*Proc. Natl. Acad. Sci. USA*
Vol. 91, pp. 9617–9621, September 1994
Developmental Biology

# A phosphatidylinositol 3-kinase is induced during soybean nodule organogenesis and is associated with membrane proliferation

ZONGLIE HONG AND DESH PAL S. VERMA*

Department of Molecular Genetics and Biotechnology Center, The Ohio State University, 1060 Carmack Road, Columbus, OH 43210-1002

*Communicated by Hans Kende, June 23, 1994 (received for review April 11, 1994)*

ABSTRACT **Phosphatidylinositol 3-kinase (PI3K) is an important component of various receptor tyrosine kinase complexes in mammalian cells and a key enzyme required for cell division and vacuolar protein sorting in yeast. To our knowledge, this enzyme has not been characterized in plants. We report the cloning and characterization of soybean PI3K cDNAs and present evidence for the induction of a distinctive form of this enzyme specific to nodule organogenesis. Expression of the root form of PI3K is repressed during nodule organogenesis and is reinduced in mature nodules. Primer-extension results showed that the gene encoding the nodule form of PI3K is highly expressed in young (12–15 day old) root nodules in parallel with membrane proliferation but is repressed in mature nodules. The root form of the PI3K cDNA (SPI3K-5) encodes a peptide of 814 amino acids and the nodule form (SPI3K-1) encodes a peptide of 812 amino acids. Both cDNAs share 98% sequence identity in the coding region but differ in the noncoding regions. The polypeptides encoded by soybean PI3K cDNAs show significant sequence homology (50–60% similarity and 20–40% identity) to both PI3Ks and phosphatidylinositol 4-kinases from mammalian and yeast cells. *Escherichia coli* expressed soybean PI3K phosphorylated phosphatidylinositol specifically at the D-3 position of the inositol ring to generate phosphatidylinositol 3-phosphate. The temporal increase of a specific PI3K activity during membrane proliferation in young nodules suggests that PI3K plays a pivotal role in development of the peribacteroid membrane forming a subcellular compartment.**

The discovery of phosphatidylinositol (PI) 3-kinase (PI3K) that specifically phosphorylates the D-3 position of the inositol ring to generate phosphatidylinositol 3-phosphate [PI(3)*P*] has identified a signaling pathway distinct from the phospholipase C-mediated secondary messengers and other signaling pathways (1–4). PI3K was originally found to be associated with the polyomavirus middle-size tumor antigen–pp60$^{c\text{-}src}$ complex that is required for viral transformation of cultured mammalian cells and for tumorigenesis in animals (5, 6). This enzyme has now been shown to be a component of a wide range of receptor tyrosine kinase complexes that transduce growth factor signal(s) for cell division (1, 4, 7). PI3K is composed of a regulatory (p85) and a catalytic (p110) subunit. The cDNAs coding for both subunits have recently been cloned from mammals (8–10). Three genes encoding PI3K homologs, *VPS34*, *TOR2*, and *PIK1*, have been cloned from budding yeast. Vps34 protein (Vps34p) possesses PI3K activity and is required for sorting of proteins to the vacuole (11–13). Tor2 protein (Tor2p), a presumed PI3K, acts in transducing signal(s) for cell cycle activation (14), and Pik1 protein (Pik1p) displays PI 4-kinase (PI4K) activity and is essential for yeast cell viability (15).

The occurrence and physiological function of PI(3)*P* in plants are still obscure (2, 16). Only two inositol phospholipids, phosphatidylinositol 4-phosphate [PI(4)*P*] and phosphatidylinositol 4,5-bisphosphate [PI(4,5)$P_2$], are present in pea leaves (17), and a small amount of presumed PI(3)*P* and PI(3,4)$P_2$ has been detected in *Spirodela* and *Commelina* (18–20). PI4K activity has also been demonstrated in plants (21, 22). To our knowledge, however, PI3K has not been characterized in plants. The PI kinase activity present in microsomal and purified plasma membrane fractions from wheat (23, 24) is more similar to mammalian PI4K than to PI3K, based on the kinetic parameters of the enzymes. Thus, it is of significance to study the role of PI3K in plants.

