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# *At*VPS34, a phosphatidylinositol 3-kinase of *Arabidopsis thaliana*, is an essential protein with homology to a calcium-dependent lipid binding domain

(vacuole/protein transport/yeast complementation/chimeric gene/antisense)

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**ABSTRACT** **The cDNA encoding phosphatidylinositol (PI) 3-kinase was cloned from *Arabidopsis thaliana*, and the derived amino acid sequence (*At*VPS34) has a significantly higher homology to yeast PI 3-kinase (VPS34) than to the mammalian (p110). The protein has two conserved domains: a catalytic site with the ATP-binding site near the C terminus and a calcium-dependent lipid-binding domain near the N terminus. The plant cDNA does not rescue a yeast *vps34* deletion mutant, but a chimeric gene in which the coding sequence for the C-terminal third of VPS34 is replaced by the corresponding sequence from the plant gene does rescue the yeast mutant. PI 3-kinase activity is detectable in extracts from plants that overexpress the plant PI 3-kinase. Expression of antisense constructs gives rise to second-generation transformed plants severely inhibited in growth and development.**

Signaling cascades involving phosphorylated phospholipids that function in mammalian and yeast cells may also operate in plant cells (1). Because of the importance of phosphatidylinositol (PI) 3-kinases in signaling cascades of mammalian cells (2) and for vacuolar targeting in yeast (3), we studied the role of PI 3-kinase in *Arabidopsis thaliana*. This enzyme has not yet been found in plant extracts, although its activity has been demonstrated by metabolic labeling of lipids with radioactive precursors (4, 5).

Targeting of vacuolar proteins requires both information within the proteins themselves (vacuolar sorting signals) and machinery for protein targeting: receptors as well as proteins on the outside of the vesicles (coat and regulatory proteins) and on the vacuolar membrane. GTPases are likely to be important in these processes (6), as well as proteins necessary for vesicle fusion with the target membrane, possibly by binding to specific phospholipids in these membranes (7). How these proteins interact to deliver vacuolar protein remains to be elucidated, but VPS34 and VPS15 have been shown to be components of a membrane-associated protein complex needed for the correct targeting of vacuolar proteins in *Saccharomyces cerevisiae* (8). *VPS15* encodes a protein kinase (9) and *VPS34* encodes a PI 3-kinase (10, 11).

The importance of PI for vesicle transport is underscored by the crucial role of PI transfer protein, the product of the *SEC14* gene in yeast, for exit of proteins from a late Golgi compartment (12). This protein transfers PI between lipid bilayers and is required to maintain the correct PI level in Golgi membranes.

The association of PI 4-kinases with the cytoskeleton (13) or their colocalization with vesicles (14) and the interaction of their products with proteins like profilin (15) also point to involvement of PI kinases in protein transport. Furthermore, PI 3-kinase is required for internalization and degradation of platelet-derived growth factor receptor (16).

Using PCR with oligonucleotides based on conserved sequences in PI 3-kinases from bovine tissues (17), yeast (9), and a PI 4-kinase of yeast (18), we identified an *A. thaliana* PI 3-kinase cDNA (*At*VPS34). We found considerable amino acid sequence identity between *At*VPS34 and yeast VPS34, as well as mammalian p110, especially in the C-terminal half with its catalytic domain. In the N-terminal half of the plant protein homology to a calcium-dependent phospholipid-binding (CaLB) domain is present, as found in proteins such as rabphilin-3A, a protein involved in fusion of synaptic vesicles to the plasma membrane (7).

## MATERIALS AND METHODS

**Molecular Biology Techniques.** Cloning, transformation, blotting, and hybridization were done by standard methods, as described (19). PCR was conducted by denaturation at 94°C for 3 min, followed by 25–40 cycles of 1-min denaturation at 94°C, 1-min annealing at 65°C, and 1-min elongation at 72°C.

