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A Small GTP-Binding Protein from *Arabidopsis thaliana* Functionally Complements the Yeast *YPT6* Null Mutant[1]

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A clone designated *A.t.RAB6* encoding a small GTP-binding protein was isolated from a cDNA library of *Arabidopsis thaliana* leaf tissue. The predicted amino acid sequence was highly homologous to the mammalian and yeast counterparts, H.Rab6 and Ryh1/Ypt6, respectively. Lesser homology was found between the predicted *Arabidopsis* protein sequence and two small GTP-binding proteins isolated from plant species (44% homology to *Zea mays* Ypt1 and 43% homology to *Nicotiana tabacum* Rab5). Conserved stretches in the deduced amino acid sequence of A.t.Rab6 include four regions involved in GTP-binding, an effector region, and C-terminal cysteine residues required for prenylation and subsequent membrane attachment. Northern blot analysis demonstrated that A.t.Rab6 mRNA was expressed in root, leaf, stem, and flower tissues from *A. thaliana* with the highest levels present in roots. *Escherichia coli* produced histidine-tagged A.t.Rab6 protein-bound GTP, whereas a mutation in one of the guanine nucleotide-binding sites (asparagine[122] to isoleucine) rendered it incapable of binding GTP. Functionally, the *A.t.RAB6* gene was able to complement the temperature-sensitive phenotype of the *YPT6* null mutant in yeast. The isolation of this gene will aid in the dissection of the machinery involved in soluble protein sorting at the *trans*-Golgi network of plants.

In eukaryotic cells, GTP-binding proteins function in a wide variety of cellular processes, including signal transduction, cytoskeletal organization, and protein transport. Genes encoding small GTP-binding proteins (21–25 kD) related to the mammalian Ras proteins have been identified in many organisms, including insects, yeast, slime molds, and plants (Barbacid et al., 1987; Terryn et al., 1993). Recent evidence supports a role for Ras-related GTP-binding proteins in the vesicular transport of secretory proteins (for review, see Pryer et al., 1992). Morphological and biochemical studies in yeast and mammalian cells have demonstrated that different small GTP-binding proteins are associated with each distinct subcompartment of the exocytic and endocytic pathways (Pryer et al., 1992). Furthermore, vesicular transport between each compartment of the secretory pathway may require several distinct small GTP-binding proteins. In the yeast cerevisiae, at least two Ras-like GTP-binding proteins, Sar1 and Ypt1, are essential for the vesicular transport between the ER and Golgi complex (Pryer et al., 1992). In vitro, Sar1 is required for vesicle formation, whereas Ypt1 is subsequently required for targeting and/or fusion of ER-derived transport vesicles with the Golgi (Pryer et al., 1992; Barlowe et al., 1993).

Members of the yeast *YPT* and mammalian *RAB* gene families share a high degree of sequence identity (Pryer et al., 1992). In fact, mammalian Rab1a and Rab6 can replace the Ypt1 and Ryh1/Ypt6 proteins, respectively, in yeast (Haubruck et al., 1989; Hengst et al., 1990). Several genes encoding small GTP-binding proteins related to the yeast *YPT* and the mammalian *RAB* genes have been identified in plants (for review, see Terryn et al., 1993). In addition, the identification of a gene from *Arabidopsis thaliana* homologous to *SAR1* from yeast (d'Enfert et al., 1992) suggests that the mechanisms regulating vesicular transport are conserved among eukaryotes.

Recently, several groups have identified and characterized targeting signals for different plant vacuolar proteins (for review see Bednarek and Raikhel, 1992; Chrispeels and Raikhel, 1992; Dombrowski et al., 1993). Similar to mammalian and yeast cells, vacuolar proteins containing these signals are sorted within the plant Golgi complex or TGN and transported via carrier vesicles to the vacuole (Bednarek and Raikhel, 1992). Given the importance of small GTP-binding proteins in vesicular trafficking of secretory proteins, we initiated a search for *trans*-Golgi-associated Ypt/Rab proteins that play a role in this process. A potential candidate is the plant homolog of the mammalian small GTP-binding protein Rab6, homologous to Ryh1 in *Schizosaccharomyces pombe* and Ypt6 in *S. cerevisiae*. Subcellular localization of Rab6 has shown it to be associated with the medial and *trans*-Golgi cisternae as well as with the TGN (Goud et al., 1990; Antony et al., 1992). Furthermore, disruption of the *S. cerevisiae* homolog of *RAB6, YPT6*, resulted in the partial missorting of vacuolar proteins (L. Hengst and D. Gallwitz,

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Abrreviations: GAP, GTPase-activating protein; TGN, *trans*-Golgi network; ts, temperature sensitive.

unpublished data). We report here the isolation and characterization of a cDNA clone termed *A.t.RAB6* from *A. thaliana* whose protein product is highly homologous to the mammalian Rab6 and yeast Ryh1/Ypt6 proteins.

