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Cell, Vol. 100, 603–606, March 17, 2000, Copyright ©2000 by Cell Press

PIP2 and PIP3: Complex Roles at the Cell Surface

Minireview

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Phosphatidylinositol (4,5)-bisphosphate (PIP2) and phosphatidylinositol (3,4,5)-trisphosphate (PIP3) represent less than 1% of membrane phospholipids, yet they function in a remarkable number of crucial cellular processes. These low-abundance polyphosphoinositides direct two major independent signaling cascades. PIP3 is the effector of multiple downstream targets of the phosphoinositide 3 kinase (PI3K) pathway (Rameh and Cantley, 1999); PIP2 is the precursor of the mediators diacylglycerol and inositol(1,4,5)P3 following its hydrolysis by hormone-sensitive phospholipase C (PLC) enzymes. New experiments are now revealing yet another signaling mode controlled by PIP2 (Toker, 1998; Honda et al., 1999; Raucher et al., 2000). This novel cascade depends on intact PIP2 rather than products of its hydrolysis. Recent work demonstrates not only new signaling functions of PIP2 but also intricate regulation of membrane phospholipids. New insights on these events highlight the cell surface membrane as a major site of action of both PIP2 and PIP3 and reveal unexpected cross-talk between these polyphosphoinositides.

PIP3 and other PI3K products control many processes at the plasma membrane, including phagocytosis, pinocytosis, regulated exocytosis, and cytoskeletal organization. Recent studies also implicate PIP2 in an impressive number of key cell surface events (Table 1). These include fundamental processes in membrane trafficking and plasma membrane-cytoskeleton linkages. Gelsolin, profilin, and other key regulators of the actin cytoskeleton associate with PIP2 through defined basic residues within their sequences (Toker, 1998). Recent results also reveal a role for PIP2 in binding and activating proteins that connect the plasma membrane to the actin cytoskeleton. A role for PIP2 in membrane ruffling requiring actin reorganization is also apparent (Honda et al., 1999). Several proteins that regulate the formation and function of clathrin-coated vesicles in endocytosis, including the AP-2 adaptor, AP180, dynamin, and synaptotagmin, also bind PIP2. Synaptojanin-1, a PIP2 phosphatase, is a constituent of clathrin-coated membranes and is required for normal endocytosis and recycling of synaptic vesicles (Cremona et al., 1999). Regulated exocytosis is also PIP2 dependent, evidenced by the requirements for enzymes that direct its synthesis in the priming/fusion step at the plasma membrane (Loyet et al., 1998).

Acute Regulation of PIP2 and PIP3 Levels in the Plasma Membrane

Until recently, direct visualization of PIP2 and PIP3 at the cell surface has been difficult. Conclusions about plasma membrane PIP3 levels have been inferred from movements of the PI3K rather than PIP3 itself. Now, effective reagents for localization of PIP2 and PIP3 within cells have become available with the discovery of pleckstrin homology (PH) domains that bind selectively to these phosphoinositides (Kavran et al., 1998). PH domains have been identified in over 100 proteins, many of which are involved in regulating the actin cytoskeleton and signaling events at the plasma membrane. PH domains are about 120 amino acid residues long, contain an invariant tryptophan, and form a structure consisting of seven beta sheets with connecting loops, representing a superfamily or superfold that also includes ligand-binding protein domains with little sequence similarity. In a survey of PH domains, most were found to bind phosphoinositides with high affinity but low selectivity (Kavran et al., 1998). Two exceptions were found; the PH domain of PLCδ and the PH domain of the ARF protein exchange factor GRP1, which bind, respectively, PIP2 and PIP3 with high affinity and selectivity. These PH domains have become powerful probes for determining the subcellular localization of these lipids.