**Plasmid Construction.** Oligonucleotides used for amplification of the *VPS34* homolog from *A. thaliana* were 2048-fold degenerated covering the following sequences: 1, CAGY-CVA/I; 2, GDDLRQD/E; and 3, HI/AD-FGH/Y/F. Sequences 1 and 3 had a *Bam*HI restriction site at their 5′ ends and amplified the complementary strand; sequence 2 had a *Pst* I site and is specific for the coding strand. All three started with GTTT to ensure function of the restriction enzyme. The resulting PCR fragment (291 bp) was isolated from an agarose gel and without further treatment cloned into pCRII (Invitrogen). pMM9.1 (*AtVPS34*) was isolated from a cDNA library of *A. thaliana* seedling mRNA in pFL61 (20). For antisense and sense expression in plants, a 2.8-kb *Not* I fragment of *AtVPS34* was cloned into *Xba* I/*Sst* I sites of pBI121 after a Klenow fragment fill-in reaction (p9.1A and p9.1S, respectively). *ScAt*I is a fusion of the N-terminal yeast and C-terminal plant clone at the single *Dra* I restriction site in *AtVPS34* conserved in *ScVPS34* yielding a fusion protein of amino acids 1–601 of the yeast and 540–815 of the plant VPS34. *ScAt*II is a fusion at the filled in *Bgl* II ends of *AtVPS34* and *Ava* I ends of *ScVPS34*, resulting in an in-frame fusion of amino acids 1–463 of *Sc*VPS34 and 402–815 of

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Abbreviations: CaLB, calcium-dependent lipid-binding; PI, phosphatidylinositol.

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*At*VPS34. *ScAt*I, *ScAt*II, and *ScVPS34* are derivatives of a single-copy yeast vector (pRS316).

**Yeast Strains.** *S. cerevisiae* strains used were SEY6210 [*MATα, leu2-3, 112 ura3-52, his3-Δ200, trp1-Δ901, lys2-801, suc2-Δ9* (ref. 21)] and the *vps34* deletion mutant PHY102 [SEY6210 *vps34Δ1::TRP1* (ref. 10)].

**Yeast Transformation.** A modified version of a published method (22) was used for transformation. The highest efficiency was achieved by applying a heat shock of 15 min at 42°C despite the heat sensitivity of the strains and using selection plates containing 1 M sorbitol.

**Plant Transformation.** Calli induced from roots of *A. thaliana* ecotype Landsberg were transformed by cocultivation with *Agrobacterium tumefaciens* LBA4044 bearing plasmids pBI121, p9.1A, and p9.1S. Transgenic plants were obtained after regeneration on kanamycin-containing medium, as described (23).

**PI 3-Kinase Assay.** A described method (3) was used for yeast protein extraction and assay conditions. For plant protein extracts, plant tissue was pulverized in liquid nitrogen. The resulting powder was suspended in yeast protein extraction buffer [15 mM Hepes, pH 7.5/100 mM KCl/3 mM EGTA/10% (vol/vol) glycerol/2 ml/g of tissue] with a proteinase inhibitor mixture (leupeptin, pepstatin, aprotenin, phenylmethylsulfonyl fluoride in concentrations between 1 and 10 ng/ml). The supernatant after centrifugation at 700 × *g* for 5 min was used for PI 3-kinase assays. Briefly, a suspension of 1.5 μg of PI in 15 mM Hepes, pH 7.5/100 mM KCl was incubated with protein extracts of yeast or plants, 10 μCi of [$\gamma$-$^{32}$P]ATP (1 Ci = 37 GBq), and 0.3 mM unlabeled ATP, in 10 mM $MgCl_2$ and 20 mM Hepes, pH 7.5, in a final 50-μl vol for 5 min at room temperature. The reaction was stopped with 80 μl of 1 M HCl; the lipids were extracted with 160 μl of $CHCl_3/CH_3OH$, 1:1, and after evaporation of the solutes and redissolving of the lipids in $CHCl_3$ separated by TLC on silica gel 60 TLC plates (Merck) with a borate buffer system (24).

**Computer Programs.** The programs used were developed by the Genetics Computer Group (25), Smith (11), Henikoff and Henikoff (ref. 26; BLOCKS search), and Altschul *et al.* (ref. 27; BLAST Network Service).

