

# Phosphoinositide Kinases

## Key References

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## Overview

The membranes of all metazoan cells contain a substantial amount of phosphatidylinositol (PtdIns) and at least seven phosphorylated PtdIns derivatives (PPI)n at 30- to 1000-fold lower concentrations. Phosphoinositide kinases synthesize the various PPI)n by adding phosphate groups to pre-existing inositol glycerophospholipids (see Figure below).

The kinase domains of the two PtdIns 4-kinase subfamilies, the various subfamilies of phosphoinositide 3-kinase (PI3K) and protein kinases are closely related. By contrast, the three phosphatidylinositol phosphate kinases (PIPkins) constitute a unique kinase family (though sharing some kinase domain motifs with other kinases), with each donating a phosphate to only one of the three isomeric PtdInsPs.

PtdIns(4,5)P<sub>2</sub> is a key phosphoinositide. It is the shared substrate of two widespread receptor-activated signaling pathways – the phospholipase C-catalyzed formation of Ins(1,4,5)P<sub>3</sub> and 1,2-diacylglycerol (DAG), and synthesis of the membrane-associated messenger molecule PtdIns(3,4,5)P<sub>3</sub> by Type I PI3Ks – and is also essential for a substantial number of other cell functions, including actin-based motility, secretory vesicle trafficking, exocytosis and phospholipase D activation.

Four kinases contribute to PtdIns(4,5)P<sub>2</sub> biosynthesis. Most is made, via PtdIns4P, by a PI4K and PIPkin-1 at the plasma membrane, secretory vesicles, Golgi and nuclei (and probably elsewhere). Cells also use another pathway, involving an uncharacterized PtdIns 5-kinase and PIPkin-II, to make a small proportion of cellular PtdIns(4,5)P<sub>2</sub> via PtdIns5P. Reasons for the coexistence of these two pathways remain uncer-

tain. There is evidence for partial nuclear localization of PIPkin-I and PIPkin-II, and some PtdIns(4,5)P<sub>2</sub> is made and undoubtedly functions in the nucleus – but how it is made and what it does remain unclear. The individual PI3K subfamilies phosphorylate different phosphoinositides: the functions of Type II PI3Ks remain uncertain. Type III PI3Ks (PI3K-IIIs) convert PtdIns to PtdIns3P. This lipid is essential for vesicle trafficking from the trans-Golgi to vacuole/lysosome compartments. It exerts most or all of its effects through interactions with secretory/endosomal target proteins that include either PtdIns3P-specific FYVE domains and/or phox (or PX) domains (see Table). This PtdIns3P is also the substrate for the synthesis by PIPkin-IIIs of PtdIns(3,5)P<sub>2</sub>. Type I PI3Ks (PI3K-Is) phosphorylate PtdIns(4,5)P<sub>2</sub> to PtdIns(3,4,5)P<sub>3</sub> and thereby transmit signals from extracellular stimuli. PI3K-IAs are mainly activated by receptor tyrosine kinases (e.g. the insulin receptor and many growth factor receptors), and PI3K-IBs by some G protein-coupled receptors (particularly, in hemopoietic cells, by receptors that influence motility and the bactericidal oxidative burst of neutrophils). PtdIns(3,4,5)P<sub>3</sub> is a remarkable membrane-associated second messenger molecule. It appears to have many direct target proteins, each of which it interacts with through highly PtdIns(3,4,5)P<sub>3</sub>-selective pleckstrin homology (PH) domains. Some of these are protein kinases (e.g. PDK1, PKB, Btk, Itk), and others include regulators of small GTPases (GTP/GDP exchange factors and GTPase-activating factors). The PtdIns(3,4,5)P<sub>3</sub>-activated protein kinases have numerous substrates, and they often initiate downstream protein kinase cascades.

In addition, there is a diverse group of 'Type IV PI3K-related kinases' (or PIKKs) – com-

prising Tor (target of rapamycin) proteins, DNA-dependent protein kinase (DNA-PK) and ATM (Ataxia Telangiectasia Mutated). Although their kinase domains are related to those of bona fide PI3Ks, they are probably protein kinases.