In order to selectively recognize PIP3 in living cells, a PH domain must exhibit an affinity for PIP3 at least one to two orders of magnitude higher than for PIP2, because, even in stimulated cells, the abundance of PIP2 is much greater than PIP3. This requirement is uniquely satisfied by the PH domain of GRP1, which exhibits an affinity for PIP3 that is two to three orders of magnitude greater than for PIP2 (Kavran et al., 1998). Importantly, the GRP1 PH domain, unlike other PH domains that bind PIP3 with high affinity, also displays a lower affinity for the other PI3K products PtdIns(3)P and PtdIns(3,4)P2. This isolated PH domain, epitope tagged or fused with GFP, acutely localizes in ruffles of the cell surface membrane upon growth factor stimulation of PI3K (Gray et al., 1999; Langille et al., 1999). Recruitment of the GRP1 PH domain to the plasma membrane is blocked by inhibitors of PI3K, consistent with its binding to PIP3. The PI3K product PtdIns(3,4)P2 also resides in the plasma membrane (Gray et al., 1999). Importantly, these results documenting plasma membrane localization of PIP3 contrasts with the apparent restriction of PtdIns(3)P to endosomes, where it may regulate endosome fusion or motility (Lawe et al., 2000). Thus, mechanisms that confine these unique 3′phosphoinositides to specific subcellular locations are likely to play a key role in defining their cellular functions.

Is PIP3 synthesized at all cellular locations where PI3Ks associate with membranes? This seems logical but is not necessarily the case. PIP2 may not be present in all locations where PI3K resides, or the kinase could localize to sites rich in PIP3 phosphatase activity. Experiments can now be performed to determine such spatial relationships between the regulated p85/p110 type PI3K, substrate PIP2, and product PIP3. Using a GFP fusion protein of p85a as a probe, Gillham et al. (1999) demonstrate cytoplasmic distribution of PI-3K and its colocalization with vinculin at peripheral focal adhesion

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Table 1. Plasma Membrane Functions that Require PIP2 and PIP3

Function	Phosphoinositide	Possible Mechanism
Membrane Trafficking		
Endocytosis	PIP2	Recruitment of AP2 to membrane initiating clathrin coating
	PIP2	Uncoating of clathrin-coated vesicles, through synaptojanin-1-mediated PIP2 hydrolysis
Regulated exocytosis	PIP2	Recruitment of CAP protein to sites of vesicle fusion
Membrane/Cytoskeletal Interface		
Micovilli formation	PIP2	Activation of ERM proteins
Membrane attachment to cytoskeleton	PIP2	Binding to gelsolin, profilin, other actin regulator proteins
Phagocytosis	PIP2/PIP3	Regulation of ARF6, PLD and actin assembly
Cell Signaling		
Protein kinase localization and activation	PIP2/PIP3	Localization of PDK1 and PKB/Akt
	PtdIns(3,4)P2/PIP3	Regulated localization of Btk tyrosine kinase
Regulation of ARF GTPases	PIP2/PIP3	Localization of ARF6 exchange factors
	PIP3	ARFGAP localization
	PIP2	Activation of ARF GAP
EGFR regulation of membrane ruffling	PIP2	Actin reorganization

complexes in several unstimulated cell types. Interestingly, PIP3 has been detected at cell–matrix and cell–cell contacts but not in focal adhesions of unstimulated cells using the GRP1 PH domain as a probe, suggesting uncoupling of PI3K localization and PIP3 generation. One possible explanation for this is the sequestration of PI3K substrate PIP2 away from these plasma membrane regions. Specialized lipid domains, or rafts, rich in glycosphingolipids and cholesterol within plasma membranes have been reported to concentrate PIP2, possibly accounting for half of the total PIP2 at the cell surface (Pike and Miller, 1998). Thus, PIP2 may be sequestered away from some pools of PI3K in the plasma membrane. Such sequestration could occur through localized generation of PIP2 or through its binding to organized protein clusters. Further studies are required to test these hypotheses.

In contrast to studies using unstimulated cells, PIP3 does seem to be present at sites to which PI3K is recruited in stimulated cells. Rapid recruitment of p85 to plasma membrane patches in response to epidermal growth factor (EGF) stimulation was observed, coincident with expressed EGF/erbB3 receptor chimera localization (Gillham et al., 1999). Direct association of the SH2 domains of p85 with membrane protein phosphotyrosines probably explains this phenomena (Fruman et al., 1998). These data demonstrate that visualization of the recruitment of p85 to plasma membrane receptors in live cells is now feasible. Future studies designed to define the spatial relationships between PI3K, PIP2, and PIP3 in the same cells will be extremely interesting.