## RESULTS

**Cloning of Plant PI 3-Kinase cDNA.** The cloning of a fragment of *At*VPS34 was achieved by using the PCR with a whole-plant *A. thaliana* cDNA library and degenerate primers derived from conserved amino acid sequence domains in the mammalian (17) and yeast PI 3-kinases (3, 9), as well as a yeast PI 4-kinase (18). Positions of the three primers are indicated in Fig. 1. The PCR conditions were optimized by probing the resulting fragments with yeast *VPS34* as probe (data not shown). Only under very stringent conditions for the PCR (65°C annealing temperature compared with a calculated melting temperature of 62°C) was amplification specific enough to yield a fragment hybridizing to the yeast probe. Moreover, only the inner primer pair (2/1, Fig. 1) could amplify the sequence. The deduced amino acid sequence of the 291-bp PCR fragment was 61% identical to the corresponding yeast VPS34 region. Identity with bovine PI 3-kinase (36%) and yeast PI 4-kinase (31%) was also significant. To get a full-length cDNA clone, this PCR fragment was used to screen an *A. thaliana* cDNA library in pFL61 (20). Out of 40,000 colonies, 12 clones were isolated. One of them (pMM9.1) yielded PCR fragments of the appropriate size with the degenerate primers; this 2.8-kb clone was sequenced in its entirety.

The cDNA clone consists of a 121-bp untranslated leader sequence, 2442-bp coding sequence, and 198-bp 3′ region followed by a stretch of poly(A) (GenBank accession no. U10669). The deduced amino acid sequence was 25% identical to the bovine p110 and 40% identical to the yeast PI 3-kinase (*Sc*VPS34 in Fig. 1). The lower identity with the mammalian peptide sequence is caused, in part, by the more variable N-terminal sequence of the p110 polypeptide. Common to the plant and mammalian PI 3-kinases is the deletion of a 60-aa stretch from the yeast sequence, 400 aa from the C terminus (Fig. 1, black box). The 60-aa insertion in the yeast protein is a serine-rich sequence with unknown function.

***At*VPS34 Contains a Lipid-Binding Domain.** A BLAST Network Service search with the entire amino acid sequence of *At*VPS34 found the closest matches to yeast VPS34, bovine and murine p110, PI 4-kinase from yeast, and TOR2, a protein involved in cell cycle control, in that order of decreasing similarity. The highest similarity was always in the ATP-binding domains found near the C terminus of these proteins. A search with parts of the N-terminal sequences (aa 85–150 of *At*VPS34) revealed a strong homology to a CaLB domain of rabphilin-3A (24% identity and 47% similarity; Fig. 2). Because of the high homology between a portion of *At*VPS34 and core stretches of the CaLB domain of rabphilin-3A (47% identity in aa 96–119 of *At*VPS34; box I, Fig. 2) and of p110 (44% identity in aa 122–139 of *At*VPS34; box II, Fig. 2), we postulate that a phospholipid-binding site is located in this region of the plant protein. In spite of the moderate identity value for these regions of the yeast and plant VPS34 proteins (21%), the good similarity scores (46%) lead us to suggest in contrast to other reports (2) that yeast VPS34 may have a similar functional domain in this region.

