11 th Calcium signaling course
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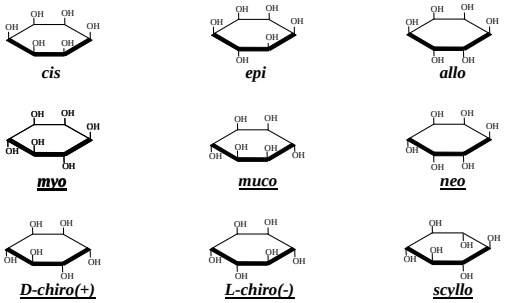
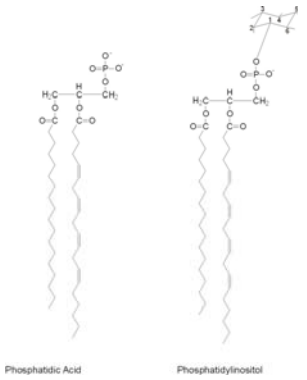
Md. Shahidul Islam, M.D., Ph.D.
Karolinska Institutet, Institutionen för klinisk forskning och utbildning, Forekningscentrum Södersjukhuset
Shaisl@ki.se

The Nine Inositol Isomers

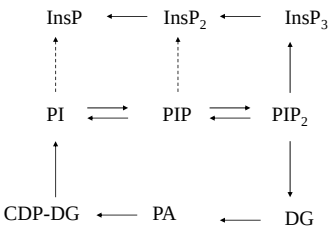




Diacylglycerol kinase

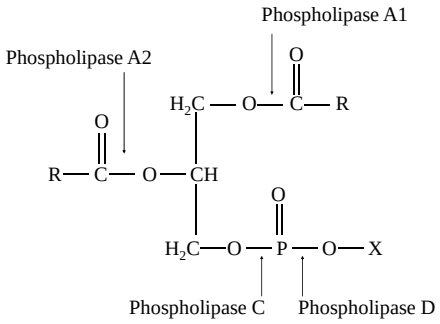
CDP-Diacylglycerol → Phosphatidylinositol

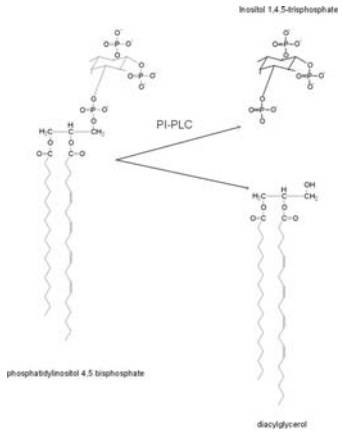
Inositol CMP

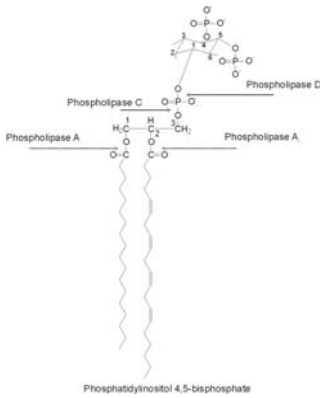


Inositol 1,4,5 trisphosphate

Cleavage sites of phospholipases





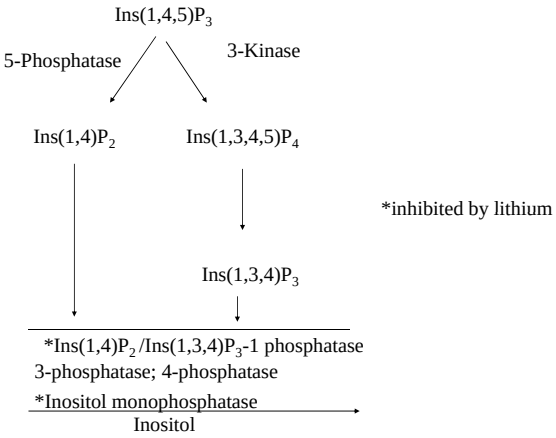


Measurement of Inositol Phospholipid Metabolism

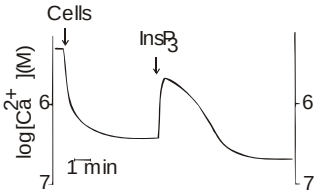
- ^{32}Pi incorporation into phospholipids
- ^{3}[H]inositol phosphate accummulation
  - Separation of ^{3}[H]inositol phosphates by Dowex anion exchange chromatography
- Measurement of mass of Ins(1,4,5)P3 by radio receptor assay

Measurement of ^{3}[H]inositol phosphate accumulation

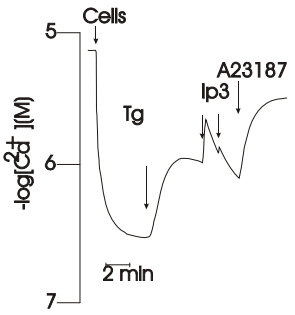
- Use inositol-free medium
  - (RPMI contain 190 μM inositol)
- Use inositol-free serum (FBS 550 μM; Horse serum 200 μM inositol)
- Add carrier inositol
- No antibiotics
- Label for prolonged period
- Use Lithium
  - Inhibits Ins-1-P/Ins(1,3,4)P_3 phosphatase and inositol monophasphatase



