PIP3 and PIP2: Complex Roles at the Cell Surface

Phosphatidylinositol (4,5)-bisphosphate (PIP2) and phosphatidylinositol (3,4,5)-trisphosphate (PIP3) are low-abundance membrane phospholipids that function in a number of crucial cellular processes, like membrane trafficking, plasma membrane-cytoskeleton linkages, second messenger signaling, cell adhesion and motility and regulation of proteins involved in phospholipid metabolism (Table1). These phosphoinositides direct two major independent signaling cascades. PIP3 is the effector of multiple downstream targets of the phosphoinositide 3 kinase (PI3K) pathway. Activation of PI3K by growth factor stimulation of cells results in PIP3 synthesis generated by phosphorylation of PIP2. PIP2 is the precursor of the second messengers in cellular signaling – Ca^{2+}-mobilizing messenger inositol (1,4,5)-trisphosphate (IP3), and the protein kinase C (PKC) activator diacylglycerol (DAG).

Table 1. Plasma membrane functions that require PIP2 and PIP3.

	Function	Phosphoinositide
Membrane Trafficking	Endocytosis	PIP2
	Regulated exocytosis	PIP2
Membrane/Cytoskeletal Interface	Microvilli formation	PIP2
	Membrane attachment to cytoskeleton	PIP2
	Phagocytosis	PIP2/ PIP3
Cell Signaling	Protein kinase localization and activation	PIP2 / PIP3
	Regulation of ARF GTPases	PIP2 / PIP3
	EGFR regulation of membrane ruffling	PIP2

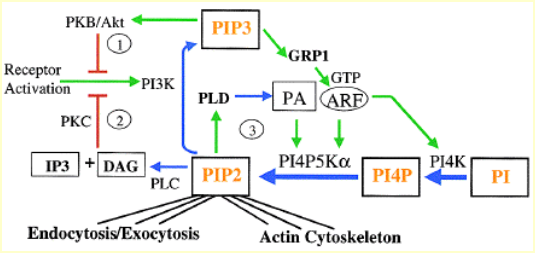
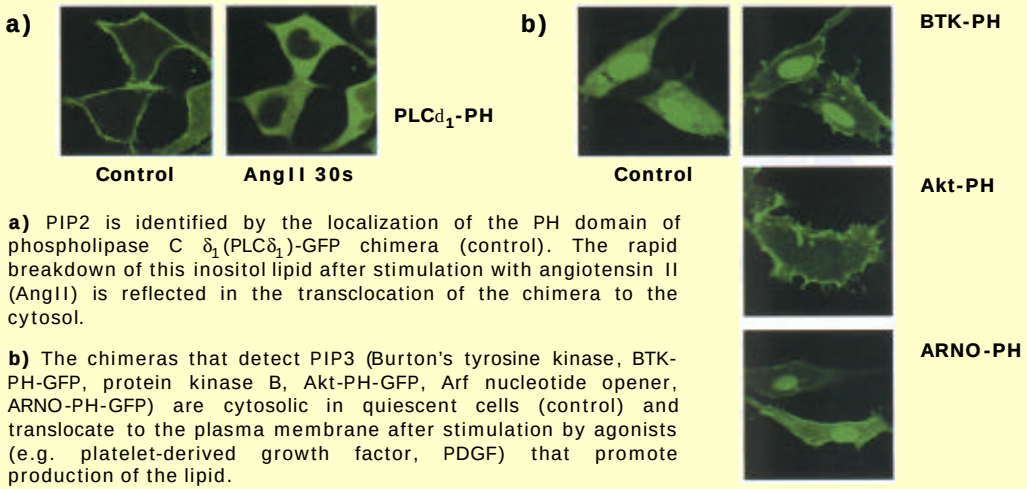


Fig. 2. Regulation of PIP2 and PIP3 synthesis.
PIP2 is synthesized from phosphatidylinositol-4-phosphate (PI4P) by PI4P 5 kinase. PI4P 5 kinase is activated by phosphatidic acid (PA), a product of phospholipase D, and the small GTPase Arf which also activates PI4 kinase. Both PIP3 and PIP2 promote PIP2 synthesis and they both connect to pathways that inhibit PI3K activation. Green arrows denote stimulatory effects; blue arrows denote synthetic pathways; red denotes inhibitory effect.

Fig.1. Visualization of phosphoinositides by protein domain-GFP chimeras in live cells.
Fusion constructs between a protein domain, such as pleckstrin homology (PH) domain, that mediate the interactions with various inositol lipids, and green fluorescent protein (GFP) represent novel tools to identify inositol lipids at their cellular sites of production and permit their dynamic imaging in living cells under a confocal microscope.



a) PIP2 is identified by the localization of the PH domain of phospholipase C δ_1 ($PLC\delta_1$)-GFP chimera (control). The rapid breakdown of this inositol lipid after stimulation with angiotensin II (AngII) is reflected in the transclocation of the chimera to the cytosol.

b) The chimeras that detect PIP3 (Burton's tyrosine kinase, BTK-PH-GFP, protein kinase B, Akt-PH-GFP, Arf nucleotide opener, ARNO-PH-GFP) are cytosolic in quiescent cells (control) and translocate to the plasma membrane after stimulation by agonists (e.g. platelet-derived growth factor, PDGF) that promote production of the lipid.

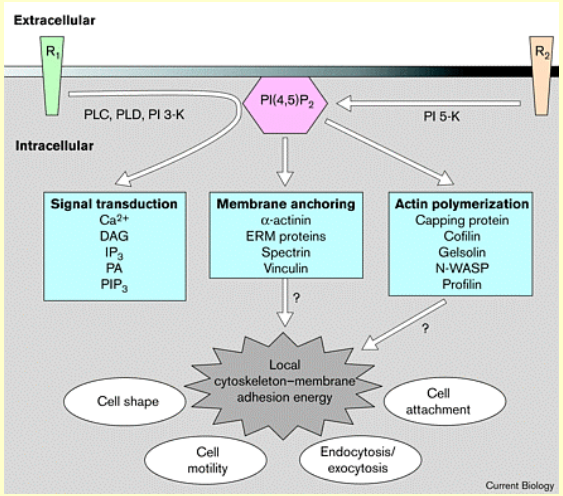


Fig. 3. How does PIP2 regulate cytoskeleton-membrane interactions in living cells?
Receptor stimuli can increase local PIP2 concentrations by activating PI5K (R2) or they can decrease local PIP2 concentration by triggering posphatidylinositide-specific phspholipase C (PLC), phospholipase D (PLD) and PI3K signaling cascades (R1). Local cytoskeleton-membrane adhesion energy controls cell shape, motility, membrane transport and attachment to the extracellular matrix and other cells.

Concluding Remarks

PIP2 along with the phosphoinositol products of PI3K are key regulators of cell surface membrane function. PIP2 has the hallmarks of a true signaling molecule-its levels are upregulated or downregulated by hormons and it serves as an effector of multiple downstream proteins. It has also been shown that PIP2 functions as a second messenger in the control of cytoskeleton-membrane adhesion. It remains to be determined how PIP2 levels are spatially and temporally regulated within the cell, and whether other PIP2-mediated cytoskeleton-membrane interactions are involved in structural transients at the cell surface.

A. Demirovic M. P. Czech, Cell **100**, 603-606 (2000); T. Balla et al., TIPS **21**, 238-241 (2000) ; T. Nebl et al., Current Biology **10**, R351-R354 (2000)