522). PtdIns5P has been linked to stress-induced nuclear signaling and apoptosis (737, 1833). The protein that responds to nuclear PtdIns5P is ING2, which specifically binds this lipid with its PHD domain. The ING2 protein associates with histone deacetylases and the chromatin (522, 837) and, hence, regulates histone acetylation. ING2 induces apoptosis via increased p53 acetylation both of which requires PtdIns5P (522). PtdIns5P levels are then regulated either by inhibition of PIPKIIβ (such as in stress responses where p38 MAPK phosphorylates PIPKIIβ (522) or by stimulation of the type I PtdIns(4,5)P$_2$ 4-phosphatase (1833). Another mechanism by which PIPKIIβ and PtdIns5P regulates nuclear functions is the association of the kinase with a nuclear ubiquitin ligase, Cul3-SPOP and stimulation of its activity (198).

In addition to these mechanisms, PtdIns(4,5)P$_2$ may have an indirect role as a precursor of other regulators. The PtdIns(3,4,5)P$_3$-Akt pathway was shown to regulate mRNA splicing, although it is not clear at what location Akt needs to be activated (148, 1709). It is generally believed that the classical PI3Ks are not associated with the nucleus (but see Refs. 831, 998), even though ample evidence suggests that PI3Ks control nuclear signaling (e.g., Ref. 781, 1809). However, the nucleus-localized inositol polyphosphate multikinase (IPMK) was shown to function as a PtdIns(4,5)P$_2$ 3-kinase (1269) capable of activating Akt (962). Still, in a somewhat convoluted manner, the nuclear IPMK lipid kinase activity requires the formation of PtdIns(3,4,5)P$_3$ in the PM by the class I PI3Ks, presumably controlling the phosphorylation state of IPMK (962). Recent studies showed that IPMK-phosphorylated PtdIns(4,5)P$_2$ was bound to the nuclear receptor steroidogenic factor 1 (SF-1) and PTEN was able to reverse this reaction (151). In the same study, IPMK was unable to phosphorylate free PtdIns(4,5)P$_2$ and, conversely, class I PI3K could not phosphorylate PtdIns(4,5)P$_2$ bound to SF-1. These results also speak to the question of the physical form(s) the lipids may take up in the nucleus.

The presence of PLC and DG kinase enzymes in the nucleus also suggest that Ins(1,4,5)P$_3$ and DG formation both can initiate further nuclear signaling events. Nuclear Ca$^{2+}$ signaling initiated from the nuclear membrane-derived intranuclear canaliculi has been demonstrated (396). However, this is not the only way Ins(1,4,5)P$_3$ can be utilized. Ins(1,4,5)P$_3$ can be phosphorylated to higher inositol phosphates, and these soluble, highly charged molecules have been found to be critical for mRNA export (1777). The enzymatic pathways of InP$_6$ formation and their importance have been first described and delineated in yeast studies (1158, 4453), but these metabolites had already been described earlier in mammalian cells (86, 610, 1466, 1776). By now all the enzymes producing them have been identified and cloned (1629, 1719). These pathways are essential in development of higher organisms (463, 1628). The higher inositol phosphates have multiple regulatory functions in the nucleus that have been recently reviewed and will not be further detailed here (28, 1324, 1375, 1390, 1455).

XI. PHOSPHOINOSITIDES IN DISEASE

A. Somatic Diseases

It may be an exaggeration, but with some efforts every human disease can be linked to altered inositol lipid metabolism. Although phosphoinositides are likely to be important in multifactorial diseases, it is easier to define the role of these lipids in disorders caused by a clearly identifiable single defect in inositol lipid metabolizing enzymes or effector proteins. Some of these are profoundly apparent, such as the combined immunodeficiency seen in Bruton’s tyrosine...
kinase patients, due to a single mutation within the PH domain of the Btk tyrosine kinase that prevents its interaction with PtdIns(3,4,5)P\(_3\) (1260). Others cause more subtle aberrations such as the activating mutations of PLC\(_\gamma\)2 in patients with spontaneous inflammations, cold urticaria, and autoimmunity (414, 1169).