**A Chimeric Gene Rescues a *S. cerevisiae* Mutant.** The *AtVPS34* gene on a 2μ plasmid expressed under the control

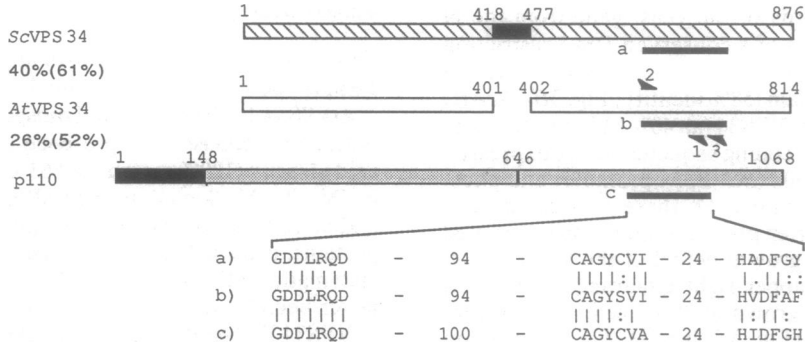


FIG. 1. PI 3-kinases of *A. thaliana* (open box), *S. cerevisiae* (hatched), and mammal (bovine p110, stippled). The amino acid sequence *At*VPS34 has 40% identity (61% similarity) to that of *Sc*VPS34 but has only 26% (52%) to p110. The N-terminal half is less conserved with 29% identity between plant (aa 1–401) and fungal (aa 1–418) domains and 22% identity between plant and mammalian (aa 148–646) polypeptides. Black boxes are regions with homology gaps >20 aa. Bars a–c indicate conserved regions used to design oligonucleotides; arrowheads indicate their orientations and positions.

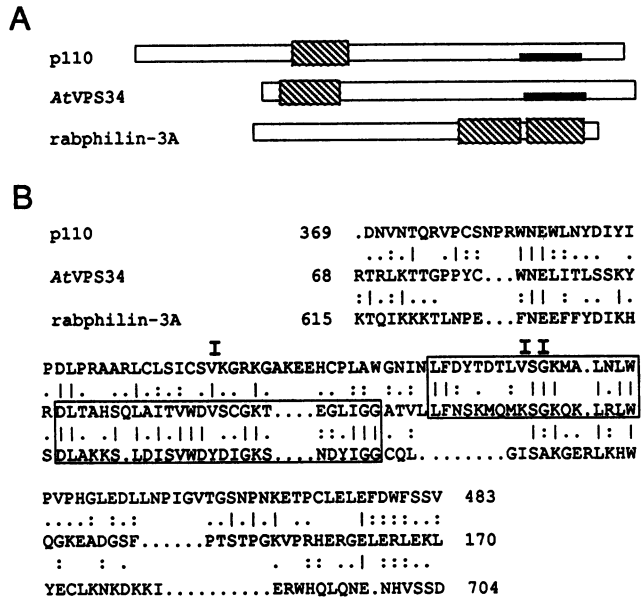


FIG. 2. Homology of *At*VPS34 CaLB-domain to p110 and rabphilin-3A. (*A*) Schematic location of CaLB domains (hatched boxes); black bars, ATP-binding domain in p110 and *At*VPS34. (*B*) Comparison of amino acid sequences of rabphilin-3A and p110 CaLB-domain to *At*VPS34. Dots represent different degrees of similarity; dashes indicate identity. Box II emphasizes regions of highest identity between *At*VPS34 and p110 (44%) CaLB-domains; box I emphasizes those between *At*VPS34 and the second CaLB-domain of rabphilin-3A (47%).