Legume root nodule organogenesis requires a precise exchange of signal molecules between rhizobia and the host plant cell. Nod factors secreted by *Rhizobium* are able to transduce a signal into the root cortical cells to initiate a cascade of gene expression leading to cell division and formation of nodule-like structures (25). In the infected cells, an organelle called peribacteroid unit is formed where the rhizobia are enclosed in host plasma membrane-derived vesicles, the peribacteroid membrane (PBM). Formation of PBM is directly affected by various mutations in the endosymbiont (26, 27). PBM is a mosaic membrane with properties common to plasma and vacuolar membranes (28). Targeting of proteins to the PBM follows the secretory pathway although the mechanisms of targeting of different PBM proteins seem to vary (28, 29). Yeast Vps34 PI3K seems to have an essential role in protein targeting in the secretory pathway (11–13). In yeast, it forms a heterodimer with another protein encoded by the *VPS15* gene (30). Mutations in the *VPS34* or *VPS15* gene result in failure to sort proteins to the yeast vacuoles (13). These studies implicate phospholipids in the regulation of vesicular trafficking. In mammalian cells, PI3K plays a role in the insulin-induced movement of internal compartments to the plasma membrane (7) and platelet-derived growth factor-stimulated membrane ruffling (3). In this communication, we report the cloning and characterization of two cDNAs† encoding PI3Ks from soybean. We present evidence that suggests the expression of a distinctive form of PI3K gene during nodule organogenesis and the recruitment of cytosolic PI3K to membranes that may be required for its activation and biological function.

## MATERIALS AND METHODS

**Plant Materials and Microbes.** Soybean (*Glycine max* L. cv. Prize) seeds were grown in vermiculite in a growth chamber at 28°C. Roots and leaves were harvested from 3- and 5-day-old seedlings, respectively. Nodules were collected 12,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PI, phosphatidylinositol; PI3K, PI 3-kinase; PI4K, PI 4-kinase; PI(3)*P*, PI(4,5)$P_2$, etc., phosphatidylinositol 3-phosphate, phosphatidylinositol 4,5-bisphosphate, etc.; PBM, peribacteroid membrane; Vps34p, Pik1p, and Tor2p, Vps34, Pik1, and Tor2 proteins, respectively.

*To whom reprint requests should be addressed.

†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. L27265 for SPI3K-5 and L29770 for SPI3K-1).

15, 21, and 35 days after inoculation with *Bradyrhizobium japonicum* 61A76 or 12 and 21 days after inoculation with a Tn*5* mutant (T8-1) (26). Strains DH5$\alpha$ and JM109 were used for expression of soybean PI3K in *Escherichia coli*. *Saccharomyces cerevisiae* SEY6210 and KTY214 (*ΔVPS34::HIS*) were kindly provided by S. Emr (University of California at La Jolla, San Diego) and were used as reference for the identification of PI(3)*P* and PI(4)*P* (11, 13).

**Isolation and Analysis of Soybean PI3K cDNAs.** Based on the conserved amino acid sequence regions of bovine p110 (10) and yeast Vps34p (12), two degenerated oligonucleotides containing cloning sites for *Kpn* I and *Bam*HI, 5′-AAGGTACCGGNGAYGAYYTNMGNCARAG-3′ and 5′-AAGGATCCRTGNCYRTCNCCNAYNCC-3′, were synthesized and used for RNA–PCR to amplify a PI3K homologue sequence from soybean nodule RNA. The amplified fragment (320 bp) was cloned at the *Bam*HI–*Kpn* I site of pUC 19 and used as a probe to screen a soybean nodule cDNA library made in λZap II. Five positive plaques were identified, and their inserts were excised *in vivo* and inserted into pBluescript SK(−). Plasmid pSPI3K-E was made by removing a *Xba* I–*Eco*RV fragment from pSPI3K-5 (having longest insert) so that the coding sequence was fused translationally in-frame to the *lacZ* promoter of the vector. pSPI3K-B was generated by cloning a *Bam*HI–*Pst* I fragment into the *Bam*HI–*Pst* I site of pBluescript II SK(−). pSPI3K-P and pSPI3K-N were constructed by releasing a *Sma* I–*Pvu* II and a *Xba* I–*Nhe* I fragment, respectively, from pSPI3K-5. An *Eco*RV–*Kpn* I fragment from pSPI3K-5 was subcloned into the *Pvu* II–*Kpn* I site of pSET-C (Invitrogen) generating pSPI3K-S.

**Primer Extension.** RNA was prepared from soybean tissues by the hot-phenol extraction method (35). Synthetic oligonucleotides corresponding to the 5′ noncoding region of pSPI3K-5 (5′-GATGAATTGATCGTCTTTG-3′) and pPI3K-1 (5′-GGTTCGTTGAATTGATGATCG-3′) were used for primer extension using a primer-extension kit (Promega) by following the manufacturer's instructions. Total RNA (10 μg) from various tissues was annealed with the primer at 60°C and the extension products were resolved on an 8% polyacrylamide gel.

