The TIP GROWTH DEFECTIVE1 S-Acyl Transferase Regulates Plant Cell Growth in Arabidopsis

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INTRODUCTION

The mechanisms controlling plant cell growth, and hence cell shape, are beginning to be elucidated (reviewed in Smith, 2003). Studying root hair mutants of Arabidopsis thaliana provides an empirical approach to finding new growth mechanisms. Root hair cells undergo dramatic shape changes involving both diffuse growth during the initial stages of root hair formation and highly polarized tip growth during root hair elongation. Numerous genes affect root hair growth (Grierson and Ketelaar, 2004), including genes with functions in auxin transport (Vicente-Agullo et al., 2004), signaling (Jones et al., 2002; Foreman et al., 2003), the cytoskeleton (Ringli et al., 2002), trafficking (Böhme et al., 2004; Zheng et al., 2004), and cell wall synthesis (Favery et al., 2001; Seifert et al., 2002).

The Arabidopsis TIP GROWTH DEFECTIVE1 (TIP1) gene was identified in visual screens for altered root hair phenotype. Two mutant alleles of TIP1 have been identified previously: tip1-1 (Schiefelbein et al., 1993) and tip1-2 (Ryan et al., 1998). The phenotype of the tip1-2 mutant is more severe than that of tip1-1, suggesting that it is the stronger allele (Schiefelbein et al., 1993, Ryan et al., 1998). The root hairs of Tip1– mutant plants are wider and approximately one-tenth the length of wild-type root hairs, and unlike wild-type root hairs, they are often branched, with up to four hairs emerging from one large initiation site. Tip1– mutants are also affected in pollen tube germination and growth, resulting in reduced male transmission (Schiefelbein et al., 1993; Ryan et al., 1998). In addition to the effects on these tip-growing cells, TIP1 also affects cell size, as shown by the tip1-2 mutant, which has shorter epidermal cells in the stem (Ryan et al., 1998), providing evidence for overlap in the processes of diffuse and tip growth in plants. TIP1 affects organ size throughout the plant, as shown by the tip1-2 mutant, which has shorter internodes, reduced height, and smaller rosettes (Ryan et al., 1998). In a large analysis of the genetic interactions during root hair morphogenesis, TIP1 was shown to contribute to root hair formation much earlier than originally thought and was the only gene of 14 studied that contributed to every stage of root hair development, from initiation through tip growth (Parker et al., 2000).

Because TIP1 plays a role in both tip and diffuse cellular growth, it is likely to play a central role in regulating plant cell shape. Here, we report the molecular identification and characterization of TIP1 and show that Tip1 is an S-acyl transferase (also known as palmitoyl transferase), demonstrating that S-acylation regulates plant cell growth.

RESULTS

Positional Cloning of the TIP1 Gene

TIP1 has previously been mapped to the top of chromosome 5, 8.6 centimorgan from CSH1 and 26.1 centimorgan from PHYC (Ryan et al., 1998). Fine-mapping followed by biolistic complementation (Kemp et al., 2001) of the phenotype conferred by the tip1-2 mutant with large insert clones spanning the TIP1 region (http://www.kazusa.or.jp/kaos/ and http://www.mpimp-golm.mpg.de/101/igf_bac_cont.html) identified two overlapping clones (Figure 1A). Further biolistic complementation using subclones identified a 22-kb subclone of BAC K19B10 that could complement the Tip1– phenotype and that contained four candidate genes. To determine which of the four genes encoded TIP1, direct
sequencing of PCR products from tip1-1 and tip1-2 mutants was performed. The nucleotide sequence of one gene, At5g20350, differed from the published wild-type sequence in tip1-1 and tip1-2 (Figure 1B). To confirm that At5g20350 was the TIP1 gene, a 6.1-kb genomic region containing At5g20350 and 1.6 kb of upstream sequence was cloned into the binary vector pCAMBIA 2200. Full-length At5g20350 cDNA was also placed under the control of the 35S promoter. Both the genomic and Pro35S:TIP1 cDNA constructs complemented the phenotype conferred by the tip1-2 mutant when transformed transiently by biolistic methods and stably by floral dipping, restoring normal root hair growth (Figure 1C), root epidermal cell size (trichoblast long axis lengths were as follows: wild type, 228 ± 43 µm; tip1-2, 131 ± 23 µm; tip1-2 Pro35S:TIP1 cDNA, 236 ± 50 µm), plant stature, and fertility (data not shown). A SALK T-DNA line with an insertion in At5g20350 had a strong Tip1/C255 phenotype similar to that of tip1-2 (Figures 2A and 2B) and did not complement tip1-2 in complementation tests. This line was designated tip1-3. Together, these data demonstrate that TIP1 is At5g20350.