A lot more human diseases are caused by excess phosphoinositides than the lack of them as exemplified by the cancer-causing elevations of PtdIns(3,4,5)P\(_3\). As detailed in section V, class I PI3K\(\alpha\) mutations are found in a large percentage of human cancers (1190, 1333), and the same is true for the loss of PTEN (210, 898), the phosphatase that converts PtdIns(3,4,5)P\(_3\) back to PtdIns(4,5)P\(_2\) (968). There is general agreement that PtdIns(3,4,5)P\(_3\) is an oncogenic lipid, and enormous information is available on how PtdIns(3,4,5)P\(_3\) can help tumor cells to survive and metastasize. First and most importantly, PtdIns(3,4,5)P\(_3\) promotes both proliferation and protects cells from apoptosis, two processes that are hallmarks of tumor cells. PtdIns(3,4,5)P\(_3\) activates numerous GEF proteins that act on small GTP binding proteins such as Ras, Rac, Rho, and Cdc42 and, therefore, can activate all of the processes that are used for invasion and metastasis, such as cell migration and attachment. The effects of PtdIns(3,4,5)P\(_3\) can be cell autonomous, but they can also affect the interaction of the cells with their tissue environment, something we still have to learn more about. This may explain why certain cancers have preferential sites of metastasis and avoid other organs. A fundamental question, however, that we still do not fully understand is whether PI3K activation per se is sufficient to transform cells. Several studies have shown that activating class I PI3K mutations are sufficient to transform cells or generate tumors in mouse models (541, 758, 873). However, often in these cancers other mutations show up that drive tumorigenesesis. It appears that cells need a driver for the transformation process, which will then be greatly aided by elevated PI3K activation. Therefore, activating PI3K mutations may just represent a sensitized state upon which any other oncogenic event can easily initiate the transformation process (1785). Especially important is the connection between K-Ras mutations and PI3Ks in cancer cell growth. K-Ras can activate all forms of class I PI3Ks, and hence is always associated with increased PI3K activity. However, PI3K inhibition alone is not very effective in K-Ras mutant-driven cancer cell lines (539), although it has been shown that K-Ras-p110\(\alpha\) interaction (539) or the presence of p85 subunits is important for K-Ras-induced carcinogenesis (404). These data suggest that there could be a difference in PI3K participation at the onset of cell transformation and during the later phase of cancer expansion and maintenance (404). Nonetheless, ample evidence suggests that PI3K inhibition is a promising way of complementing cancer therapy. The close connection between PI3K activation and mTORC1 (and -2) (1830) and the characteristic metabolic realignment of cancer cells (930, 1608, 1769) explain why PI3K inhibitors and mTOR inhibitors are both in the frontiers as potential cancer therapies and that combined PI3K/mTOR inhibitors are also gaining significant momentum (600, 926, 1727).

Apart from the class IA PI3Ks, human diseases are rarely caused by activating mutations of inositide lipid kinases. In contrast, an increasing number of rare human diseases are linked to phosphoinositide phosphatase defects. These have been discussed in the respective individual sections but will be listed here in Table 5.

The general lesson learned from these studies is that these phosphatase defects cause very specific phenotypes because the affected enzymes control a very specific pool of the inositol lipid, and for these narrowly defined biochemical events, the other enzymes do not provide alternative coverage in spite of their identical enzymatic activities. These

<table>
<thead>
<tr>
<th>Disease</th>
<th>Enzyme Defect</th>
<th>Main Symptoms and Pathology</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCRL</td>
<td>OCRL 5-phosphatase</td>
<td>Early cataract, mental retardation, renal tubular acidosis, aminoaciduria</td>
<td>61, 1483</td>
</tr>
<tr>
<td>Dent disease</td>
<td>OCRL 5-phosphatase</td>
<td>Variable, milder forms of Lowe's syndrome</td>
<td>647</td>
</tr>
<tr>
<td>ALS, PLS [small %]</td>
<td>Sac3/Fig4</td>
<td>Progressive degeneration of motor neurons</td>
<td>252</td>
</tr>
<tr>
<td>Charcot-Marie-Tooth type CMT4J</td>
<td>Sac3/Fig4</td>
<td>Peripheral sensorimotor neuropathy</td>
<td>253</td>
</tr>
<tr>
<td>Downs syndrome??</td>
<td>Synaptotagmin 1 trisomy?</td>
<td>Multiple neurological symptoms in CNS</td>
<td>1647</td>
</tr>
<tr>
<td>Joubert syndrome</td>
<td>INPP5E/Pharbin</td>
<td>Midbrain-hindbrain malformation, retinodystrophy, nephronophthisis, liver cirrhosis, polydactyly</td>
<td>145</td>
</tr>
<tr>
<td>X-linked myotubular myopathy</td>
<td>MTM1</td>
<td>Skeletal muscle weakness, centronuclear myopathy</td>
<td>858</td>
</tr>
<tr>
<td>Charcot-Marie-Tooth disease type CMT4B1</td>
<td>MTMR2</td>
<td>Peripheral neuropathy with demyelination</td>
<td>159, 653</td>
</tr>
<tr>
<td>Charcot-Marie-Tooth disease type CMT4B1</td>
<td>MTMR13</td>
<td>Peripheral neuropathy with demyelination</td>
<td>69</td>
</tr>
<tr>
<td>Autosomal recessive centronuclear myopathy</td>
<td>MTMR14/hJUMPY</td>
<td>Centronuclear myopathy</td>
<td>1566</td>
</tr>
</tbody>
</table>
susceptibilities may depend on species and probably even on other genetic modifiers as perfectly exemplified by the OCRL disease that is not easily reproducible in mice (173) and in which the apparently same enzyme defect can produce a range of symptoms with different severities (647).