**Subcellular Fractionation and PI3K Assays.** Fresh plant tissues were homogenized in liquid nitrogen with mortar and pestle and extracted with buffer A (10 mM Tris·HCl, pH 7.5/250 mM sucrose/1 mM EDTA/1 mM 2-mercaptoethanol/0.5 mM phenylmethylsulfonyl fluoride). The homogenate was filtered through two layers of Miracloth and centrifuged at 10,000 × *g* for 10 min. The resulting supernatant was further centrifuged at 100,000 × *g* for 1 h. The pellet was resuspended in buffer B (50 mM Hepes·NaOH, pH 7.4/1 mM EDTA/5 mM $MgCl_2$/0.5 mM phenylmethylsulfonyl fluoride). The supernatant was dialyzed against buffer B. *E. coli* cells harboring various plasmids (see below) grown in the presence of 1 mM isopropyl β-D-thiogalactoside were centrifuged, resuspended in buffer B, and sonicated. The lysate was centrifuged at 10,000 × *g* for 5 min, and the resulting supernatant was used for PI3K assay. Yeast soluble and particulate fractions were prepared as described (11, 13). Total protein (2 μg) from each fraction was assayed for PI3K activity using [$\gamma$-$^{32}$P]ATP (5, 6, 13). The labeled phosphatidylinositol phosphates were separated on silica TLC plates (Merck) with a borate buffer system (36). The plates were autoradiographed or quantified by PhosphorImager (Molecular Dynamics).

## RESULTS AND DISCUSSION

**Cloning of Soybean PI3K cDNAs.** Bovine p110 and yeast Vps34p kinases have two highly conserved regions (GDDLRQD and LGVGDRH) in a domain identified as a lipid kinase consensus motif (Fig. 1 *A* and *C*). Two degenerate oligonucleotides from these regions were used to PCR-amplify a sequence by using as template cDNAs reverse-transcribed from soybean nodule mRNA. An amplified fragment with the expected size of ≈320 bp was cloned at *Bam*HI–*Kpn* I sites of pUC19. The DNA sequence of this insert was determined, and the deduced amino acid sequence showed significant homology with yeast Vps34p and bovine p110 (10, 12). By

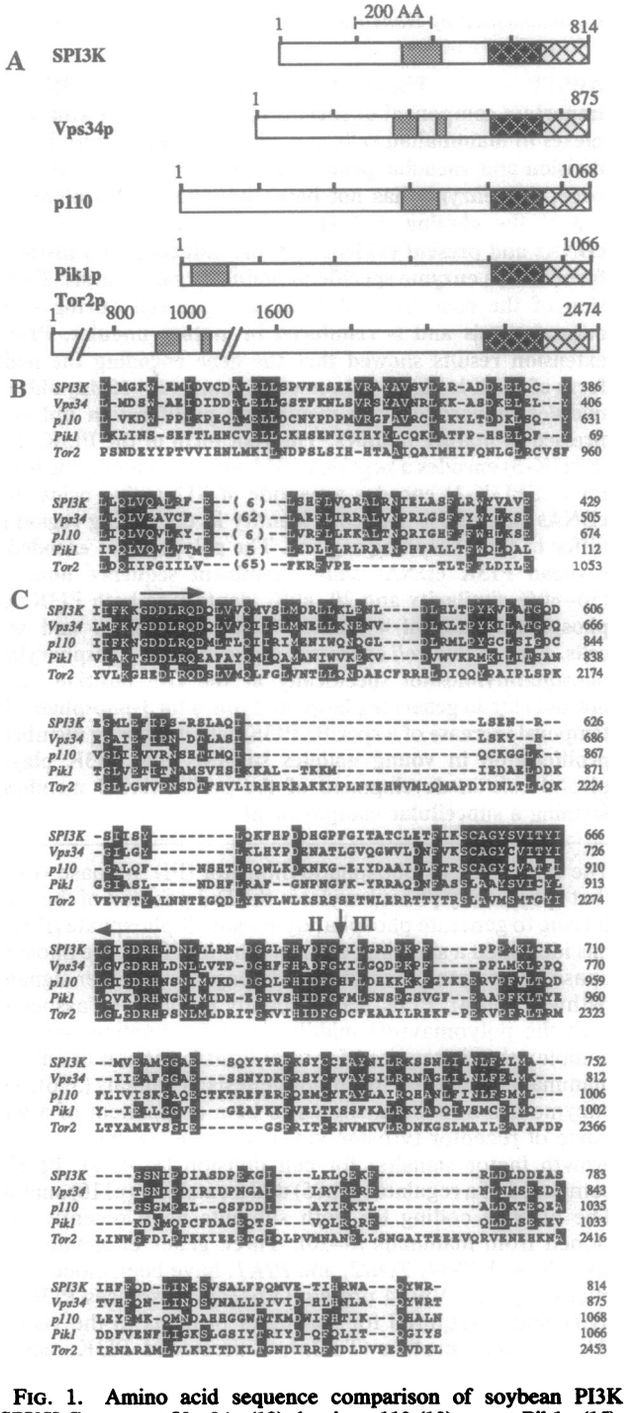


FIG. 1. Amino acid sequence comparison of soybean PI3K (SPI3K-5) to yeast Vps34p (12), bovine p110 (10), yeast Pik1p (15), and Tor2p (14). (*A*) Schematic representation of amino acid homology among members of PI kinase family. (*B*) PI kinase conserved region I (stippled boxes in *A*). (*C*) PI kinase conserved regions II (solid boxes with cross-hatches in *A*) and III (cross-hatched boxes in *A*). Regions used to design degenerate oligonucleotides for PCR are indicated by arrows [for comparison with SPI3K-1, see full sequence (GenBank accession no. L29770)].