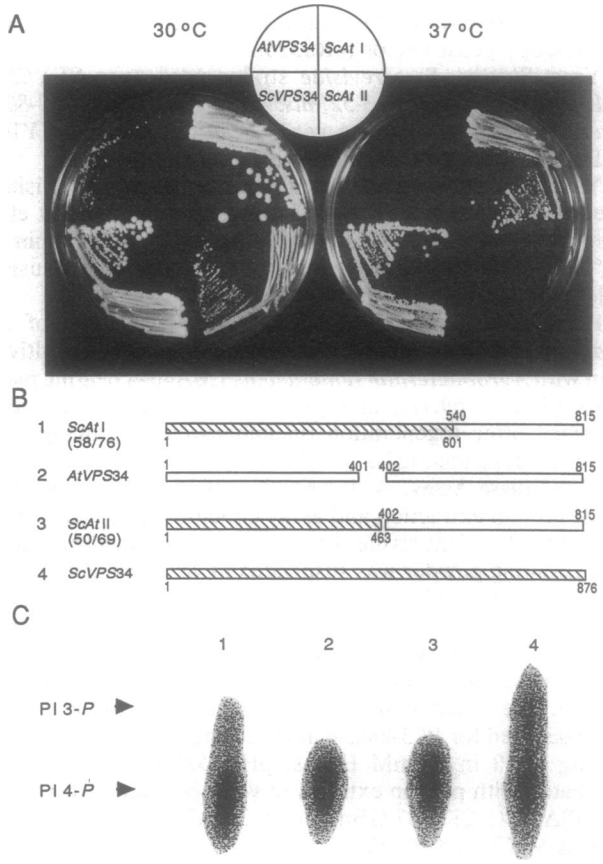


FIG. 3. Growth at different temperatures and PI 3-kinase assay for a *vps34* deletion mutant of *S. cerevisiae* (PHY102) transformed with *ScVPS34*, *AtVPS34*, and chimeric genes of both. (*A*) Growth of PHY102 transformed with *AtVPS34*, *ScVPS34*, *ScAt*II, and *ScAt*I on yeast extract/peptone/dextrose at 30° and 37°C. The *Sc*VPS34 and the *ScAt*I fusion complemented the temperature-sensitive phenotype of the yeast mutant. PHY102 transformed with *ScAt*II or with *AtVPS34* were unable to grow at 37°C. [Fewer colonies in the upper left of the plate grown at 30° (*AtVPS34*) is not caused by the gene presence and was not found on other plates.] (*B*) Schematic drawing of PI 3-kinases of yeast and *A. thaliana* and chimeric fusions tested in PHY102 complementation. Numbers above and below bars denote amino acids at point of fusion, respectively, in *Arabidopsis* and yeast proteins; in brackets are percentage identity/percentage similarity ratios of the exchanged *Arabidopsis* segment to its yeast counterpart. (*C*) PI 3-kinase assay of protein extracts of the strains described above grown in liquid medium at 30°C. Corresponding to the results of the growth phenotype, the strains able to grow at 37°C, PHY102/*Sc*VPS34 (lane 4) and PHY102/*ScAt*I (lane 1), have PI 3-kinase activity (PI 3-*P*), whereas no PI 3-kinase activity was detectable in PHY102/*At*VPS34 (lane 2) and PHY102/*ScAt*II (lane 3). PI 4-*P*, PI 4-kinase activity.

of the phosphoglycerate kinase promoter and terminator (20) failed to rescue a *vps34* deletion mutant that is temperature sensitive for growth at 37°C (Fig. 3*A*, upper left quadrant). Extracts of the cells grown at 30°C expressed *At*VPS34 (as shown in Northern blots) but contained no PI 3-kinase activity (Fig. 3*C*, lane 2).

To test whether the phosphoglycerate kinase promoter was responsible for the lack of enzyme activity, we made a construct in which the plant coding sequence was controlled by yeast *VPS34* promoter. This construct, which contained the proper yeast expression signals, also failed to rescue the yeast deletion mutant (data not shown).

The extent of homology in the N-terminal half of the VPS34 proteins is relatively low (29%, Fig. 1). Therefore, the plant protein may not associate with the necessary effector proteins, such as VPS15. To test this hypothesis, we made chimeric yeast–plant cDNAs in which domains between the two proteins were swapped. Two of these are shown in Fig. 3*B*. In the construct *ScAt*I the terminal third of the plant cDNA is fused to two-thirds of the yeast gene from the N terminus. Taking advantage of a conserved *Dra* I restriction site in both genes, this fusion was made at the N-terminal end of the most conserved domain (58% identity, Fig. 3*B*). *ScAt*II has the C-terminal half of *At*VPS34 (aa 402–815) and aa 1–463 of *Sc*VPS34 (Fig. 3*B*). Both coding sequences were expressed with yeast expression signals (*Sc*VPS34 promoter and phosphoglycerate kinase terminator). Of these two constructs, only the one with the shorter plant coding segment rescued the temperature-sensitive growth defect of the yeast *vps34*-deletion strain (Fig. 3*A*, upper right quadrant). When this complemented strain was grown at 30°C, cell extracts contained PI 3-kinase activity (Fig. 3*C*, lane 1). The substitution in *ScAt*I involves the most conserved domains of the yeast and plant proteins (76% similarity; 58% identity), and this domain contains the protein catalytic activity (3). When a slightly larger part of the plant protein was present in the chimeric construct, complementation failed (Fig. 3*A*, lower right quadrant), and cells grown at 30°C had no detectable PI 3-kinase activity (Fig. 3*C*, lane 3).