Several stable transformants carrying Pro35S:TIP1 had significantly longer root hairs than did the wild type (average length: wild type, 622 ± 148 µm; Pro35S:TIP1, 1002 ± 101 µm), indicating that TIP1 overexpression can increase root hair length.
Sequence and Expression of *TIP1* in Mutant and Wild-Type Plants

*TIP1* cDNA was sequenced, and exons 5 and 6 were both longer than predicted by public databases (Figure 1B), making the *TIP1* cDNA 1863 bp long and predicted to encode a 68-kD protein of 620 amino acids (see Supplemental Figure 1 online). The predicted *TIP1* protein contains an N-terminal region with six ankyrin repeats, four transmembrane domains, and a DHHC Cys-rich domain (DHHC-CRD) between transmembrane domains 3 and 4, as predicted by CDART (http://www.ncbi.nlm.nih.gov/structure/...\n
Figure 3. *TIP1* Shows Homology with Other Proteins from Yeast and Mammals.

The greatest homology is observed around the region of the DHHC motif (indicated by asterisks). *TIP1* (*Arabidopsis*), HIP14 (human), AKR1 (yeast), ERF2 (yeast), GODZ (mouse). *TIP1* shows greater homology with HIP14, ERF2, and GODZ within the DHHC-CRD than to AKR1p. Residues that are identical in three or more of the five sequences are shaded in black. Conservative substitutions at an amino acid position that occur in three or more of the five sequences are shaded in gray.
TIP1 is homologous with various proteins in animals, plants, and fungi, all of which are predicted to contain six ankyrin repeats and a DHHC-CRD and to be integral membrane proteins. The DHHC-CRD appears to be highly conserved across all species studied (Figure 3). TIP1 appears to be the only ankyrin repeat-containing DHHC-CRD gene in the Arabidopsis genome. Two adjacent predicted genes on chromosome 2, At2g14250 and At2g14255, show strong homology with the 5’ and 3’ regions of TIP1, respectively. Two alternatively spliced transcripts, each containing both putative genes, are detectable by PCR using oligo(dT)-primed cDNA, indicating that these two predicted genes are actually transcribed as a single unit, but sequencing shows that the transcripts contain numerous in-frame stop codons, suggesting that this is a transcribed pseudogene (data not shown). Arabidopsis contains 22 predicted DHHC-CRD genes lacking the ankyrin repeats plus a predicted alternative splice form of one of the genes organized into two large groups, plus two pairs of similar genes and five less closely related genes (see Supplemental Figure 2 online). All of the DHHC-CRD genes in Arabidopsis show high homology around the DHHC-CRD domain but show variation outside of this domain (see Supplemental Figure 3 online). TIP1 also shows homology with DHHC-CRD proteins from other eukaryotic organisms, such as AKR1p and ERFp from yeast (Saccharomyces cerevisiae), HIP14 from human (Homo sapiens), and GODZ from mouse (Mus musculus) (Figure 3).

Genomic sequencing of TIP1 in the three mutants revealed that tip1-1 carries a G/A transition in the splice site between exon 10 and intron 10 and tip1-2 carries a Y41stop nonsense mutation. tip1-3 contains a SALK T-DNA insertion in the 3’ end of the DHHC-CRD (Figure 1B).

TIP1 is expressed in root, leaf, inflorescence stem, and floral tissue of wild-type plants, as determined by RNA gel blot analysis (Figure 4A). As every organ in tip1-2 mutants is smaller than that in the wild type (Ryan et al., 1998), it is likely that TIP1 is expressed throughout the plant. This conclusion is supported by the publicly available microarray experiments held by The Arabidopsis Information Resource (TAIR; http://www.arabidopsis.org). tip1-1 produces a wild-type sequence cDNA, but transcript levels are greatly reduced compared with those of the wild type. tip1-2 also shows a second, slightly larger transcript detectable by RNA gel blot analysis (Figure 4) and RT-PCR (data not shown) of the size expected if intron 10 splicing is inefficient. Full-length tip1-2 cDNA can be retrieved by RT-PCR and contains the Y41stop mutation observed in the genomic DNA (data not shown). Very little tip1-2 transcript is detectable by RNA gel blot (Figure 4B), suggesting that the premature stop codon may lead to nonsense-mediated decay (reviewed in Baker and Parker, 2004). tip1-3 has no detectable transcript in RNA gel blots (Figure 4B) or by RT-PCR (data not shown). Therefore, tip1-2 and tip1-3 are probably null alleles.