The cellular regulation of cellular phosphoinositide levels, and of the sizes of the phosphoinositide pools at particular intracellular sites, are as dependent on the activities of the diverse PPI)n phosphatases as on the kinases discussed above.

# Phosphoinositide Kinases

PHOSPHOINOSITIDE KINASE	PtdIns 4-kinase (PI4K)	PtdIns4P 5-kinase (PIPkin-I)	PtdIns5P 4-kinase (PIPkin-II)	PtdIns3P 5-kinase (PIPkin-III)
REACTION <i>IN VIVO</i>	PtdIns ( <b>P8443</b> , <b>P2517</b> ) > PtdIns4P ( <b>P9638</b> )	PtdIns4P ( <b>P9638</b> ) > PtdIns(4,5)P <sub>2</sub> ( <b>P9763</b> )	PtdIns5P > PtdIns(4,5)P <sub>2</sub> ( <b>P9763</b> )	PtdIns3P > PtdIns(3,5)P <sub>2</sub> ( <b>P9763</b> )
STRUCTURE	Two subfamilies: Type II: 100-230 kDa (some have SH3 and PH) – inc. yeast Pik1p and Stt2p Type III: ~50-70 kDa – inc. yeast Lsb6	~60-90 kDa: multiple forms – include yeast Mss4p	~45 kDa: multiple forms	~160-250 kDa: includes FYVE, CCP1-chaperone-like and kinase domains - prototype is yeast Fab1p
LOCATION/CONTROL/COMMENTS	Some types may be regulated by phosphorylation	Major route for PtdIns(4,5)P <sub>2</sub> synthesis. Stimulated by phosphatidate ( <b>P9511</b> )	Minor route for PtdIns(4,5)P <sub>2</sub> synthesis. May be regulated by phosphorylation	Hyperosmotic stress activates in yeast. This involves Vac7 and Vac14; not understood in animal cells
INHIBITORS	Adenosine ( <b>A9251</b> ) Wortmannin for some forms ( <b>W1628</b> )	Not known	Heparin ( <b>H3393</b> (p), <b>H0777</b> (b))	Not known
TISSUE EXPRESSION	Ubiquitous	Ubiquitous	Ubiquitous?	Ubiquitous? Abundant in adipocytes.
PHYSIOLOGICAL FUNCTION	PtdIns4P synthesis. PtdIns4P is precursor of PtdIns(4,5)P <sub>2</sub> and PtdIns(3,4,5)P <sub>3</sub> . Other functions may include involvement in protein traffic from the trans-Golgi	PtdIns(4,5)P <sub>2</sub> synthesis. Thus supports signaling, actin cytoskeleton function, exocytosis, etc.	Implicated in tissue sensitivity to insulin	Protein trafficking to and membrane recycling from lysosomes/yeast vacuoles.
DISEASE RELEVANCE	Not known	Not known	Not known	Loss of PtdIns3P/PtdIns(3,5)P <sub>2</sub> phosphatases (MTM-type) causes X-linked myotubular myopathy and Charcot-Marie-Tooth (CMT) neuropathy Types 4B1 and 4B2