**AtVPS34 Is Expressed in Transgenic Cells and Encodes an Active PI 3-Kinase.** The presence of two stretches of amino acid identity (100% match, aa 563–577 and 665–676), defined in Prosite 11 (keyed to SwissProt 27) as specific for PI 3-kinases in *At*VPS34 and the high homology between these proteins, especially in the catalytic domain, suggest a similar enzymatic activity for *At*VPS34. PI 3-kinase activity has never been found in plant protein extracts. To demonstrate *At*VPS34 PI 3-kinase activity in plant extracts, the gene was overexpressed in plants. *A. thaliana* root explants were transformed with an *AtVPS34* construct in which expression was driven by the cauliflower mosaic virus 35S promoter (p9.1S) or with a control vector (pBI121) that lacked the *AtVPS34* gene. The resulting transformed cells were propa-

gated as a callus and also regenerated into plants. These plants appeared normal and had normal seed set; the seeds germinated on kanamycin-containing medium. RNA was extracted from control, pBI121-transformed, and AtVPS34-transformed plants, and a Northern blot was probed with *At*VPS34 (Fig. 4*A*). The same blot was probed with a β-tubulin cDNA as control. Transformed plants highly expressed a 2.8-kb RNA, for which a dilution series is shown in lanes 1–3. Control plants and vector-transformed plants had much lower levels of this mRNA (lanes 5 and 6, respectively). The mRNA was also readily detected in the transformed callus cells at a level considerably above that in control callus (data not shown). Extracts of control and transformed callus were assayed for PI 3-kinase activity (Fig. 4*B*). *AtVPS34*-transformed callus cells contained low, but detectable, levels of PI 3-kinase (lane 3, the radioactivity applied represents enzyme activity 2.5 times more protein for plant extract than for yeast extract). No enzyme activity was found in cells transformed with pBI121 vector alone (lane 2) or in wild-type cells, even when 8 times more protein was used (data not shown). No other differences were detected in the radioactivity-labeled lipids when comparing extracts of control and overexpressing plants. PI 3-kinase activity was very abundant in extracts of yeast cells (lane 1).

***AtVPS34* Encodes an Essential Protein.** To gain insight into the role of *AtVPS34* in plants, the plant gene was inactivated by expressing the cDNA in *A. thaliana* in the antisense orientation (28) using the cauliflower mosaic virus 35S promoter and kanamycin as the selectable marker. Plants expressing the antisense gene are used to determine the phenotype resulting from a lower level or the absence of PI 3-kinase.

Characteristic of the initial transformants ($T_o$) with the antisense construct (p9.1A) was the slower regeneration of shoots (6 weeks instead of 4 weeks) and fewer shoots arising from the calli. About 20 independent shoots were obtained. From seven of these, callus lines were generated and maintained. The regenerated plantlets flowered and after selfing produced seeds; seed set was poor, as was germination of the seeds; from only three plants were fertile seeds obtained. With kanamycin (50 μg/ml) seedlings died within 2 weeks. Without kanamycin, we recovered 11 plants, and the transgene in these plants was shown by PCR.

The three most severely affected $T_1$ plants had leaves that grew more or less normally at first, but the petioles failed to elongate, and no stem was formed. The leaf edges curled