It is notable that loss of function of inositol lipid kinases is rarely seen (or have not been yet recognized) in human diseases. This either means that such mutations are incompatible with normal development [see the only report on an inactivating mutation within the PIP 5-kinase 1y gene that causes severe developmental abnormalities with perinatal lethality (1119)], or there is enough redundancy in the system to overcome such defects. However, it is quite likely that more subtle functional defects, either structural or regulatory, contribute to the development of human diseases. Given the role of the PI4Ks and class III PI3K in ER-Golgi and endosomal functions, respectively, it would be almost surprising if they did not have a role in diseases where protein misfolding and quality control problems are part of the etiology such as in neurodegenerative diseases. A few examples have started to appear in the literature: for example, mutations in one allele of the Sac3/Fig4 phosphatase have been detected in 2% of ALS (amyotrophic lateral sclerosis or Lou Gehrig’s disease) and PLS (primary lateral sclerosis) patients (252). As discussed in section VI, this phosphatase also impacts the function of the type III PIP kinase PIKfyve. PI4K2A gene-trapped mice develop late-onset spinocerebellar degeneration (1408). Notably, though, no mutations in the human PI4K2A gene were found in patients suffering from a similar condition (270). Given the slow onset of many of these diseases (like Alzheimer’s or Parkinson’s disease) and their multifactorial background, one would not expect to see a very drastic change attributed to a phosphoinositide signaling defect but rather a subtle one that will have a gradual and compounding effect on protein trafficking and folding. Other subtle differences could cause developmental alterations in the brain that do not manifest in any recognizable anatomical abnormality, but manifests in diseases such as schizophrenia, depression, or autism. For example, the PI4K4A gene is located in chromosome 22 in the q11.2 region that is often deleted in “22q11.2 deletion syndromes” such as in DeGeorge syndrome or Velocardiofacial syndrome (808). A strong association has been observed between 22q11.2 deletion syndromes and schizophrenia (55, 111). Several obvious schizophrenia susceptibility genes can be found in this chromosomal region, such as the catecholamine metabolizing COMT, the DeGeorge critical region gene (DGCR6) and the proline dehydrogenase gene (PRODH2) (227, 1730) or SNAP29 whose promoter region also showed association with schizophrenia (1729). However, some studies also found association with an SNP in the PI4KA promoter region with the schizophrenia phenotype (1326, 1648) and suggested that the PI4KA gene could be a modifier in cases of schizophrenia associated with 22q11.2 deletion syndromes (1648). It is almost certain that an increasing number of neurological and psychiatric diseases will be linked to altered phosphoinositide metabolism (269).