using this fragment as a probe, five positive plaques were isolated from a soybean nodule cDNA library. Sequence analysis of these clones suggested the presence of two types of PI3K mRNAs in the nodule. The inserts in pSPI3K-5 (3.0 kb), pSPI3K-14 (3.0 kb), pSPI3K-4 (1.7 kb, lack of 5′ end), and pSPI3K-9 (0.9 kb, lack of 5′ end) were almost identical, suggesting that these cDNAs represent the same gene in soybean genome. Clone pSPI3K-1 (2.9 kb) shared 98% sequence homology in the coding region with pSPI3K-5 but had significant differences in both 5′ and 3′ noncoding regions with pSPI3K-5, indicating that pSPI3K-1 is derived from a different PI3K gene. Southern blot analysis confirmed the existence of more than one copy of the PI3K gene in soybean genomic DNA (unpublished data). By using soybean PI3K cDNA as probe, homologous sequences were also found in genomic DNA from two other legume plants, *Vigna* and *Lotus* (unpublished data), indicating the evolutionary conservation of these genes.

**Plant PI3Ks Are Similar to Those from Yeast and Animals.** Plasmid pSPI3K-5 contains a 3010-bp insert with an open reading frame to encode a polypeptide of 814 amino acids with a predicted molecular mass of 93 kDa. There is an in-frame stop codon 36 bp 5′ of the open reading frame and a long (500 bp) 3′ noncoding region with a poly(A) tail, indicating that it may be a full-length clone. The 2848-bp insert of pSPI3K-1 has an open reading frame to encode an 812-amino acid peptide. The deduced amino acid sequences of SPI3K-5 and SPI3K-1 differ by the deletion of 2 amino acids and 15 amino acid substitutions. One of the amino acid substitutions creates a potential phosphorylation site for cAMP-dependent protein kinase (31) and Ca/calmodulin-dependent protein kinase II (32). Both clones showed PI3K activity when expressed in *E. coli* (see below). The deduced amino acid sequence of soybean PI3Ks showed significant homology (Fig. 1) to yeast Vps34p (41% identity and 61% similarity), bovine p110 (25% identity and 53% similarity), and the C-terminal half of the yeast Tor2p (21% identity and 49% similarity) (10, 12, 14). Homology also exists with a recently described PI4K, Pik1p (15). Three conserved regions were identified as PI kinase conserved regions I–III (Fig. 1*A*). Conserved region I is located in the middle of soybean SPI3K, Vps34p, and p110 molecules. In Pik1p, a yeast PI4K, this region is located in the N-terminal end of the protein, and in Tor2p, a presumed PI3K, it is in the middle of the protein (Fig. 1*A*). Conserved region II showed highest homology and has been designated as a lipid kinase consensus motif (14). This region contains a part of the protein kinase consensus sequence implicated in the ATP binding and phosphotransferase activity (33). Conserved region III is located in the C-terminal end of all PI3Ks and has less homology than other regions. Soybean PI3Ks are also Leu-rich (13%), a property shared by other PI3Ks (14% in yeast Vps34p, 13% in yeast Tor2p, and 12% in bovine p110).

**Catalytic Activity of Soybean PI3Ks and Identification of Domains Required for this Activity.** To examine whether the cloned soybean PI3Ks possess catalytic activity or whether other subunits are required for activity, we expressed the soybean PI3K proteins in *E. coli* and assayed for the activity. Crude *E. coli* extract from cells harboring pSPI3K-1, pSPI3K-14, and pSPI3K-5 phosphorylated PI to PI(3)*P* in the presence of [$\gamma$-$^{32}$P]ATP (Fig. 2*B*, lanes 1–3), suggesting that the *E. coli*-expressed protein alone is sufficient for catalytic activity in the presence of appropriate substrates. Both pSPI3K-14 and pSPI3K-5 exhibited high PI3K activity, but lower activity was observed for pSPI3K-1 (Fig. 2*B*, lanes 1–3). To dissect the domains required for PI3K catalytic activity, we deleted sequence from the 5′ region of pSPI3K-5 cDNA. The peptide encoded by pSPI3K-B was about one-third shorter than the original protein encoded by pSPI3K-5 but was still active although at a lower level (Fig. 2*B*, lane 4).