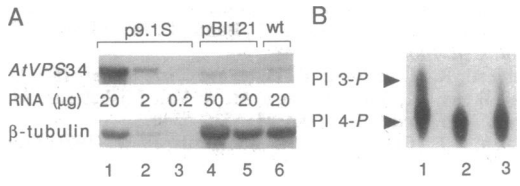


FIG. 4. (*A*) Detection of *At*VPS34 by Northern blotting. Total RNA of shoots of wild-type and transgenic plants were probed with a 1.6-kb C-terminal *Sac* I fragment of *AtVPS34*, detecting a 2.8-kb band. The dilution series to evaluate extent of overexpression (lanes 1–3) suggest a 20- to 50-fold higher *At*VPS34 expression in p9.1S (lane 1) compared with the same amount of RNA in transgenic control (lane 5) or wild-type (lane 6) plants. The blot was reprobed with β-tubulin as internal control. The callus tissue of p9.1S showed a similar increase in *At*VPS34 expression as regenerated p9.1S plants. (*B*) PI 3-kinase activity (PI 3-*P*) of callus tissue overexpressing *At*VPS34. Protein extracts of kanamycin-resistant calli from a transformation with a p9.1S and a control plasmid were assayed for PI 3-kinase activity, and labeled lipids were separated by TLC. Overexpressing *At*p9.1S calli (lane 3) show four times less PI 3-kinase activity than yeast cells (lane 1) normalized to equal PI 4-kinase activity (PI 4-*P*). PI 3-kinase activity is below detection limit in extracts of control calli (lane 2).

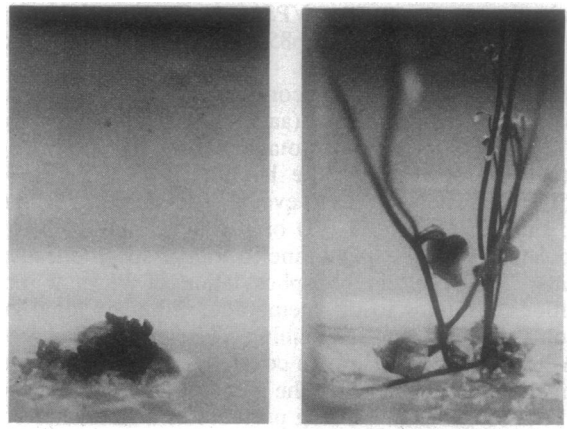

FIG. 5. Phenotype of *At*VPS34 antisense plants. Shown are 3-mo-old plants germinated from seeds of regenerated transgenic plants derived from a transformation with p9.1A. (*Left*) Most severe viable phenotype without shoot elongation. (*Right*) Plant with nearly wild-type phenotype.

inward and dipped into the agar. Then growth stopped, although a callus eventually was formed where small leaves grow out (Fig. 5 *Left*). In a less severe phenotype, (four plants) the leaves were less rolled, and a short shoot formed. In both types, the leaves appeared shiny, and water covered the leaf surface. One plant containing the antisense gene exhibited a nearly wild-type phenotype (Fig. 5 *Right*). The other four plants with wild-type phenotypes were not tested by PCR.

## DISCUSSION

In plant, mammalian, and yeast cells, proteins are synthesized on the endoplasmic reticulum, sorted in the Golgi body, and delivered to the extracellular space or to vacuoles (lysosomes), depending on specific targeting information. This targeting information differs among organisms, but some components of the secretory machinery appear conserved. Indeed, some proteins of the secretory system of plant cells have been identified by complementing yeast secretion mutants (29, 30). The enzyme PI 3-kinase, which catalyzes phosphorylation of PI, is involved in protein sorting in both mammalian and yeast cells (3, 16). In yeast, vps34 mutants secrete vacuolar enzymes, and the protein encoded by *VPS34*, PI 3-kinase, binds directly to a membrane-associated protein kinase encoded by *VPS15*. This protein complex is postulated to regulate the sorting/packaging of vacuolar hydrolases in a late Golgi compartment (8). In mammals, PI 3-kinase plays a role in the internalization of human platelet-derived growth factor receptors and other receptor tyrosine kinases (16).

**Structure and Function of *At*VPS34, an *A. thaliana* PI 3-Kinase.** In addition to size similarity, the homology of *A. thaliana* PI 3-kinase to yeast VPS34 (40% identity) is significantly higher than to mammalian p110 (26% identity). This result favors the conclusion that *At*VPS34 has similar properties as *Sc*VPS34. The C-terminal domains of both proteins contain ATP-binding and kinase motifs that define the presumed catalytic domain. Point mutations in this part of yeast VPS34 abolish any detectable PI 3-kinase activity and do not complement a yeast *vps34* deletion mutant (3). Exchange of the last 255 aa of the yeast protein for the plant sequence created a fusion protein able to restore temperature resistance and PI 3-kinase activity to a yeast *vps34* deletion mutant (Fig. 3).