### TIPT1 Is an S-Acyl Transferase

#### TIPT1 Complements Yeast Cells Deficient in the AKR1p S-Acyl Transferase

TIP1 is a plant member of an evolutionarily conserved group of proteins that contains six ankyrin repeats and a DHHC-CRD and that are predicted to be integral membrane proteins. Two members of this group, S. cerevisiae AKR1p and human HIP14, have been shown to be S-acetyltransferases (Roth et al., 2002; Ducker et al., 2004). S-acetylation is commonly known as palmitoylation, but because other types of acylation have been elucidated, such as the modification of human Sonic hedgehog by an amide-linked palmitoyl group (Pepinsky et al., 1998) and the ability of so-called palmitoyltransferases to use a wide range of acyl-CoA substrates, it is now more accurately termed S-acylation. To determine whether TIP1 might also be an S-acetyltransferase, we tested whether TIP1 could rescue the defects of yeast akr1Δ loss-of-function mutants. The defects include reduced growth, aberrant morphology, and mislocalization of the casein kinase YCK2p to the cytoplasm. The growth and morphological defects are temperature sensitive, with normal growth occurring at 25°C and reduced and aberrant growth occurring at 30°C (Kao et al., 1996; Pryciak and Hartwell, 1996; Feng and Davis, 2000). TIP1 cDNA and a mutant lacking the DHHC Cys (TIP1 C401A) were placed under the control of the GAL1 yeast promoter and introduced into akr1Δ yeast cells. Single colonies were streaked on complete minimal medium and grown at 25 and 30°C (Figure 5A). At 25°C, all genotypes grew well, but with pPB575 (a yeast Akr1p genomic fragment on a high-copy-number vector with a 2μ autonomous replication sequence origin of replication), complemented yeast and akr1Δ TIP1 yeast showed stronger growth than akr1Δ and akr1Δ TIP1 C401A. At 30°C, akr1Δ TIP1 yeast grew much better than akr1Δ yeast and as well as akr1Δ pPB575. TIP1 C401A failed to complement the growth defects of akr1Δ on solid medium at 30°C. Similar results were obtained across three replicates. Figure 5B shows the morphology of yeast cells of each genotype observed using a light microscope. TIP1 cDNA complemented the morphological defects observed in the akr1Δ mutant grown at 30°C. Again, TIP1 C401A failed to complement the akr1Δ growth defects observed at 30°C.

YCK2p localizes sites of polar growth in an Akr1p-dependent manner and is anchored to the membrane by two acylated C-terminal Cys residues. The acylation of YCK2p is disturbed in

**Figure 4. RNA Gel Blot Analysis of TIP1 Expression in Wild-Type and Mutant Plants.**

(A) TIP1 is expressed throughout the plant. R, root; L, leaf; I, inflorescence; F, flower.

(B) The Tip1 mutants all show reduced levels of transcript compared with the wild type. The arrowhead indicates a second larger transcript in tip1-1. No transcript is detectable in tip1-3.
akr1Δ mutants and shows a diffuse cytoplasmic distribution of YCK2p (Babu et al., 2004). AKR1 yeast or akr1Δ yeast expressing TIP1 and a YCK2:green fluorescent protein (GFP) fusion show a defined localization of YCK2:GFP to the plasma membrane and an internal compartment. akr1Δ yeast or akr1Δ yeast expressing TIP1 C401A and YCK2:GFP show a cytoplasmic distribution of YCK2:GFP (Figure 5C). The promotion of membrane association of YCK2p by TIP1 indicates that TIP1 is able to acylate YCK2p in vivo and that the DHHC Cys is required for acylation to occur.