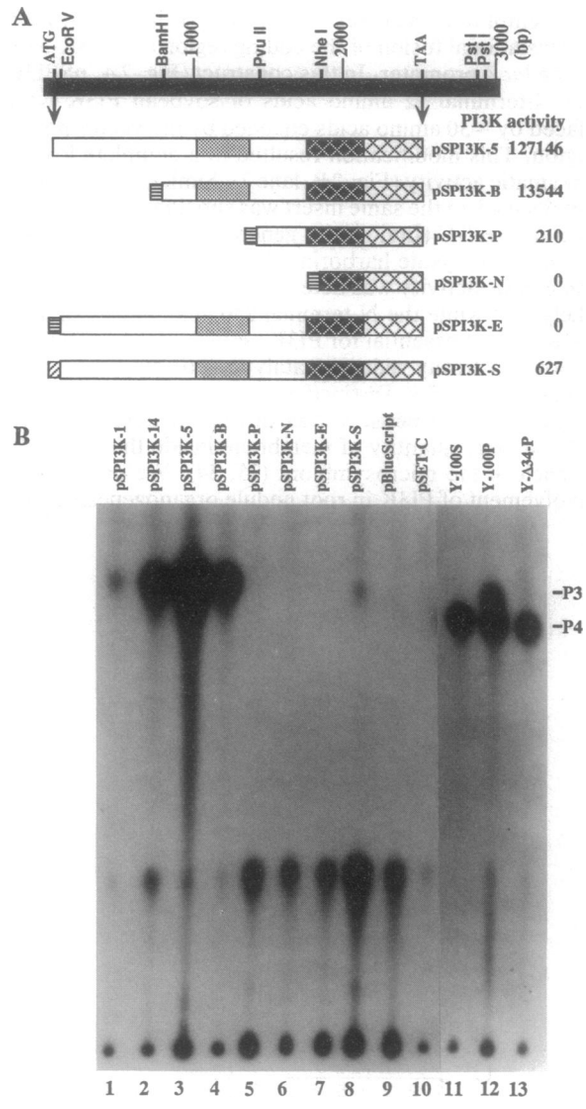


FIG. 2. Identification of amino acid sequence regions important for PI3K catalytic activity. (*A*) Schematic representation of different deletion constructs of a soybean PI3K cDNA and the enzymatic activity levels. Plasmid pSPI3K-5 was used for these experiments. The PI kinase conserved regions I, II, and III are indicated by stippled and cross-hatched solid and open boxes, respectively. The boxes with horizontal bars and the hatched box indicate polypeptide derived from polylinker region of vector pBluescript SK(−) and pSET-C, respectively. The relative activity of PI3K was measured by quantification of PI(3)*P* spots on TLC plates using a PhosphorImager. (*B*) PI3K activity of *E. coli* extracts expressing various soybean cDNA clones (lanes 1–3) and deletion constructs derived from pSPI3K-5 (lanes 4–8). Cloning vectors pBluescript and pSET-C were used for blank controls (lanes 9 and 10). Yeast soluble (Y-100S, lane 11) and particulate (Y-100P, lane 12) fractions, and a membrane fraction (Y-Δ34-P, lane 13) from *VPS34* deletion mutant cells were used to generate reference markers for PI(3)*P* (band P3) and PI(4)*P* (band P4) (see refs. 11 and 13).

This suggests that the N-terminal 250 amino acids are not essential for the catalytic activity of this enzyme. Further deletion of consensus region I (amino acids 342–429) resulted in a drastic reduction of activity (Fig. 2, pSPI3K-P). Deletion up to the highly conserved region II (pSPI3K-N) completely abolished enzymatic activity (Fig. 2*B*, lane 6), suggesting that these two consensus regions are required for PI3K activity. Because of the presence of a stop codon (TAA) in the 5′ noncoding region, the peptide expressed by pSPI3K-5 should not contain amino acids encoded by the polylinker region. In

an attempt to overexpress soybean PI3K in *E. coli*, we made a translational fusion of the coding region of SPI3K-5 cDNA to the *lacZ* promoter. In this construct (Fig. 2*A*, pSPI3K-E), the N-terminal 12 amino acids of soybean PI3K were replaced by ≈30 amino acids encoded by the vector polylinker region. This modification resulted in a complete loss of the enzymatic activity (Fig. 2*B*, lane 7). Similar results were also obtained when the same insert was subcloned into expression vector pSET-C (Invitrogen) generating pSPI3K-S (Fig. 2*A*). In an *E. coli* lysate harboring this plasmid (pSPI3K-S), only trace PI3K activity was detected (Fig. 2*B*, lane 8). Thus, it is likely that while the N-terminal 250 amino acids of soybean PI3K are not essential for PI3K activity, modification of this region drastically reduces catalytic activity.