MATERIALS AND METHODS

All standard recombinant DNA techniques were performed as described by Sambrook et al. (1989) unless otherwise noted.

PCR, cDNA Library Screening, and DNA Sequencing

An *Arabidopsis thaliana* leaf cDNA library constructed in λZap II was a generous gift of Eric Ward (Ciba-Geigy). λZAP II phage DNA was isolated for PCR, and degenerate oligonucleotide sequences used for PCR were derived from conserved regions of human Rab6 and *Schizosaccharomyces pombe* Ryh1. Nucleotide sequences of the "effector region" primer and the amino acid stretch (LWIDEV) were as follows: (a) 5′ GAA TTC TA(CT) CA(AG) GC(AGCT) AC(AGCT) AT(ACT) GG 3′, (b) 5′ TAT AGG ATC CAG (AG)TC (AGCT)TC (AGT)AT CCA (CT)TT 3′. A 209-bp PCR product was purified from low melting point agarose and subcloned into pUC119 (Vieira and Messing, 1987) for further analysis. The *A. thaliana* library was screened using the radiolabeled (^{32}P) 209-bp PCR product prepared by random primer labeling. Recombinant plaques were screened by plaque hybridization, and λZAP II clones were converted to pBluescript phagemids (Stratagene). Nucleotide sequences of isolated clones and mutant constructs were determined by the dideoxy chain termination method (Sanger et al., 1977).

Northern and Southern Analysis

A. thaliana plant genomic DNA was isolated according to the method of Dellaporta et al. (1983), and DNA hybridization was performed using high-stringency conditions (Lerner and Raikhel, 1992). Total RNA from different organs of *A. thaliana* was isolated according to the method of Newman et al. (1993), and subsequent isolation of poly(A^+) RNA was performed using the QuickPrep mRNA purification kit (Pharmacia). Root RNA was isolated from plants grown in liquid culture. mRNA (5 μg) was run on 1.2% formaldehyde-agarose gels and transferred to Hybond-N nylon filters (Amersham). Filters were hybridized with ^{32}P random primer-labeled *A.t.RAB6* cDNA as described by Lerner and Raikhel (1992).

A.t.Rab6 Antibody Production

For expression in *Escherichia coli,* the 209-bp PCR product was subcloned into pET14b (Novagen). In addition to the His leader, the fusion protein also contained an additional C-terminal segment that was encoded by the expression vector. The construction was transformed into *E. coli* strain BL21(DE3)LysS. Following isopropylthio-β-galactoside induction, the His-tagged fusion protein formed inclusion bodies. Solubilization from inclusion bodies and affinity purification of the fusion protein under denaturing conditions on immobilized nickel was performed according to manufacturer's instructions (Novagen).

The purified fusion protein containing the polypeptide encoded by the *A.t.RAB6* 209-bp PCR product was used as an antigen to raise polyclonal antisera in rabbits. Rabbits were immunized by subcutaneous injection with 200 μg of protein in Hunters Titer Max (CytRx Corp.) and a booster injection was given every 2 weeks.

Mutagenesis and Bacterial Overexpression

A.t.rab6 mutant constructs were prepared by site-directed mutagenesis (Kunkel et al., 1987; Dombrowski et al., 1993), and mutations were confirmed by sequencing. To facilitate subcloning of wild-type *A.t.RAB6* and mutant *A.t.rab6* in the *E. coli* expression vector pET14b, *Nde*I and *Bgl*II restriction sites were introduced by site-directed mutagenesis at the 5′ and 3′ ends, respectively. Constructions in pET14b were expressed in *E. coli* strain BL21(DE3)LysS following induction by isopropylthio-β-galactoside. N-terminal His-tagged A.t.Rab6 fusion proteins were purified according to manufacturer's instructions (Novagen) for soluble proteins.