**Inhibiting Acylation Phenocopies the Tip1− Mutant Phenotype**

TIP1 complemented the akr1Δ S-acyl transferase mutant, suggesting that TIP1 is likely to be an S-acyl transferase. The palmitate analogue 2-bromopalmitate is known to be an inhibitor of S-acyl transferase activity (Webb et al., 2000). To test whether a change in acyl transferase activity could be responsible for the Tip1− phenotype, wild-type roots were treated with varying concentrations of 2-bromopalmitate. Figure 6 shows that mock treatments with solvent (0.1% methanol) had no effect on Arabidopsis root hair morphology. Treatment with 10 μM 2-bromopalmitate did not significantly affect tip1-2, but treated wild-type plants showed a strong Tip1− root hair phenotype (Figure 6). Mean root hair lengths were as follows: wild-type untreated, 669 ± 106 μm; wild-type treated, 72 ± 19 μm; tip1-2 untreated, 82 ± 40 μm; tip1-2 treated, 73 ± 18 μm. All wild-type roots recovered normal root hair growth after leaving the zone of treatment. Only developing root hair morphology was sensitive to 2-bromopalmitate, as the morphology of fully developed root hairs was unaffected (data not shown).

**TIP1 Binds Palmitate**

S-acyl transferases such as AKR1p bind acyl groups such as palmitate to the Cys of their DHHC motif before transfer to the target protein. This intermediate form is detectable by labeling in vivo with [3H]palmitic acid (Roth et al., 2002). The DHHC motif Cys has been shown to be essential for acyl transferase activity in AKR1p (Roth et al., 2002) and ERF2p (Lobo et al., 2002). TIP1 is labeled by [3H]palmitic acid when expressed in yeast. TIP1 is not acylated when the DHHC motif Cys, Cys-401, is mutated to Ala (Figure 7). Treatment of yeast cells with 2-bromopalmitate during labeling reduced the labeling of TIP1 (data not shown). Treatment of TIP1 with DTT after labeling removed the label, indicating that palmitic acid is attached via a labile thioester bond to Cys-401 (data not shown). These results show that TIP1 can bind palmitic acid, that binding is reliant on the DHHC Cys, and that palmitic acid is attached via a thioester bond. These are indicative features of an S-acyl transferase (Roth et al., 2002; Ducker et al., 2004).

**DISCUSSION**

We have identified the Arabidopsis TIP1 gene and shown that it encodes an S-acyl transferase. We have shown that TIP1 can bind palmitic acid and functionally substitute for the S-acyl transferase AKR1p in yeast and that inhibition of acyl transferase...
activity in *Arabidopsis* roots with 2-bromopalmitate phenocopies the Tip1− root hair phenotype. These results demonstrate that TIP1 regulates root hair growth by acting as an S-acyl transferase.

S-acylation is the addition of acyl moieties, frequently the C16 lipid palmitic acid, to the thiol groups of Cys residues. As with other fatty acid modifications of proteins, such as myristylation, farnesylation, and geranylgeranylation, this increases the hydrophobicity of the modified protein and promotes the association with membranes (Yalovsky et al., 1999; Thompson and Okuyama, 2000). Unlike other hydrophobic lipid modifications, S-acylation is reversible. Rapid addition and removal of acyl moieties from the thiol group of Cys residues enhances or decreases, respectively, the association with membranes and probably also aids in hydrophobic protein–protein interactions (Smotrys and Linder, 2004). This allows swift, flexible control of protein hydrophobicity and membrane association. To date, no consensus motif has been found for S-acylation, and it is thought that each S-acyltransferase recognizes a specific set of target proteins via an unknown mechanism (Smotrys and Linder, 2004).

Acylation has been implicated in protein sorting into lipid rafts, which are cholesterol-rich regions of the plasma membrane thought to be important for polarity determination (Bagnat and Simons, 2002), and cell signaling (Lai, 2003). A wide variety of proteins are known to be acylated, including small GTPases such as the *Arabidopsis* type II ROP, ROP10 (Lavy et al., 2002), human H-Ras and N-Ras (Hancock et al., 1989), and yeast Ras2 (Lobo et al., 2002). All type II ROPs (ROP9, ROP10, and ROP11) are thought to be palmitoylated at the C terminus (Lavy et al., 2002), as are their maize (*Zea mays*) homologues ROP6 and ROP7 (Ivanchenko et al., 2000). The higher plant–specific RAB5 homologue ARA6, with a suggested role in endosomal fusion, is palmitoylated and myristoylated at the N terminus (Ueda et al., 2001). Some G protein α subunits (Degtyarev et al., 1994), casein kinases (Roth et al., 2002), phospholipases (Sugars et al., 1999), and regulators of G-protein signaling (Tu et al., 1999) have also been shown to be palmitoylated. The *Arabidopsis* membrane-associated calcineurin B–like calcium-sensing proteins (AtCBL1, AtCBL4, AtCBL5, and AtCBL9) are N-terminally myristoylated and contain an adjacent Cys that may be palmitoylated, although this awaits experimental verification (Kolukisaoglu et al., 2004). Calcium–dependent protein kinases (CDPKs) are the most abundant Ser/Thr kinases in plants, and the rice (*Oryza sativa*) CDPK OsCPK2 has been shown to be N-terminally myristoylated and palmitoylated (Martin and Busconi, 2000), and sequence