**Expression of a Distinctive Form of PI3K Gene During Nodule Organogenesis.** Nodule development requires synthesis of a large quantity of membrane inside the infected cells to enclose the microsymbiont (25, 34). We determined the involvement of PI3K in root nodule organogenesis and PBM

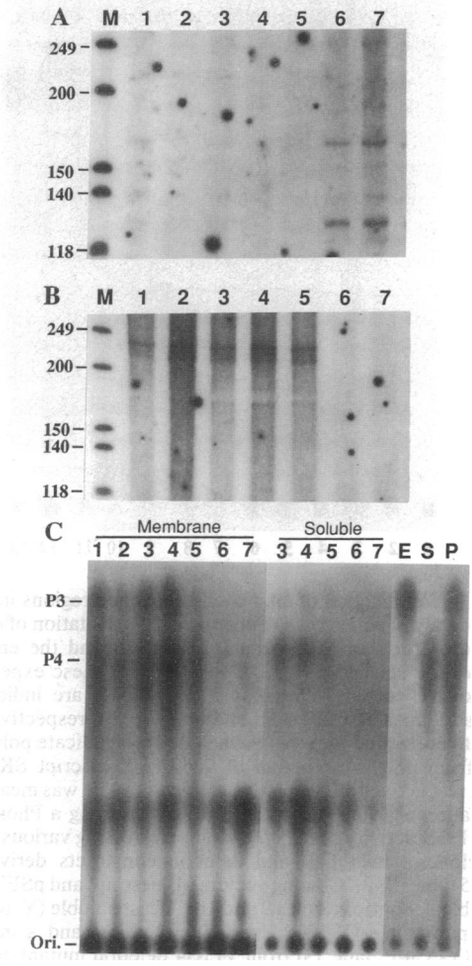


FIG. 3. Differential PI3K gene expression and enzyme induction during nodule organogenesis. (*A*) Extension products of the primer corresponding to soybean pSPI3K-5 clone. Lanes: 1 and 2, RNA from 12- and 21-day-old nodules infected with a *Rhizobium* mutant strain (T8-1, ref. 26); 3–6, RNA from 12-, 15-, 21-, and 35-day-old nodules infected with a wild-type *Rhizobium* strain; 7, RNA from uninfected roots. (*B*) Extension products of the primer corresponding to soybean pSPI3K-1 clone. RNA samples were the same as in *A*. (*C*) Induction of PI3K activity. PI3K activity (lanes 1–7) was assayed in the membrane fraction prepared from the same tissues used for the primer extension (*A* and *B*, lanes 1–7). Total protein (2 μg) from the membrane or soluble fractions was used for enzyme assay. Lanes: E, crude extract of *E. coli* cells expressing soybean pSPI3K-5; S, yeast soluble fraction; P, yeast particulate fraction.

synthesis by monitoring the expression of each of the soybean PI3K genes in roots and nodules at different stages of development. Because the two soybean PI3K clones differed in the 5′ noncoding regions, two oligonucleotide primers corresponding to SPI3K-1 and SPI3K-5 cDNAs were synthesized and used for primer extension. Total RNA was prepared from 12-, 15-, 21-, and 35-day-old nodules and from uninfected roots. With the SPI3K-5-specific primer, two major products of about 125 and 165 bp in size were extended using RNA from roots and old nodules (Fig. 3*A*, lanes 6 and 7) but not from young nodules (lanes 1–5). When the SPI3K-1-specific primer was used, three major extension products of about 220, 230, and 240 bp in size were detected in RNA from young nodules (Fig. 3*B*, lanes 1–5) but not from old nodules and roots (lanes 6 and 7). These data suggest that a switch in the expression of PI3K gene from SPI3K-5 to SPI3K-1 takes place when nodules are initiated. The induction of SPI3K-1 gene, a nodule form, was followed by concomitant repression of SPI3K-5, a root form of PI3K. This induction and repression is directly correlated with membrane proliferation in root nodules (34). *Rhizobium* mutant T8-1 (*Nod*$^+$ *end*$^-$ Tn*5* mutant, ref. 26), which fails to endocytose but initiates membrane proliferation and nodule development, showed the same switching pattern of PI3K gene expression (Fig. 3 *A* and *B*, lanes 1 and 2), suggesting that the switch of PI3K gene expression is triggered along with the nodule developmental program and is not regulated by nitrogen fixation or endocytosis of bacteria. Signals necessary for membrane proliferation are apparently transduced in T8-1-induced nodules because the infected cells in these nodules contain many vesicles and form normal-size nodules (26).