The N-terminal part of the protein is thought to have a ligand binding and/or regulatory role by interacting with other proteins, such as VPS15 or p85. The low sequence conservation in this domain may account for our failure to complement the yeast mutant, as plant VPS34 may not

efficiently recognize yeast VPS15. The interaction of p110 with its regulatory subunit p85 also maps to this less conserved N-terminal region (2).

Our finding that *At*VPS34 contains a putative lipid-binding domain at its N-terminal end (aa 39–170) similar to that found in p110, rabphilin-3A, synaptotagmin (p65), and several other proteins (2) strengthens the hypothesis that *At*VPS34 is involved in vesicle-transport events. This CaLB domain may help bind the substrate (PI) or it may permit PI 3-kinase association with the membrane to trigger specific transport events. The resulting phosphorylation of PI may recruit additional proteins to the membrane, either by ionic interaction as for the CaLB-containing GTPase activating protein (GAP) protein (2) or through covalent binding as shown for elongation factor (EF) 1$\alpha$. The membrane-bound portion of the pool of this GTP-binding protein has a PI anchor covalently attached to an internal amino acid (31). Another function of this protein is the binding and bundling of F-actin. The recent finding that an EF-1$\alpha$ plant homolog is also a membrane-bound activator of PI 4-kinase (32), together with reports of PI 4-kinases found on the surface of vesicles (14) and isolated together with F-actin (33), may indicate that both kinase activities are crucial in vesicle budding processes, as shown for the internalization of tyrosine receptor kinases by endocytotic events in mammalian cells (2).

**PI 3-Kinase Activity in Plants.** PI 3-kinase activity has so far not been found in plant protein extracts (13), and we also failed to detect this activity in control plant extracts (Fig. 4*B*, lane 2). The existence of PI 3-kinase could be inferred from labeling patterns of lipids extracted from duckweed (5) or guard cells of *Commelina communis* (34) labeled with appropriate radioactive precursors. We detected very low levels of PI 3-kinase activity (compared with PI 4-kinase activity) in transgenic callus cells overexpressing *At*VPS34 (Fig. 4*B*). PI 3-phosphate and PI 4-phosphate were not completely separated as unique spots in this chromatographic system. Nevertheless, we believe that the spot identified as PI 3 is not caused by "fronting" from the PI 4 spot because increased loading (5-fold) did not produce such a spot when extract from an untransformed plant was used. Headgroup analysis, as done for yeast (3), will be necessary to confirm unequivocally that this spot represents PI 3-kinase activity. mRNA for the enzyme was more readily detected than enzyme activity (Fig. 4*A*). The low level of active enzyme may be caused by rapid inactivation in cell extracts or the absence of a required activation step, leaving most of the enzyme inactive, as occurs in *Sc*VPS34-overexpressing yeast cells (3). *Sc*VPS34 appears to be activated by the VPS15 protein kinase (8). If plants also contain such a protein kinase, its function may be impaired in the cell extracts or it may be present in very low levels, insufficient to fully activate the overexpressed *At*VPS34.

**What Is the Role of PI 3-Kinase?** To gain insight into the role of PI 3-kinase, we made plants that express antisense *At*VPS34 mRNA. Seedlings grown of such plants grew extremely poorly, and in many cases the plants died, indicating that loss of *At*VPS34 function results in a lethal phenotype. According to what is known about PI 3-kinases in yeast and mammals, such a phenotype could have two different underlying causes. In mammals, PI 3-kinase has been associated mostly with signal-transduction pathways because it is essential for internalization and degradation of tyrosine receptor kinases (16) and has high affinity for phosphatidyl 4,5-bisphosphate, the precursor of inositol 1,4,5-trisphosphate (2). Therefore, the lethal phenotype of the plants may indicate a serious disruption of essential signal-transduction pathways. These results with animal cells implicate PI 3-kinase in vesicle transport: the platelet-derived growth factor receptor must be complexed with PI 3-kinase for proper endocytosis of these receptors (16). In yeast, PI 3-kinase is essential for vesicle-mediated delivery of proteins to the vacuole (3). Therefore, *At*VPS34 may serve an essential role in similar targeting events and, as a consequence of their defect, prevent normal plant development and growth in antisense plants.

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