B. Infectious Diseases

It has been increasingly recognized that various intracellular parasites utilize the host’s intrinsic cellular machinery to enter the cells, move around, and evade the natural protective mechanisms (1204, 1225). In addition, many organisms, especially viruses, generate a platform for the reproduction of the organism and then get released from the cell interior (38). Identification of host proteins that these pathogens require at any point of their life cycle is an important goal that may uncover new therapeutic opportunities (777, 1239). Phosphoinositides, again, are important regulators of many of these processes. For example, the endocytosis of several pathogens uses the same routes by which cell surface proteins enter the endocytic pathways and hence rely upon PtdIns(4,5)P2 as detailed in section IX.D. Examples include the entry of bacteria such as Listeria (1226, 1548), Yersinia (1340), enterohemorrhagic E. coli (1348), Salmonella (1544), or viruses such as hepatitis C (1577), HIV (1069), or influenza (472), just to name a few from an ever-increasing list. Another role of PtdIns(4,5)P2 and PtdIns(3,4,5)P3 has been reported in filopod formation, actin polymerization, and movement of Listeria within the infected cells (1405). PtdIns4P is also utilized by several pathogens to shape their intracellular milieu. Legionella pneumophila uses several of its virulence proteins, such as SidC and SidM that bind PtdIns4P, to form its replicative vacuole within the cell (188, 613, 1248, 1492, 1824). Another intracellular bacteria Clamydia uses both type II PI4Ks and the OCRL 5-phosphatase to modify its vacuole for production of infectious progeny (1080). Most recently it was discovered that hepatitis C virus requires PtdIns4P, specifically made by PI4KA for replication (122, 1513, 1577, 1597). It is not clear for what purpose PI4KA is needed, but most likely generation of the “membranous webb,” which serves as the replication platform of the virus (1082), requires the PI4KA enzyme whose activity is stimulated by one of the viral proteins, NS5A (38, 910, 1267, 1514). Some enteroviruses, such as Polio or Coxsackie, need PtdIns4P made by PI4KB to generate their replication organelle (656). Development of specific PI4K inhibitors now attracts a lot more interest than it did a few years ago. Another process where phosphoinositides can be important is the budding of viruses when they leave the cell. This process uses several of the same lipid and protein regulators that are required for the biogenesis of the MVB during the formation of inwardly budding vesicles (1016), and inhibition of PIKfyve-catalyzed formation of PtdIns(3,5)P2 was found to inhibit retroviral budding (686).

Other pathogens do not rely upon the host cell’s phosphoinositide metabolizing enzymes to create the lipid environ-
ment that is required for infection or survival. For example, *Listeria* produces a PLC enzyme as an important virulence factor that helps the bacterium escape from the vacuole of infected cells (1235). *Salmonella enterica* and *Shigella flexneri* inject an enzyme, called SigD/SopB and IpgD in the two strains, respectively, into the prospective host cells that induce active membrane remodeling to help the bacterial invasion at the entry process and, in the case of *Shigella*, its intracellular release from the endocytic compartment. Both of these enzymes show homology to mammalian inositol lipid phosphatases with a relatively broad in vitro specificity (993, 1151). However, inside the cells, these enzymes appear to hydrolyze primarily PtdIns(4,5)P$_2$ with production of the rare and enigmatic PtdIns5P (607, 1130, 1544). These enzymes also lead to the production of 3-phosphorylated lipids (1130, 1206) by mechanisms that are not all that clear and, curiously, are not inhibited by classical PI3K inhibitors in the case of *Salmonella* SigD/SopB (975, 993). A similar phosphatase acting upon PtdIns(4,5)P$_2$, called VPA0450, was identified in *Vibrio parahaemolyticus* (185), and a previously described acid phosphatase of *Legionella* that inhibits superoxide production of neutrophil cells is also capable of PtdIns(4,5)P$_2$ hydrolysis (1323). The intracellular parasite, *Plasmodium falciparum* that causes malaria also produces its own inositol lipid kinases, a class III PI3K ortholog PfPI3K (1533, 1596), a PI4K ortholog PFE0485w (826), and a PIP 5-kinase (864) and also in- 

PI3K ortholog PfPI3K (1533, 1596), a PI4K ortholog PFE0485w (826), and a PIP 5-kinase (864) and also increases the levels of several inositol lipid isomers in the infected red blood cells (399, 1533). A plasmidium PLC enzyme has also been described (1245), and activation of Ca$^{2+}$ signaling within the parasite has also been observed (37). *Mycobacterium tuberculosis* uses its own tricks to stay dormant in macrophages and evade the lysosome. It uses its own PtdIns-mannoside to inhibit PtdIns3P production (256) but also secretes a phosphatase, called SapM capable of dephosphorylating PtdIns3P (1630). Another phosphatase, MpptB, which is an important virulence factor of *Mycobacterium tuberculosis* (1411), is a trifunctional enzyme that hydrolyzes phosphoserine/threonines, phosphotyrosines, and phosphoinositides (120, 121). While inhibiting PtdIns3P production of the host, *Mycobacteria* also synthesize PtdIns3P (1085), apparently as an intermediate of PtdIns synthesis and not as a result of PtdIns phosphorylation (1084).