**Temporal Coordination of PI3K Activity with Membrane Proliferation During Nodule Organogenesis.** One of the major events during nodule organogenesis is a proliferation of the membrane system that is essential for the establishment of symbiosis between *Rhizobium* and the legume host (25). To determine the temporal pattern of PI3K activity with PBM proliferation, we measured PI3K activity at various stages of nodule development. One part of the sample was used for primer extension (Fig. 3 *A* and *B*) and the other was assayed for PI3K activity. The PI3K activity in 12-day-old nodules (Fig. 3*C*, lane 3) was much higher than in uninfected roots (lane 7). The activity continued to increase during early nodule development (15-day-old nodule, lane 4). In 21-day-old nodules, which were fully developed, PI3K activity started to decrease (lane 5). A low activity was observed in soluble fractions and did not change during nodule development (Fig. 3*C*, soluble, lanes 3–5). The activity in 35-day-old nodules declined to a basal level observed in uninfected roots

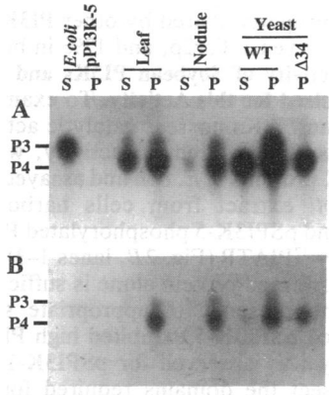


FIG. 4. Subcellular distribution of PI3K in soluble (lanes S) and particulate (lanes P) fractions (*A*) and availability of PI in the fractions (*B*). The enzyme assays in *A* and *B* were identical except for the exclusion of exogenous PI in the reaction mixture in *B*.

(Fig. 3C, lanes 6 and 7). Similar temporal patterns of PI3K activity were observed with T8-1 nodules when the same amount of membrane was assayed for the enzyme activity (Fig. 3, lanes 1 and 2), although these nodules contain very few bacteria and are ineffective in nitrogen fixation. This temporal pattern of PI3K activity was well coordinated with the pattern of membrane proliferation during nodule organogenesis (25). Membrane synthesis is reduced in mature nodules (34). These results suggest that PI3K may play a pivotal role in the biogenesis of membranes leading to the formation of an endosymbiotic compartment.

**Distribution of PI3K and Enzyme Activation by Association with Membrane Components.** Analysis of the deduced amino acid sequence of soybean PI3Ks did not reveal any transmembrane domains or consensus sequences for prenylation, myristoylation, or lipid binding. The *E. coli*-expressed soybean PI3K was present in the soluble fraction (Fig. 4A, lanes 1 and 2). However, PI3K activity was detected in both soluble and particulate fractions in leaves and nodules with the highest activity in the membrane fraction (Fig. 4A, lanes 3–6). Although there may be another type of PI3K that contains transmembrane domains and whose cDNA has not been cloned, it is more likely that the same PI3K exists in two forms, one soluble and the other membrane bound. To evaluate the relative biological importance of each of these two forms, we tested the availability of its substrate, PI, by assaying PI3K activity in the cytosolic and particulate fractions. While exogenous PI had little effect on PI3K activity in the particulate fraction (Fig. 4B, lanes 4 and 6), no activity was detected in the soluble fraction in the absence of exogenous PI (lanes 3 and 5), suggesting that the cytosolic form of PI3K may be inactive *in vivo* due to the lack of substrate (PI) in the cytosol. The results in Fig. 3B revealed a switch in transcription between root and nodule forms of PI3K genes during nodule organogenesis. Between 12 and 21 days of nodule development, the level of transcription remained almost the same while a drastic change in PI3K activity occurred (Fig. 3C, lanes 1–5). Thus there appear to be at least two levels of control for PI3K activity in root nodules, a transcriptional switch in expression between root and nodule forms of PI3K genes and the binding of cytosolic PI3K to the membrane, which may activate the enzyme to catalyze the phosphorylation of PI. PI3K has been implicated in signal transduction and vesicular transport in animal cells (1, 3, 4, 7) and in the targeting of vacuolar proteins in yeast (11–13). In root nodules, a coordination between proliferation of bacteria and membrane synthesis to enclose the newly divided bacteria would require both signal exchange and control of membrane flow. The synthesis of PBM, a membrane with properties of vacuolar and plasma membranes, and a concomitant increase in PI3K activity suggest that the nodule form of PI3K is primarily involved in the biogenesis of PBM compartment. This is consistent with data that in mature nodules, where PBM synthesis is reduced (34), expression of the nodule-specific PI3K gene is repressed. It should be of interest to determine how this enzyme participates in membrane protein targeting and signal transduction in plants.

We thank Drs. K. Peters and G.-H. Miao for comments on this manuscript and Dr. S. Emr for kindly providing us with yeast strains used in this study. This work was supported by a National Science Foundation grant (DCB-8819399).