Immunoblot Analysis and GTP-Binding Assay of A.t.Rab6 and Mutant Proteins

Microsomal membranes of *A. thaliana* were prepared (Lawson et al., 1989), and proteins were separated by SDS-PAGE, electroblotted onto nitrocellulose, and reacted with the A.t.Rab6 antibody (1:750 dilution). The GTP-binding assays were performed according to the procedure of Wagner et al. (1992).

Complementation of the Yeast *YPT6* Mutant

Wild-type (*A.t.RAB6*) and mutant (*A.t.rab6* [N^{122} to I]) *Nde*I/*Xho*I fragments were blunt-end ligated into the *Sal*I site of the YEPH vector. This vector was produced from Yep51 (Broach et al., 1983) by deleting the *Leu2* gene with PflM1 and inserting the *His3 Bam*HI fragment. In this construct, the cloned genes are under the control of the GAL10 promoter. For the yeast transformation (Ito et al., 1983), the strains GL72 (MATα,*leu2,his3,YPT6::LEU2*) and GL74 (MATα,*leu2,his3,YPT6*) were used. For testing the ts phenotype, the strains were grown at 30 or 37°C on 15 g L^{-1} of agar, 10 g L^{-1} of yeast extract, 20 g L^{-1} of peptone, and 20 g L^{-1} of Glc or Gal for 3 d.

RESULTS

Isolation of a cDNA Clone *A.t.RAB6*

Ras and the Ras-related proteins encoded by the *YPT* and *RAB* gene families display a high degree of sequence conservation. These proteins share four highly conserved regions that form the GTP-binding site and a conserved C terminus containing Cys residues essential for membrane association (Balch, 1990). In addition, these proteins contain an effector region that in H-Ras (p21) has been shown to be at the surface of the protein (Pai et al., 1989) and to interact with GAPs (Bourne et al., 1990, 1991). GTP-binding proteins that functionally complement one another such as Ryh1/Ypt6 and H.Rab6 share identical effector regions. To identify the

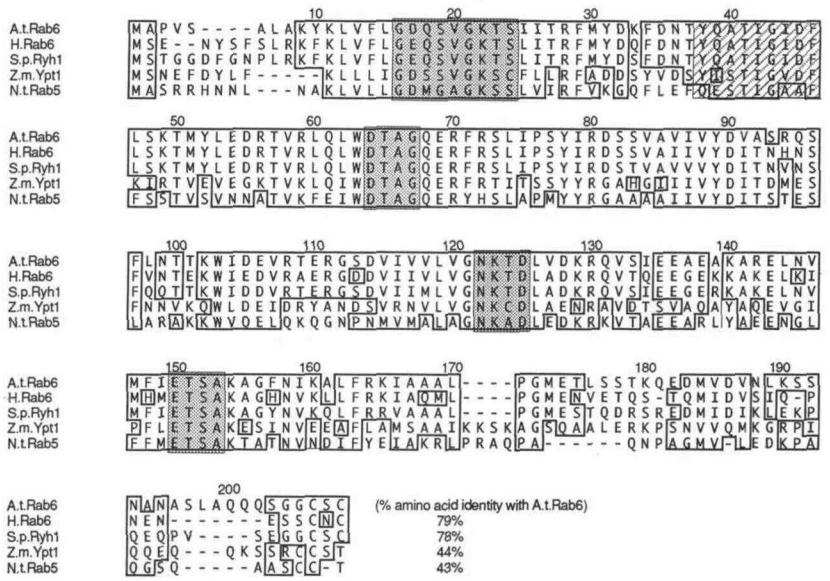

Figure 1. Alignment of *A. thaliana* (A.t.) Rab6 protein sequence with the human (H.) Rab6, *S. pombe* (S.p.) Ryh1, *Z. mays* (Z.m.) Ypt1, and *N. tabacum* (N.t.) Rab5 proteins. Alignments were generated using CLUSTAL V multiple sequence alignment software. Percentages of amino acid identities between protein sequences were calculated using the BLAST algorithm developed by the National Center for Biotechnology Information at the National Library of Medicine. Sets of identical or conservative residues are boxed. Amino acid sequences that interact with the bound guanine nucleotide are shaded, and the effector region residues are hatched. The reference numbering is that of A.t.Rab6.

plant homolog of *H.RAB6* (Zahraoui et al., 1989) and yeast *RYH1/YPT6* (Hengst et al., 1990), two degenerate oligonucleotide primers specific for the *RAB6/RYH1* effector-coding region and another conserved amino acid stretch, amino acids 102 to 107 (KWIDEV), were used to prime PCR amplification of DNA prepared from a λZap II *Arabidopsis* leaf cDNA library. Sequence analysis revealed that the PCR product encoded a polypeptide with extensive homology to regions from H.Rab6 and *S. pombe* Ryh1.

