

## Plant Gene Register

# An *Arabidopsis* cDNA Related to Animal Phosphoinositide-Specific Phospholipase C Genes<sup>1</sup>

Yuri T. Yamamoto\*, Mark A. Conkling, Ian M. Sussex, and Vivian F. Irish

Department of Biology, Yale University, New Haven, Connecticut 06520–8104 (Y.T.Y., I.M.S., V.F.I.);  
Department of Genetics, North Carolina State University, Raleigh, North Carolina 27695–7614 (Y.T.Y., M.A.C.);  
and Department of Plant Biology, University of California, Berkeley, California 94720 (I.M.S.)

We have cloned an *Arabidopsis thaliana* cDNA and corresponding genomic clones exhibiting a high degree of sequence similarity to animal PI-PLC genes (Table I).

A PCR strategy was designed to clone genes expressed in *Arabidopsis* shoots during floral induction. *Arabidopsis* plants grown under SD conditions (8 h of light/16 h of dark) were transferred to LD conditions (16 h of light/8 h of dark) to induce flowering. Shoots were harvested during the first 3 d after the transfer and divided into expanded leaves and the remainder of the shoots. Vegetative shoots were also harvested before the transfer. Poly(A)<sup>+</sup> RNA was isolated from each tissue, and the corresponding cDNAs were used as templates for PCR amplification. Degenerate primers deduced from conserved regions of *CDC25* and *SCD25* (*Saccharomyces cerevisiae*) and *ste6* (*Schizosaccharomyces pombe*) were used. A 165-bp fragment that appeared specific to the shoot without leaves during the transition to flowering was isolated and used to identify *Arabidopsis* genomic clones. Six genomic clones were recovered, and all of them contained a *Hind*III fragment of approximately 700 bp that hybridized to the PCR product. The nucleotide sequence of the *Hind*III fragment did not exhibit any similarity to the *CDC25* gene. Instead, it contained an ORF highly similar to animal PI-PLC genes.

Approximately 100,000 clones of a primary cDNA library constructed from poly(A)<sup>+</sup> RNA isolated from *Arabidopsis* shoots that had begun floral evocation were screened using the 703-bp genomic *Hind*III fragment as a probe, and one positive clone was recovered. Sequencing of the cDNA confirmed that it corresponded to the transcript from which the original PCR product was amplified, and the *Hind*III genomic fragment contained three exons of the gene. The cDNA is 1861 bp and contains an ORF of 533 amino acids and 20 A residues at the 3' end. The ORF contains regions similar to two conserved domains of PI-PLCs in animals, slime mold, and yeast (Berridge, 1993). Deletion and expression studies of mammalian PI-PLCs in *Escherichia coli* and COS cells demonstrated that these domains are essential for the catalytic activity (Bristol et al.,

**Table I.** Characteristics of the *AtPLC1* gene

|                             |   |
|-----------------------------|---|
| Organism:                   | <i>Arabidopsis thaliana</i> Landsberg <i>erecta</i> .   |
| Gene Product:               | Phosphoinositide-specific phospholipase C.  |
| Genome Location:            | Nuclear genome; chromosome location unknown.  |
| Gene Copy Number:           | Single copy.  |
| Technique:                  | The materials for PCR were collected from <i>Arabidopsis</i> plants that were transferred from SD growth conditions to LD growth conditions to induce flowering. A cDNA fragment specific to the shoots without expanded leaves harvested after the transfer was amplified using two primers, 5'-MGNYTBVR-NAARACHTGG-3', corresponding to RL(D/K/R)KTW, and 5'-TANACNCCNARRAAHGGNACRCA-3', complementary to the coding region of CVPF(L/F)GVY. The primer sequences were selected from conserved regions of the genes related to <i>CDC25</i> of <i>S. cerevisiae</i> . (Degenerate amino acids are shown in parentheses.) The ratio of the two primers in the amplification was adjusted to 4:1, respectively, because of the higher T <sub>m</sub> of the second primer. Degeneracies of these primers are 6912- and 6144-fold, respectively. The expected size of the <i>CDC25</i> -like product was about 165 bp. Subsequently, the corresponding genomic clones and a cDNA were cloned. ExonucleaseIII/nuclease S1 deletions and specific primers were used to determine the nucleotide sequences using a Sequenase kit (United States Biochemical). |
| Source:                     | A cDNA library in pBSKSII+ (Stratagene) was constructed from poly(A) <sup>+</sup> RNA from <i>Arabidopsis</i> shoots with developing flower primordia.<br>The genomic library of <i>Arabidopsis</i> Landsberg <i>erecta</i> was kindly provided by Dr. Brian Hauge.   |
| Method of Identification:   | National Center for Biotechnology Information BlastP search (Altschul et al., 1990) revealed extensive similarities between the <i>Arabidopsis</i> gene product and PI-PLC sequences of animals, slime mold, and yeast.   |
| Expression Characteristics: | The expression of <i>AtPLC1</i> is not detectable in young seedlings. Plants transferred from SD conditions to LD conditions express the gene in shoots without expanded leaves. The expression levels in whole shoots with flower primordia are approximately 0.001%.  |

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\* Corresponding author; e-mail yuri@unity.ncsu.edu; fax 1-919-515-3355.

Abbreviations: ORF, open reading frame; PI-PLC, phosphoinositide-specific phospholipase C.

1988; Emori et al., 1989). Thus, the *Arabidopsis* gene probably encodes a functional PI-PLC and was named *AtPLC1*. High- and low-stringency Southern hybridization of the *AtPLC1* cDNA to *Arabidopsis* genomic DNA revealed that this gene is single copy. The *AtPLC1* message is detectable at low levels in shoots and even lower levels in expanded leaves during the SD to LD transition period. The *AtPLC1* message could not be detected in poly(A)<sup>+</sup> RNA from 2-week-old seedlings.

Animal PI-PLCs play key roles in signal transduction by hydrolyzing phosphatidylinositol bisphosphate into two second messengers, inositol 1,4,5-trisphosphate and diacylglycerol. Inositol 1,4,5-trisphosphate induces the increase in Ca<sup>2+</sup> levels, and diacylglycerol activates protein kinase Cs. The activation of PI-PLCs requires complex interactions with various receptors and G proteins (reviewed by Berridge, 1993). In addition to the highly conserved catalytic domains, animal PI-PLCs contain divergent regulatory domains. Based on these structural differences, the PI-PLCs are classified into three groups:  $\beta$ ,  $\gamma$ , and  $\delta$  (Berridge, 1993). The  $\beta$  type interacts with G proteins, and the  $\gamma$  type interacts with Tyr kinase-type receptors. The signal transduction cascade with which the  $\delta$ -type PI-PLCs interact is not well understood. Gene disruption studies of *S. cerevisiae PLC1* indicate that this PI-PLC $\delta$  is essential for growth (Yoko-o et al., 1993). On the other hand, disruption of the *Dictyostelium discoideum plc* gene resulted in loss of PI-PLC activity, but cells developed normally (Drayer et al., 1994). The *AtPLC1* ORF is most closely related to the  $\delta$  type of PI-PLCs.

PI-PLC also plays important roles in plant signal transduction (recent reviews by Coté and Crain, 1993; Drøbak, 1993; Gross and Boss, 1993). The presence of PI-PLC activities in various plant systems and changes in their activities in association with physiological responses have been demonstrated. Cloning and sequencing of the *AtPLC1* gene, to our knowledge the first plant PI-PLC gene, reported here demonstrates that at least some plant PI-PLCs are related

to the PI-PLCs found in other systems. Characterization of the *AtPLC1* gene and its gene product should contribute to further understanding of plant signal transduction.

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