	On switch	Off switch
cAMP	Adenelyl cyclase	cAMP PDEs
$Ins(1,4,5)P_3$		5-Phosphatase 3-Kinase



Islam ms et al 1991, FEBS lett. 288:27



Members of PI-PLC superfamily

- Four mammalian PI-PLC β (β1-β4)
- Two PI-PLCγ (γ1 and γ2)
- Four PI-PLC δ (δ1-δ4)
- PI-PLC ε
- PLCp21 and PLCnorpA are drosophila homolog of PLCβ
- PI-PLC β regulated by G-Proteins
- PI-PLC γ regulated by tyrosine kinases
- PI-PLCδ regulated by Ca^{2+}
- PI-PLCε activated by GTP-Ras

Pleckstrin/PH domain

- Pleckstrin: platelet protein; substrate of PKC
- PH domain: about 100 amino acid module
- PH domain containing proteins

PI-PLC	Many KInases
GTPases	GTPase activating proteins
Nucleotide exchange factors	

- Many PH containing proteins interact with G-proteins and membrane phospholipids

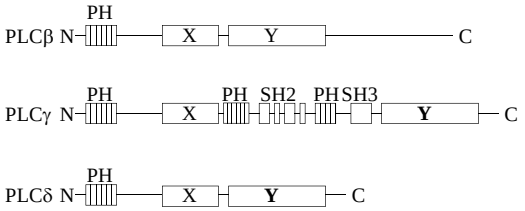
SH2 Domain

- About 100 aa domains: homology to the sequences found in nonreceptor tyrosine kinases of *src* family
- Respond to tyrosine phosphorylation by binding to the phosphorylated sequences
- On protein-protein interaction through SH2 domains, one of the protein may be relocalized

SH3 domain

- 60-85 aa stretches
- Frequently occur together with SH2 domain
- Involved in interaction with proteins containing proline-rich sequences
- SH3 domain on PLCγ may associate with actin network

Domain structure of PI-PLC family



Activation of PI-PLC β by α subunit of G protein

- alpha subunits of Gq family: Gq, G11, G14-16
- bind to C terminal part of PI-PLC: G-box
- activate mainly PI-PLC β1 and β3

Regulation of PI-PLC

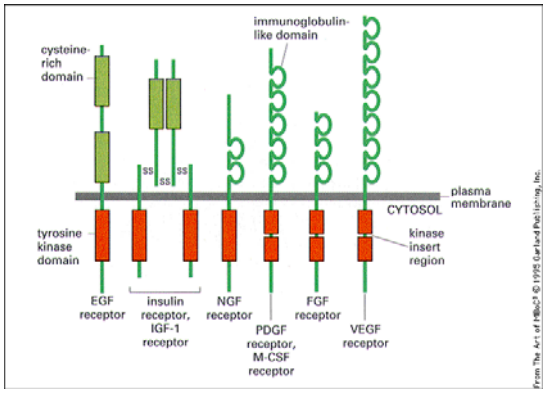
- PI-PLC β by G proteins
- PI-PLC γ by tyrosine kinase receptors
- PI-PLC δ by Ca^{2+} ?

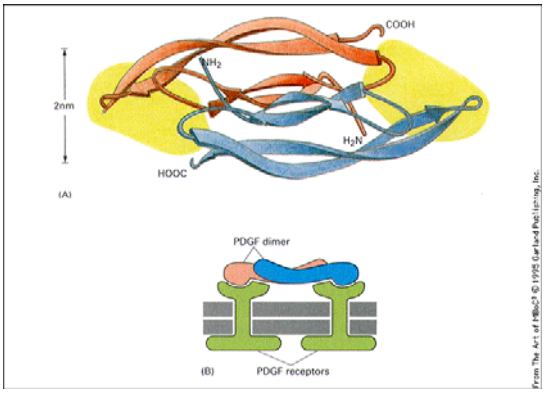
Activation of PI-PLC β by βγ subunit of G protein

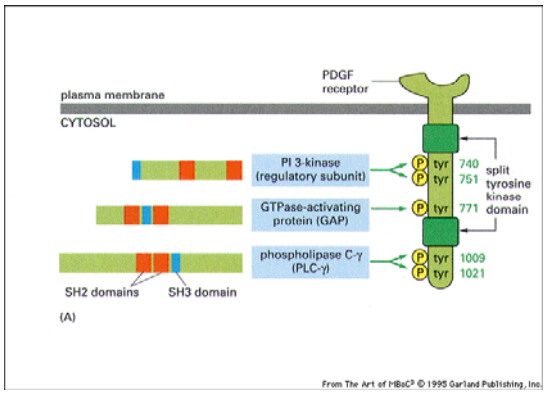
- Gβγ released from G-proteins, specially from G_1
- Binds at the N terminal part of PI-PLC (PH domain)
- Activates mainly PI-PLC β2 and β3

PI-PLC γ is activated by tyrosine phosphorylation

- Tyrosine kinase receptors e.g. PDGF
- Ligand binding, receptor dimerization
- Mutual transphosphorylation of tyrosines on the receptor
- SH2 domain of PI-PLCγ docks on to the phosphorylated tyrosines
- PI-PLCγ is phosphorylated on tyrosine residues and this activates PI-PLC







Activation of PI-PLCγ requires two events

1. Association with the tyrosine phosphorylated receptor through SH2 domain
2. Phosphorylation of specific tyrosine residues on the PI-PLCγ