To isolate a full-length cDNA clone, the 209-bp PCR product was used as a probe to screen an *Arabidopsis* cDNA library. Nucleotide sequence analysis revealed a 513-nucleotide open reading frame with 104 bp flanking the 5′ region and 285 bp at the 3′ end (data not shown, accession No. L29684). The motifs AAAAAAT and AAAAT [located 217 and 163 nucleotides upstream, respectively, of the poly(A) tail] are possible polyadenylation signals. Sequence analysis of a clone, designated *A.t.RAB6*, revealed a long open reading frame coding for a 208-amino acid protein of predicted molecular mass 23,129 D.

Sequence Analysis of the Protein Encoded by *A.t.RAB6*

Predicted amino acid sequences of the A.t.Rab6 protein were aligned with human Rab6, *S. pombe* Ryh1, *Zea mays* Ypt1 (Palme et al., 1992), and *Nicotiana tabacum* Rab5 (Dallmann et al., 1992) using CLUSTAL V multiple sequence alignment software (Fig. 1). A.t.Rab6 shared 79 and 78% amino acid identity with its human and fission yeast counterparts, Rab6 and Ryh1 proteins, respectively. The degree of similarity was lower for the two plant GTP-binding proteins; *Zea mays* Ypt1 (44% amino acid identity), and *N. tabacum* Rab5 (43% amino acid identity). Similarly, lower sequence identities were observed between members of the Ypt/Rab protein families that perform different functions. The four regions required for nucleotide binding (Fig. 1, shaded area), characteristic of Rab proteins in sequence and spacing, are present in the A.t.Rab6 protein. These sequence segments, GDQSVGKTS (amino acids 16–24), DTAG (amino acids 64–67), NKTD (amino acids 122–125), and ETSA (amino acids 150–153), were identical with those in the other four proteins.

The effector region for the A.t.Rab6, H.Rab6, and Ryh1 sequences are completely alike. Amino acids that encode the effector region (38–46; YQATIGIDF, Fig. 1, hatched region) are thought to interact with the GAP specific for the Rab6 and Ypt6 class of GTP-binding proteins (Strom et al., 1993). Also, the C-terminal region of A.t.Rab6 possesses the CXC motif (X denotes any other amino acid) common to other Ypt/Rab proteins, which serves as a substrate for posttranslational modification through isoprenylation (Khosravi-Far et al., 1991).

Northern and Southern Analyses

The organ-specific expression of the *A.t.RAB6* was examined by RNA gel blot analysis of mRNA from leaf, root, stem, and flower tissues of *Arabidopsis*. A single 1-kb band corresponding to *A.t.RAB6* mRNA was detected in all tissues, and

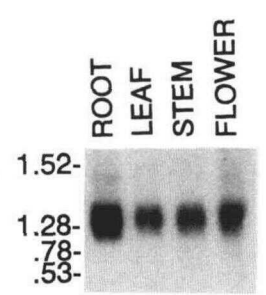

Figure 2. Organ-specific expression of A.t.Rab6 mRNA. *A. thaliana* poly(A$^+$) RNA (5 μg) was separated in a formaldehyde gel, transferred to a nylon Hybond N filter, and hybridized with a ^{32}P-labeled cDNA insert of A.t.*RAB6*. Molecular mass markers (kb) are shown to the left.

highest expression was present in root tissue from liquid culture-grown roots (Fig. 2).