1. Cantley, L. C., Auger, K. R., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R. & Soltoff, S. (1991) *Cell* **64**, 281–302.
2. Drobak, B. K. (1992) *Biochem. J.* **288**, 697–712.
3. Wennstrom, S., Hawkins, P., Cooke, F., Hara, K., Yonezawa, K., Kasuga, M., Jackson, T., Claesson-Welsh, L. & Stephens, L. (1994) *Curr. Biol.* **4**, 385–393.
4. Valius, M. & Kazlauskas, A. (1993) *Cell* **73**, 321–334.
5. Whitman, M., Kaplan, D. R., Schaffhausen, B., Cantley, L. & Roberts, T. M. (1985) *Nature (London)* **315**, 239–242.
6. Whitman, M., Downes, P. C., Keeler, M., Keller, T. & Cantley, L. (1988) *Nature (London)* **332**, 644–646.
7. Kelly, K. L., Ruderman, N. B. & Chen, K. S. (1992) *J. Biol. Chem.* **267**, 3423–3428.
8. Escobedo, J. A., Navankasattusas, S., Kavanaugh, W. M., Miflay, D., Fried, V. A. & Williams, L. T. (1991) *Cell* **65**, 75–82.
9. Skolnik, E. Y., Magolis, B., Mohammadi, M., Lowenstein, E., Fischer, R., Drepps, A., Ullrich, A. & Schlessinger, J. (1991) *Cell* **65**, 83–90.
10. Hiles, I. D., Otsu, M., Volinia, S., Fry, M. J., Gout, I., Dhand, R., Panayotou, G., Ruiz-Larrea, F., Thompson, A., Totty, N. F., Hsuan, J. J., Courtneidge, S. A., Parker, P. J. & Waterfield, J. D. (1992) *Cell* **70**, 419–429.
11. Schu, P. V., Takegawa, K., Fry, M. J., Stack, J. H., Waterfield, M. D. & Emr, S. D. (1993) *Science* **260**, 88–91.
12. Herman, P. K. & Emr, S. D. (1990) *Mol. Cell. Biol.* **10**, 6742–6754.
13. Stack, J. H., Herman, P. K., Schu, P. & Emr, S. D. (1993) *EMBO J.* **12**, 2195–2204.
14. Kunz, J., Henriquez, R., Schneider, U., Deuter-Reinhard, M., Movva, N. R. & Hall, M. N. (1993) *Cell* **73**, 585–596.
15. Flanagan, C. A., Schnieders, E. A., Emerick, A. W., Kunisawa, R., Admon, A. & Thorner, J. (1993) *Science* **262**, 1444–1448.
16. Gross, W. & Boss, W. F. (1993) in *Control of Plant Gene Expression*, ed., Verma, D. P. S. (CRC, Boca Raton, FL), pp. 17–32.
17. Irvine, R. F., Letcher, A. J., Lander, D. J., Drobak, B. K., Dawson, A. P. & Musgrave, A. (1989) *Plant Physiol.* **89**, 888–892.
18. Brearley, C. A. & Hanke, D. E. (1992) *Biochem. J.* **283**, 255–260.
19. Brearley, C. A. & Hanke, D. E. (1993) *Biochem. J.* **290**, 145–150.
20. Parmar, P. N. & Brearley, C. A. (1993) *Plant J.* **4**, 255–263.
21. Xu, P., Lloyd, C. W., Staiger, C. J. & Drobak, B. K. (1992) *Plant Cell* **4**, 941–951.
22. Young, W., Burkhart, W., Cavallius, J., Merrick, W. C. & Boss, W. F. (1993) *J. Biol. Chem.* **268**, 392–398.
23. Sandelius, A. S. & Sommarin, M. (1986) *FEBS Lett.* **201**, 282–286.
24. Sommarin, M. & Sandelius, A. S. (1988) *Biochim. Biophys. Acta* **958**, 268–278.
25. Verma, D. P. S. (1992) *Plant Cell* **4**, 373–382.
26. Morrison, N. & Verma, D. P. S. (1987) *Plant Mol. Biol.* **9**, 185–196.
27. Roth, L. E. & Stacey, G. (1989) *Eur. J. Cell Biol.* **49**, 24–32.
28. Miao, G.-H., Hong, Z. & Verma, D. P. S. (1992) *J. Cell Biol.* **118**, 481–490.
29. Cheon, C.-I., Hong, Z. & Verma, D. P. S. (1994) *J. Biol. Chem.* **269**, 6598–6602.
30. Herman, P. K., Stack, J. H., DeModena, J. A. & Emr, S. D. (1991) *Cell* **64**, 425–437.
31. Taylor, S. (1989) *J. Biol. Chem.* **264**, 8443–8446.
32. Edelman, A., Blumenthal, D. & Krebs, E. (1987) *Annu. Rev. Biochem.* **56**, 567–613.
33. Hanks, S. K., Quinn, A. M. & Hunter, T. (1988) *Science* **241**, 42–52.
34. Verma, D. P. S., Kazazian, V., Zogbi, V. & Bal, A. K. (1978) *J. Cell Biol.* **78**, 919–936.
35. Verwoerd, T. C., Dekker, B. M. M. & Hoekema, A. (1989) *Nucleic Acids Res.* **17**, 2362.
36. Walsh, J. P., Caldwell, K. K. & Majerus, P. W. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9184–9187.