## Phosphoinositide Kinase (continued)

PHOSPHOINOSITIDE KINASE	PtdIns(4,5)P <sub>2</sub> 3-kinase (PI3K-IA)	PtdIns(4,5)P <sub>2</sub> 3-kinase (PI3K-IB)	PtdIns4P 3-kinase (PI3K-II)	PtdIns 3-kinase (PI3K-III)
REACTION <i>IN VIVO</i>	PtdIns(4,5)P <sub>2</sub> ( <b>P9763</b> ) > PtdIns(3,4,5)P <sub>3</sub> (also has protein kinase activity)	PtdIns(4,5)P <sub>2</sub> ( <b>P9763</b> ) > PtdIns(3,4,5)P <sub>3</sub>	PtdIns4P ( <b>P9638</b> ) > PtdIns(3,4)P <sub>2</sub>	PtdIns ( <b>P8443</b> , <b>P2517</b> ) > PtdIns3P
STRUCTURE	p85(α or β) or p55 (α or γ regulatory subunit; p110 (α, β or δ) catalytic subunit	p101 regulatory subunit; p110γ catalytic subunit	170 kDa, C-terminal C2 domain, multiple (α,β,γ) forms	~80-110 kDa – include yeast Vps34p
LOCATION/CONTROL/COMMENTS	p85 SH2 domain interacts with P-Tyr residues, giving p85*. p110 is activated both by p85* and by activated Ras	The p101 regulatory subunit is activated by the Gβγ-complexes liberated from activated G <sub>i</sub> and/or G <sub>o</sub> . It, in turn, activates p110γ	Some tyrosine kinases may activate?	Activated via N-terminally myristoylated ~160 kDa protein serine kinase - prototype is yeast Vps15
INHIBITORS	Wortmannin ( <b>W1628</b> ), LY-294002 ( <b>L9908</b> )	Wortmannin ( <b>W1628</b> )	Not known	Not known
TISSUE EXPRESSION	αβ ubiquitous; δ mainly leukocytes	Mainly hemopoietic cells	α,β ubiquitous; γ mainly liver	Ubiquitous?

## FOOTNOTES

## Phosphoinositide Kinases

<b>PHYSIOLOGICAL FUNCTION</b>	Makes the membrane-associated 'second messenger' PtdIns(3,4,5)P <sub>3</sub> . PtdIns(3,4,5)P <sub>3</sub> interacts with multiple targets, mainly through PH domains – these include the PDK1/PKB kinase cascade, Tec family kinases, phosphoinositidase-C $\gamma$ and Arf-GEFs	Not known	Protein trafficking to and membrane recycling from lysosomes/yeast vacuoles
<b>DISEASE RELEVANCE</b>	Genetic loss of the PtdIns(3,4,5)P <sub>3</sub> 3-phosphatase PTEN predisposes to multiple cancers. PtdIns(3,4,5)P <sub>3</sub> -unresponsive mutants of Tec family kinases (e.g. Btk, Itk) cause hemopoietic and immune deficiencies	Not known	Loss of PtdIns3P/PtdIns(3,5)P <sub>2</sub> phosphatases (MTM-type) causes X-linked myotubular myopathy and Charcot-Marie-Tooth (CMT) neuropathy Types 4B1 and 4B2
	P110 $\alpha$ over-expression promotes cancer. Knockout of p110 $\delta$ or partial KO of p85 $\alpha$ curtails B-lymphocyte development and function. p110 or p85 $\alpha$ ablation kills before birth	Knockout curtails neutrophil and monocyte function, dendritic cell migration and T cell-mediated immunity, also pancreatic $\beta$ -cell function	

### Abbreviations

**PI3K:** Phosphoinositide 3-kinase

**PIK:** PtdIns kinase

**PIP:** Phosphatidylinositol phosphate

**PIPkin:** PtdInsP kinase

**PPI:** Polyphosphoinositides

**PtdIns:** Phosphatidylinositol

**PtdIns3P:** PtdIns 3-phosphate

**PtdIns4P:** PtdIns 4-phosphate PtdIns5P: PtdIns 5-phosphate

**PtdIns(3,4)P<sub>2</sub>:** PtdIns 3,4-bisphosphate

**PtdIns(3,5)P<sub>2</sub>:** PtdIns 3,5-bisphosphate

**PtdIns(4,5)P<sub>2</sub>:** PtdIns 4,5-bisphosphate

**PtdIns(3,4,5)P<sub>3</sub>:** PtdIns 3,4,5-trisphosphate

**Ins(1,4,5)P<sub>3</sub>:** Inositol 1,4,5-trisphosphate

## FOOTNOTES