To investigate the number of related genes in *Arabidopsis*, Southern blot analysis was performed. Total genomic DNA of *A. thaliana* was digested with the restriction enzymes *Eco*RI, *Bam*HI, *Hin*dIII, and *Pvu*II and hybridized with the ^{32}P-labeled cDNA probe under stringent washing conditions (Fig. 3). The *A.t.RAB6* cDNA has one internal *Pvu*II restriction site, whereas *Eco*RI, *Hin*dIII, and *Bam*HI do not cut within the sequence. The *Bam*HI and *Hin*dIII restrictions resulted in one band, and the *Pvu*II restriction gave two bands. However, a low molecular mass band appeared in the *Eco*RI digestion, indicative of the presence of a small multigene family or an intron in the genomic DNA sequence. Previously, strong hybridization signals were seen in *S. pombe* genomic DNA digestions, using a *RYH1* cDNA probe, that were later shown to be a *SEC4* gene (L. Hengst, personal communication). The presence of many highly conserved DNA sequences in *YPT/RAB* genes may explain observed cross-hybridizations. Further restriction analyses will be performed to distinguish between these possibilities.

Membrane Association of A.t.Rab6 in *Arabidopsis* Tissue

Antibodies specific for A.t.Rab6 were raised against an *E. coli*-produced fusion protein containing amino acids 38 to 107 of A.t.Rab6, which include the effector region and the second GTP-binding domain. The polyclonal sera detected the *E. coli*-produced fusion protein as well as a protein of approximately 24 kD in a microsomal membrane fraction from *Arabidopsis* leaves (Fig. 4a). This increase in molecular mass over the expected 23 kD may be attributed to the addition of a His leader in the *E. coli*-produced protein and the presence of a prenylated C terminus in the *A. thaliana* membrane fraction. Previous results have shown that mammalian Rab6 is tightly associated with membranes (Goud et al., 1990). Like the mammalian and yeast Ypt/Rab proteins, A.t.Rab6 contains a C-terminal CXC motif (206–208) that will likely serve as a substrate for posttranslational prenylation that promotes membrane association (Khosravi-Far et al., 1991). The association of A.t.Rab6 with different membrane fractions and the function of membrane association is currently under investigation.

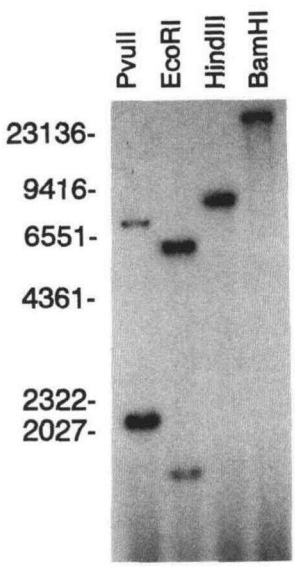

Figure 3. Southern blot analysis of *A. thaliana* genomic DNA. *Arabidopsis* DNA (2 μg) was digested with *Pvu*II, *Eco*RI, *Bam*HI, and *Hin*dIII, separated on a 0.7% agarose gel, and transferred to Hybond N filter. A ^{32}P-labeled cDNA insert of *A.t.RAB6* was hybridized to the filter under high-stringency conditions. Molecular mass markers (kb) are shown on the left.

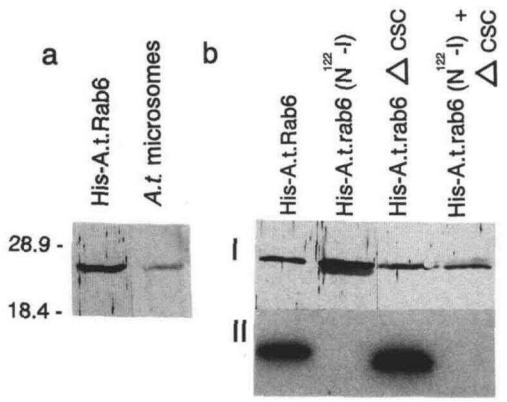

Figure 4. Fusion proteins of the wild-type (His-A.t.Rab6) contruct, the mutant His-A.t.rab6 (Asn^{122}–Ile), the mutant His-A.t.rab6 ΔCSC, and the double mutant [His-A.t.rab6 (Asn^{122}–Ile) plus ΔCSC] were produced in *E. coli*. a, Immunodetection of His-A.t.Rab6 protein and a microsomal membrane fraction from *A. thaliana* using the A.t.Rab6 antibody. Molecular mass markers (kD) are shown on the left. b, *E. coli*-produced proteins were separated by SDS-PAGE, electroblotted onto nitrocellulose, and reacted with (I) A.t.Rab6 antibody or (II) [α-^{32}P]GTP as described by Palme et al. (1992).