The ability to measure PIP3 levels directly at the plasma membrane of both fixed and live cells has already clarified an important paradox in PI3K signaling. Both insulin and platelet derived growth factor (PDGF) cause equivalent recruitment of p85/p110-type PI3Ks to membrane-bound phosphotyrosine containing proteins in 3T3-L1 adipocytes, but only insulin causes activation of downstream targets of PI3K products such as protein kinase B/Akt and regulated exocytosis of GLUT4 glucose transporters. Using the expressed GFP fusion protein of the GRP1 PH domain as probe, it was revealed that insulin but not PDGF acutely elevates PIP3 levels in the plasma membrane of these cells (Oatey et al., 1999). These new data suggest that PIP3 synthesis can be controlled at a step distal to the recruitment of PI3Ks to protein phosphotyrosines by an unknown mechanism.

Just as the PH domain of GRP1 allows visualization of PIP3, PIP2 can now be visualized using the PH domain of PLCδ. A GFP fusion protein of this PH domain localizes to plasma membranes in the basal state and is released into the cytoplasm upon hydrolysis of intracellular PIP2 in response to the calcium ionophore ionomycin (Varnai and Balla, 1998). Minutes after washout of ionomycin, the PLCδ PH domain concentrates in the golgi, where de novo synthesis of PIP2 is thought to occur. This localization shifts back to the plasma membrane with time. Importantly, stimulation of endogenous angiotensin II receptors in primary cultures of bovine adrenal glomerulosa cells also transiently releases the PIP2 probe into the cytoplasm, coincident with the rapid decline of total cellular levels of PIP2 assayed by biochemical methods. Similar studies performed with rat basophilic leukemia cells transfected with platelet activation factor (PAF) receptors also suggest rapid ablation of plasma membrane PIP2 upon receptor stimulation (Stauffer et al., 1998; movies of these PIP2 dynamics are available at http://biomednet.com/elecref/0960982200800343). Taken together, these data demonstrate visualization of the phospholipase C–sensitve pool of PIP2 at the plasma membrane of cells can be accomplished with the PLCδ PH domain as a probe.

Some caveats are apparent in the use of PH domains or anti-PIP2 antibodies (Honda et al., 1999) as detectors of PIP2 and PIP3 in intact cells. For example, competition by endogenous proteins for binding to these phosphoinositides could severely limit the detection sensitivity. Complicating this further are possible changes in expression or activity of such endogenous proteins during an experiment. Another potential problem is binding of inositol(1,4,5)P3, released through the phospholipase C reaction, to the PLCδ PH domain, dampening its binding to PIP2. Thus, further development of methods used

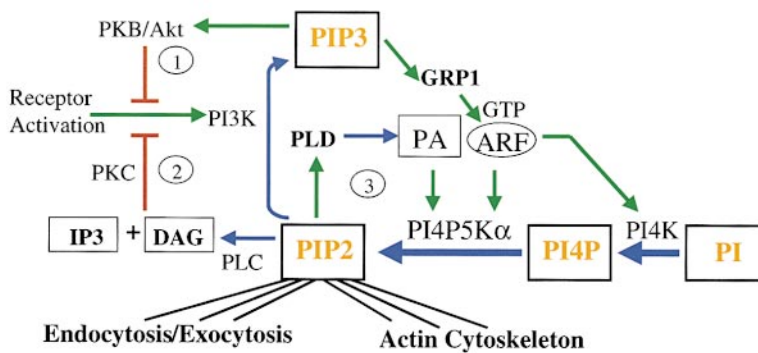

Figure 1. Regulation of PIP2 and PIP3 Synthesis

Green arrows denote stimulatory effects; blue arrows denote synthetic pathways; red denotes inhibitory effect. Feedback inhibitory loop (1). Cross-talk between receptor signaling pathways (2). Feed-forward loop (3).

to detect PIP2 and PIP3 in intact cells would clearly be useful.

