

Phospholipase C (PLC) genes from *Physcomitrella patens*: Physiology and molecular biology

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Phospholipid metabolism plays an important role in signal transduction pathways of plants. We could isolate all genes of the biochemical pathway of phospholipids (Fig. 1). I will report only about Phospholipase C. PLC has the main regulatory function in the phospholipid signal cascade (1,2,3). We isolated 4 PLC-genes from *Physcomitrella patens*. PpPLC1 and PpPLC2 were cloned in full length, and PpPLC3, PpPLC4 as ESTs. The sequences of PLC1 and PLC2 show an interesting sequence difference. PLC1 and PLC2 have no PH-motive, what is typical for all PLCs so far known from plants. PpPLC2 has in contrast to PpPLC1 an EF-hand (Fig. 2). We expect different functions of both enzymes. PLC1 and PLC2 were expressed in *E. coli* and the enzyme activity determined. The intracellular distribution was visualized by GFP as reporter gene. The distribution of GFP-labelling suggests membrane association and interaction with chloroplasts. The knock-out of PpPLC1 could be performed by homologues recombination. It was no significant phenotype distinguishable, but a significantly reduced chlorophyll content in the knock-out mutant. Northern technique could be installed and expression patterns determined. We are especially interested in the phytochrome regulation, which is involved in the phototropic response of moss protonemata.

(1) Caroni, P. (2001) Actin cytoskeleton regulation through modulation of PI(4,5)P2 rafts. EMBO J. 20, 4332-4336

(2) Rhee, Sue Goo (2001) Regulation of phosphoinositide-specific phospholipase C. Ann.Rev.Biochem. 70, 281-312

(3) Hartmann, E. and Pfaffmann, H. (1990) Phosphatidyl inositols and phytochrome-mediated phototropism of moss protonemal tip cells; in: Inositol Metabolism in Plants eds. Morre, J., Boss, W.F. and Loewus, F.A., Wiley-Liss, New York Toronto, pp. 239-275

Fig.1 Genes of phospholipid metabolism isolated from *Physcomitrella patens*

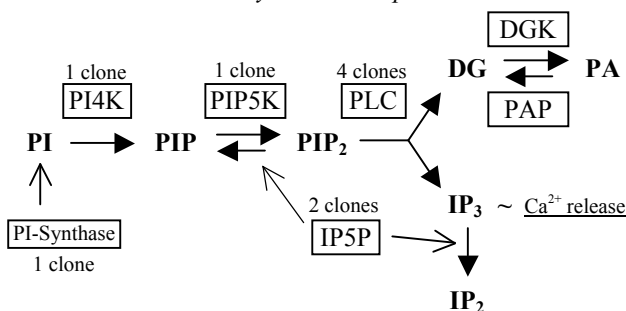


Fig. 3 GFP-Labeling of Phospholipase C (PpPLC1) from *Physcomitrella patens*

Laser scanning microscopy (LSM)

1. transmission image of regenerating protonema
2. GFP-fluorescence



Fig.2 Sequence comparisons of plant and animal PLCs

PpPLC1 of *Physcomitrella patens*
 PpPLC2 of *Physcomitrella patens*
 AtPLC1S of *Arabidopsis thaliana*
 PnPLC1□ of *Rana esculenta*