Expression of A.t.Rab6 Protein in *E. coli*

GTP-binding proteins possess the biochemical property of binding and hydrolyzing GTP. The A.t.Rab6 protein shares extensive homology to known GTP-binding proteins in regions thought to be involved in nucleotide binding and GTPase activity (Fig. 1). The ability of A.t.Rab6 to bind GTP was analyzed on blots of immobilized protein produced in *E. coli* (His-A.t.Rab6). As shown in Figure 4a, guanine nucleotide binding could be observed with the purified His-A.t.Rab6 protein. Mutational and structural analysis of the Ras superfamily of GTP-binding proteins have identified essential amino acid residues required for guanine nucleotide binding, nucleotide exchange, and hydrolysis of GTP and membrane attachment (Bourne et al., 1990, 1991). Three mutations were made to the wild-type *A.t.RAB6* gene: (a) a mutant that converted Asn^{122} to Ile in the third GTP-binding domain, (b) a mutant in which the membrane attachment sequence at the C-terminal region was deleted, and (c) a double mutant containing both of the above mutations. Mutant proteins containing a His leader were overproduced in *E. coli* and purified by Ni^{2+} affinity chromatography. All His-A.t.Rab6 proteins were recognized by the antibody on western blots

(Fig. 4b, panel I). In addition, analysis of guanine nucleotide binding of immobilized A.t.Rab6 mutants indicated that the (Asn^{122}–Ile) and double mutation rendered the protein unable to bind GTP, whereas the C-terminal mutant retained GTP-binding capability (Fig. 4b, panel II). The mutations of this protein result in characteristic alterations in the biochemical properties of a plant protein that have been observed for mutations of comparable mammalian and yeast counterparts (Wagner et al., 1987; Walworth et al., 1989; Tisdale et al., 1992).

A.t.RAB6 Functionally Complements the Yeast *YPT6* Mutant

The ts phenotype, resulting from *S. pombe RYH1* and *Saccharomyces cerevisiae YPT6* gene disruption, can be suppressed by the human Rab6 protein (Hengst et al., 1990; L. Hengst and D. Gallwitz, unpublished data). Therefore, it was of considerable interest to determine the functional conservation of the *A. thaliana* protein. The *A.t.RAB6* gene and the *A.t.rab6* (Asn^{122}–Ile) mutant were expressed in yeast under the control of the GAL10 promoter. Both constructs were transformed into the *S. cerevisiae* strain G172 to obtain the strains GAL10-*A.t.RAB6* and GAL10-*A.t.rab6*. The *YPT6* null mutant cells were unable to grow at 37°C on either Glc- or Gal-containing media but were unaffected on both media at 30°C. When the GAL10-*A.t.RAB6* strain was grown on Glc-containing media at 37°C, no observable growth was detected; yet plating onto Gal-containing media resulted in the induction of the Gal-inducible promoter and subsequent survival of the strain due to complementation of the null mutant yeast strain with the A.t.Rab6 protein (Fig. 5). Expression of the mutant protein (Asn^{122}–Ile) on Gal-containing media at 37°C did not result in recovery of the phenotype. Therefore, the A.t.Rab6 protein was able to functionally complement the *S. cerevisiae YPT6* null mutant.

DISCUSSION

We have isolated a cDNA clone, *A.t.RAB6*, from an *Arabidopsis* cDNA library encoding a protein with high homology to the mammalian Rab6 and yeast Ryh1/Ypt6 proteins. *A.t.RAB6* encoded a 208-amino acid polypeptide with a corresponding molecular mass of 23.1 kD. The amino acid sequence contains the Ypt/Rab consensus motifs required for GTP binding and hydrolysis. Biochemically, we have demonstrated that the *E. coli*-expressed A.t.Rab6 was able to bind GTP. Mutational and structural analysis of the Ras superfamily of GTP-binding proteins have indicated that an Asn residue corresponding to Asn^{122} in the H.Rab6 protein interacts with the guanine nucleotide ring (Pai et al., 1989). Substitution of the highly conserved Asn^{122} for Ile rendered the protein incapable of binding GTP, suggesting that A.t.Rab6 shared similar structural properties with other Ras-like GTP-binding proteins.