PIP2 as a Signaling Molecule

Using the technology described above that confirmed rapid breakdown of plasma membrane PIP2 by PLCβ in response to angiotensin II and PAF, other studies have found acutely elevated plasma membrane PIP2 levels in HeLa cells in response to EGF (Honda et al., 1999). This activity of EGF apparently occurs through enhanced synthesis of PIP2. Figure 1 illustrates a hypothetical basis of these findings as well as other recently identified pathways whereby PIP2 and PIP3 levels in the cell surface membrane are modulated. PIP2 is synthesized from PI4P by PI4P 5 kinases α and β (Fruman et al., 1998). A second, less well-studied pathway of PIP2 synthesis (data not shown in Figure 1) involves phosphorylation of PI5P on the 4′ position by PIP4Kα and β. The PI4P 5 kinases appear to be somewhat promiscuous in terms of substrate specificity, but they have preference for PI4P. PI4P 5 kinase is activated by phosphatidic acid, a product of phospholipase D. PI4P 5 kinase α is also activated by the small GTPase ARF6 in a GTP- and phosphatidic acid-dependent manner (Honda et al., 1999). The small GTPase Rac1 may also play a role in the regulation of PI4P 5 kinase. Interestingly, ARF proteins have also recently been implicated in the activation of PI4 kinase, which produces the PIP2 precursor PI4P (Godi et al., 1999). Taken together, these findings show concerted actions of ARF proteins to enhance the two sequential synthetic reactions for PIP2 synthesis from PI.

The requirements for both phosphatidic acid and ARF:GTP complexes to generate PIP2 acutely through modulation of PI4P 5 kinase suggests the potential for an explosive feed-forward regulatory loop driven by PIP2 itself. First, PIP2 is a positive regulator of phospholipase D and acts synergistically with activated ARF on this enzyme (Figure 1). Thus, elevated PIP2 acutely generates increased phosphatidic acid, further stimulating PI4P 5 kinase to produce PIP2. Second, PIP2, like PIP3, may itself activate ARF proteins by virtue of its recruitment of ARF exchange factors, many of which have PH domains. GTP loading of ARF proteins under the influence of PIP2 thus further stimulates PI4P 5 kinase, producing more PIP2. Recent results also suggest PIP3- and PIP2-mediated attenuation of this process in that the PH domain of ARFGAP centaurin-α1 is recruited to the plasma membrane by a PI3K-dependent pathway while the ASAP1 ARFGAP is stimulated by PIP2 (Brown et al., 1998; Venkateswarlu et al., 1999).

The acute increase in cell surface PIP2 due to EGF action is displayed as enhanced localization of the PLCδ PH domain in membrane ruffles (Honda et al., 1999). EGF action also concentrates ARF6, PI4P 5 kinase, and phospholipase D2 in these same plasma membrane regions, consistent with engagement of the mechanisms described above for rapid PIP2 synthesis. ARF6 appears necessary for EGF-mediated PI4P 5 kinase recruitment to the cell surface. Conversely, dominant inhibitory ARF6 (N122I) blocks recruitment of PI4P 5 kinase and localization of PIP2 in membrane ruffles, suggesting ARF6 acts between EGF receptor and PI4P 5 kinase. A simple explanation of these data is that an ARF6 exchange activity is enhanced through the action of the EGF receptor tyrosine kinase, consistent with the recent demonstration that the ARF6 exchange factor GRP1 is recruited to membrane ruffles by this growth factor (Langille et al., 1999). Importantly, in this latter study, association of GRP1 with membrane ruffles of cells treated with EGF is blocked by PI3K inhibition, suggesting that ARF6 activation by EGF may be PI3K dependent (Langille et al., 1999). These considerations theoretically connect PIP3 generation to GTP loading of ARF6, and to acute, localized PIP2 synthesis through activation of PI4P 5 kinase. Receptor activation of PI3Ks may therefore lead to a coincident rise in both PIP3 and PIP2 levels that are localized to specific plasma membrane regions, while total cellular PIP2 levels do not detectably change.