A lot more details about these processes can be found in several recent reviews (290, 613, 1204). Nevertheless, even this short summary makes it clear that pharmacological targeting of phosphoinositide metabolizing enzymes could be beneficial in fighting a variety of infectious agents and, indeed, has already attracted significant attention (38, 144, 243).

**XII. CONCLUDING REMARKS**

Even a lengthy review like this is unable to cover all of what has been learned about phosphoinositides over the last 50 years. It is clear that these lipids are fundamental in the regulation of all aspects of membrane dynamics in eukaryotic cells. However, it is also clear that we do not have a clear understanding of the overall underlying principles of how these lipids work and why we run into them wherever we look in biological research and medicine. The arrival of such a fundamental understanding will be marked when a single short paper can summarize much of what is covered here in hundreds of pages. From an evolutionary and teleological standpoint, phosphorylation of lipids (as well as proteins) is a way for simple organisms to store phosphate, a uniquely important commodity, whether a component of DNA or of the energy currency ATP. The inositol ring is a very useful structure for this purpose as it can take several phosphate molecules, especially in its soluble form, and indeed, it has been recognized as a phosphate storage molecule in seeds (1246). Phosphorylated inositides, therefore, must have served as signals to indicate the phosphate status of simple organisms and molecules capable of recognizing the phosphorylated headgroups must have developed to communicate this information to the processes that responded to the availability of phosphate from external sources. These primitive information circuits have evolved and been utilized in a number of new ways creating the seven inositol lipid isomers, produced by the multiple kinases and phosphatases and talking to the myriad of effector molecules. The permutation of these elements provides ample variations to serve the signaling needs of a variety of cellular processes discussed in this review.

As in any other scientific discipline, the progress in inositide research has been tightly linked with methodological advances. Much of what was learned from the metabolic studies using radioactive tracers and bulk lipid separation techniques paved the road for subsequent work on isolation and cloning of the enzymes. Progress in molecular biology techniques and the sequencing of the various genomes gave an enormous boost to these efforts and helped identify many of the inositol converting enzymes known today. Advances in genetics and positional cloning identified enzyme defects in animal models for better understanding the physiological and pathological roles of these lipids and their selected metabolic enzymes. As we understand more and more about the importance of spatial and temporal organization of inositol lipid signals, it has become clear that we needed to follow lipid changes in live cells with subcellular resolution (94). Moreover, now we want to artificially induce inositol lipid changes in localized compartments to see the consequences for specific cellular processes (437, 1489, 1586, 1617). These techniques are now being extended to achieve super-resolution level imaging (141, 917). These advances will surely help us better understand the biology of phosphoinositides.
Lastly, a word about how public health has benefitted from phosphoinositide research. As we look back on the long road that has been traveled from the first description of phosphoinositides in the brain to the PI3K inhibitors that are currently in clinical trials as anti-cancer or anti-inflammatory drugs, we cannot help but recognize the principles of how humankind benefits from basic research. It is quite unlikely that those early scientists thought about the “usefulness” of their research for immediately palpable results to improve human conditions. They were driven primarily by scientific curiosity and, sadly, might not have been funded in the current milieu of short-sighted demand for foreseeable immediate return by funding agencies. The impact of their research was not predictable, and that is the essence of basic research. And yet, wanted or not, the moments have come when those efforts, pieced together, outlined a larger picture that had tremendous relevance to human physiology and disease. Miraculously, without much organized intervention, the Pharma industry reacted and a translational aspect of phosphoinositide research has naturally evolved, yielding drugs that may see the market really soon, and this process is being extended to other inositides and diseases. We can only hope that these examples will serve as reminders that high basic quality basic research is worth pursuing and funding even when the outcome is less than predictable or exactly because of its unpredictability.

ACKNOWLEDGMENTS

I thank Dr. Robert Michell for his kind reading and suggestions on the historical overview and Drs. Tibor Rohacs and Gerald Hammond for their valuable comments. I am grateful to Anna Mezey-Brownstein for proofreading the article.

Address for reprint requests and other correspondence: T. Balla, National Institutes of Health, Bldg. 49, Rm. 5A22, 49 Convent Dr., Bethesda, MD 20892 (e-mail: ballat@mail.nih.gov).

GRANTS

The author’s research is supported by the Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

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