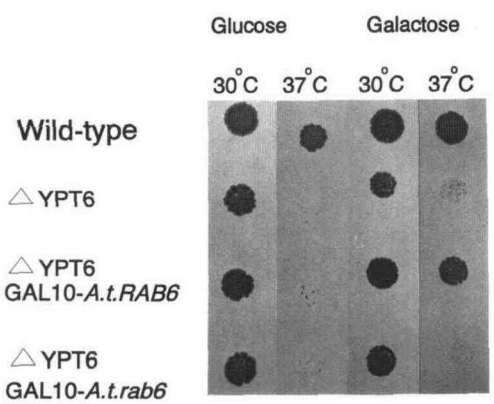

Figure 5. Complementation of the ts phenotype of *S. cerevisiae YPT6* cells by the A.t.Rab6 protein. Wild-type cells, Δ*YPT6* null mutant cells, and Δ*YPT6* mutant cells carrying GAL10-*A.t.RAB6* and Gal10-*A.t.rab6* were cultured on Glc- and Gal-containing medium at 30 or 37°C.

The observed membrane association of A.t.Rab6 indicated that the C-terminal CXC of A.t.Rab6 may serve as a substrate for posttranslational isoprenylation and subsequent membrane attachment. The majority of prenylated proteins, including the Rab proteins in mammalian cells, are modified by C_{20} geranylgeranyl groups (Farnsworth et al., 1990; Khosravi-Far et al., 1991); however the isoprenyl modifications of proteins in plants have yet to be characterized. The A.t.Rab6 mutant that lacked the C-terminal CXC residues did not have altered guanine nucleotide-binding properties. However, previous experiments have shown that the C-terminal Cys residues were required for membrane association and biological activity of Ypt/Rab proteins in vivo (Khosravi-Far et al., 1991).

The C-terminal regions (termed hypervariable) of Ras-like GTP-binding proteins vary greatly in both sequence and length, and they function as signals for the localization of Rab proteins to their appropriate target membranes (Chavrier et al., 1991). The homology between C-terminal regions (amino acids 195–209) of A.t.Rab6, Z.m.Ypt1 and N.t.Rab5, which are likely to have different subcellular locations in the plant secretory pathway, was low, 29 and 21%, respectively. Although the A.t.Rab6, H.Rab6, and Ryh1/Ypt6 proteins shared 80% homology throughout the length of the polypeptide, the C-terminal regions of A.t.Rab6, H.Rab6, and Ryh1/Ypt6 shared 36 and 42% homology, respectively. Despite these differences, A.t.Rab6 could functionally complement the *ypt6* mutant.

Another region, the effector domain of A.t.Rab6, was identical with its human and yeast counterparts. Interaction of the effector domain with GAPs was required for the regulation of GTP hydrolysis (Bourne et al., 1991). The gene encoding the GAP specific for Ypt6 from *S. cerevisiae* has recently been cloned (Strom et al., 1993). It will be of interest to determine whether the Ypt6 GAP, Gyp6, can regulate GTP hydrolysis of A.t.Rab6 and to determine the subcellular location and function in plant secretory protein transport.

Overexpression of mutant GTP-binding defective mutants of Ypt/Rab proteins, which exhibit *trans*-dominant secretory defects (Tisdale et al., 1992), has been used to define a functional role in secretion. Subcellular localization of H.Rab6 has shown that it is distributed from the medial Golgi to the TGN (Goud et al., 1990; Antony et al., 1992). Overexpression of a dominant rab6 mutant protein, however, failed to inhibit vesicular transport of stomatitis virus glycoprotein through the mammalian Golgi apparatus to the *trans*-Golgi compart-

ment (Tisdale et al., 1992). This suggests that Rab6 may not function in transport within distinct compartments of the Golgi. Furthermore, Ryh1 and Ypt6 are not essential for cell viability, and secretion of invertase is not blocked in *S. pombe* ryh1 and *S. cerevisiae* ypt6 mutants (Hengst et al., 1990; L. Hengst and D. Gallwitz, unpublished data).

To test whether A.t.Rab6 is functioning in protein sorting to the plant vacuole, we will try to disrupt targeting using the Asn^{122}–Ile mutant introduced in this paper as well as antisense constructs of the wild-type sequence stably transformed into tobacco and *Arabidopsis* containing barley lectin as a reporter protein. Using these plants, we will determine whether barley lectin is correctly sorted to the vacuole.

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