**Figure 6.** Inhibition of Acylation with 2-Bromopalmitate Phenocopies the Tip1− Phenotype.

(A) Growing roots were treated with 2-bromopalmitate solution or mock-treated with solvent. The white arrows denote the region of root growth and root hair expansion in the presence of 2-bromopalmitate over the subsequent 6 h before imaging. Bar = 500 μm.

(B) Magnified images of tip1-2, mock-treated wild-type, and treated wild-type roots show that 10 μM 2-bromopalmitate treatment produces a root hair phenotype comparable to that of tip1-2. Bar = 400 μm.

**Figure 7.** TIP1 Is Labeled with [3H]Palmitic Acid in Yeast.

S-acyl transferases can be labeled with [3H]palmitic acid ([H]PA) in vivo. TIP1 carrying a C401A mutation within the DHHC active site motif fails to label with [3H]palmitic acid. TIP1 and TIP1 C401A were expressed as C-terminal FLAG epitope tag fusions under the GAL1 promoter.
homology suggests that the same may be true for some Arabidopsis CDPKs (Hrabak et al., 2003).

The yeast and human S-acyl transferases AKR1p and HIP14 localize to the Golgi apparatus and late endosome and do not rely on endocytosis to localize there (Roth et al., 2002; Singaraja et al., 2002; Harada et al., 2003). As TIP1 is capable of functionally replacing AKR1p in yeast, it is tempting to speculate that TIP1 may have a similar subcellular localization, and the results of preliminary experiments using a functional ProTip1::TIP1:GFP fusion in plants support this view (data not shown). AKR1p directs YCK2p to the yeast plasma membrane (Roth et al., 2002), so it seems likely that TIP1 regulates root hair growth by directing proteins to discrete areas of the plasma membrane. TIP1 may play a role in directing vesicle traffic and the assembly of the cytoskeleton, leading to an axis of polarity, but the precise targets for TIP1, and hence the detailed way in which TIP1 regulates growth, remain to be identified. We are taking a combined genetic and biochemical approach to identify protein targets for acylation by TIP1 and to investigate how these might influence root hair growth.

BLAST searches within the Arabidopsis genome have revealed no homologues of TIP1, making it likely that TIP1 is the only DHH-CRD ankyrin repeat–containing protein in Arabidopsis. We have identified a related pseudogene on chromosome 2, but this contains numerous stop codons. There are 22 other genes present in Arabidopsis that contain a DHH-CRD but lack ankyrin repeats. Two proteins with this structure, mouse GODZ (Keller et al., 2004) and yeast ERF2p (Lobo et al., 2002), are S-acyl transferases, so it is likely that Arabidopsis has additional S-acyl transferases of this related but distinct type. Outside the core DHH-CRD motif, all of the Arabidopsis DHH-CRD proteins show variation, and it will be interesting to determine whether, and how, this variation contributes to activity and the specificity of function. The phylogenetic analysis places TIP1 with a group of ankyrin repeatless DHH-CRD proteins, suggesting that ankyrin repeat DHH-CRD proteins such as TIP1, HIP14, and AKR1p arose after the diversification of early acyl transferases but before the split of eukaryotes into the distinct kingdoms that we recognize today. It is clear from the strong phenotype of Tip1− mutants that these other Arabidopsis genes are not capable of regulating growth as effectively as TIP1 or are involved in regulating different aspects of growth and development. It will be interesting to discover the features and substrates of TIP1 that enable it to have such a powerful effect on plant cell growth.