While both PIP3 and PIP2 may promote PIP2 synthesis as described above, both PIP2 and PIP3 connect to pathways that tend to inhibit PI3K activation (Figure 1). PIP3 and PtdIns(3,4)P2 activate protein kinase B/Akt, which negatively regulates at least one receptor tyrosine kinase substrate that in turn desensitizes PI3K (Li et al., 1999). Similarly, diacylglycerol formed from the hydrolysis of PIP2 activates protein kinase C, which can also inhibit PI3K activation. This latter pathway represents receptor cross-talk that may coordinate cell responses to hormones with antagonistic actions.

Within the last 3 months, exciting data supporting a signaling role for PIP2 in controlling cortical plasma membrane-cytoskeleton structure have been reported (Raucher et al., 2000). In these studies, the adhesion energy between the plasma membrane and underlying cytoskeleton was measured using tether force measurements with optical tweezers. A thin membrane tether is

pulled from the cell surface with an attached IgG-coated polystyrene bead and the force on the bead determined. Sequestration of plasma membrane PIP2 in NIH-3T3 fibroblasts by the expressed PLCδ PH domain dramatically lowered the plasma membrane-cytoskeletal adhesion energy. Expression of a membrane targeted construct of the yeast Inp54p PIP2-specific 5′phosphatase similarly reduced adhesion energy. Importantly, activation of either PLC by PAF or PDGF also decreased the plasma membrane-cytoskeletal adhesion energy. These observations reveal a physiological mechanism driven by PIP2 that dramatically controls membrane dynamics.

How does PIP2 control connections between the plasma membrane and the cytoskeleton? Direct interactions between PIP2 molecules on the inner leaflet of the plasma membrane and anchoring proteins of the actin filament network is theoretically possible. However, indirect regulation of cortical actin by PIP2 was suggested because reduced total cellular polymerized F-actin is found upon expression of the PIP2 5′phosphatase (Raucher et al, 2000). Further, the actin polymerization drug jasplakinolide increases adhesion energy in these experiments, while cytochalasin D has the opposite effect. Taken together, these studies provide a model for PIP2 regulation of cell shape in which localized separation of the plasma membrane from the cytoskeleton occurs when PIP2 and adhesion energy is lowered, leading to the formation of membrane blebs.

The above studies have broad implications for many cell surface functions that require shape changes such as cell motility and endocytosis. For example, PIP2 is required for clathrin-coated vesicle function through regulation of the adaptor protein AP-2 (Gaidarov and Keen, 1999). Consistent with this concept are recent studies showing that gene ablation of mouse synaptojanin-1—a protein enriched in neurons that contains a central PIP2 and PIP3 5′ phosphatase domain and an NH 2-terminal domain that can hydrolyze PI(3)P, PI(4)P, and PI(3,5)P2—causes increased clathrin-coated vesicles in nerve endings (Cremona et al., 1999). Thus, uncoating of these vesicles appears to require PIP2 hydrolysis. What is particularly intriguing here is the potential connection between these PIP2 functions in neuronal endocytosis and the actin cytoskeleton. Actin is strongly implicated in endocytosis and enriched at active sites of clathrin-coated membrane internalization in neurons. Increased PIP2 levels in the absence of synaptojanin-1 may mobilize assembled actin that interacts with coated vesicles and contributes to their abnormal function.

Based on the work reviewed here, PIP2 has emerged as a highly versatile signaling molecule in its own right. It has the hallmarks of a true signaling intermediate—its levels are acutely upregulated or downregulated by hormones and it serves as an effector of multiple downstream proteins. It deservedly takes its place along with the polyphosphinositide products of PI3K as a key regulator of cell surface membrane function. PIP2 likely plays major roles in a variety of functions at other intracellular locations as well. Major challenges include how to investigate the potential role of membrane microdomains in PIP2 and PIP3 actions and how the functions of the proteins that interact with these lipids are coordinated. Further details of the interrelationships between PIP2 and PIP3, and of the cell surface functions that PIP2 and PIP3 execute, should surely command our continued attention.

Selected Reading

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