Work on TIP1 and related S-acyl transferases has potential implications beyond our understanding of plant cell growth. The human S-acyl transferase HIP14 and TIP1 are more similar to each other and to the ankyrin repeatless ERF2p and GODZ within the DHH-CRD than any of them are to yeast AKR1p. HIP14 S-acylates huntingtin (htt) (Huang et al., 2004) and shows decreased interaction with mutant htt, the causative agent of Huntington’s disease (Harada et al., 2003). HIP14 and its homologues have been implicated in endocytosis and intracellular trafficking (Givan and Sprague, 1997; Feng and Davis, 2000; Harada et al., 2003), and the decreased interaction between HIP14 and htt may affect transport and endocytosis within neurons, leading to neuronal degeneration. HIP14 was also recently shown to be oncogenic when overexpressed in 3T3 cells in culture or introduced subcutaneously into mice. The exact mechanism by which this is achieved is not known but may involve constitutive H-Ras and N-Ras acylation, leading to constitutive activity (Ducker et al., 2004). In an interesting parallel, we found that overexpression of TIP1 in Arabidopsis led to longer root hairs, possibly by moving the equilibrium of S-acylation/deacylation toward excess S-acylation of factors involved in the promotion of tip growth. Further detailed characterization in Arabidopsis and yeast of TIP1 and related proteins could shed light on the mechanisms affecting Ras-based cancer and Huntington’s disease.

METHODS

Plant Materials and Growth Conditions

Seeds of the Arabidopsis thaliana Columbia and Landsberg erecta ecotypes were obtained from the Nottingham Arabidopsis Stock Center. Seeds of tip1-1 and tip1-2 were gifts from John Schiefelbein and Liam Dolan, respectively. Arabidopsis lines bearing insertions in TIP1 were obtained from the Salk Institute Genomic Analysis Laboratory (Alonso et al., 2003). Growth conditions were as described previously (Grierson et al., 1997). Transformed plants were selected on 0.5× Murashige and Skoog medium (Sigma-Aldrich), 1% agarose, 200 µg/mL cefotaxime, and 50 µg/mL kanamycin (pCAMBIA 2300–based vectors) or 15 µg/mL hygromycin B (pCAMBIA 1300–based vectors).

Genetic Mapping and Complementation

A mapping population was created from a cross between tip1-2 (Columbia background) and the wild type (Landsberg erecta ecotype). Self seeds from one F1 plant were used for genetic mapping. Genomic DNA was extracted from 235 F2 plants displaying the phenotype conferred by tip1-2. All information regarding the genetic markers used was obtained from TAIR (http://www.arabidopsis.org/). The results were analyzed with Mapmaker 3.0 (Lander et al., 1987).

Complementation tests were performed either transiently (Kemp et al., 2001) or stably through Agrobacterium tumefaciens–mediated transformation (Clough and Bent, 1998). Large insert clones were obtained from the Mitsui Plant Biotechnology Research Institute (transformation-competent artificial chromosome clones) or from the ABRC (P1 and BAC clones). pCAMBIA vectors were obtained from the Centre for the Application of Molecular Biology to International Agriculture (http://www.cambia.org.au/). TIP1 cDNA was isolated from a Col-4 whole seedling cDNA library (a gift from C. Lazarus) using Pfx polymerase (Invitrogen), cloned into pENTR/SD/D-TOPO (Invitrogen), and recombined into the Gateway binary vector pCAMBIA 1300-35S EC (a gift from C. Lazarus). A. tumefaciens strain GV3101+pGw (Koncz and Schell, 1986) was used throughout.

Sequencing of Tip1-1 Alleles

Genomic DNA was prepared from tip1-1, tip1-2, and tip1-3 seedlings using a Plant DNAeasy kit (Qiagen). PCR products for sequencing were produced with Advantage II DNA polymerase (BD Clontech). PCR products covering At5g20320, At5g20330, At5g20340, and At5g20350 were sequenced directly (Qiagen). Mutations were verified by sequencing of cDNA produced from total root RNA using Omniscript reverse transcriptase (Qiagen) and Advantage II DNA polymerase. PCR sequence fragments were assembled using Sequencher version 5.0. The T-DNA insertion site in tip1-3 was sequenced directly from PCR products using TIP1-specific primers and border-specific T-DNA primers. Details of each mutation have been logged with TAIR (http://www.arabidopsis.org).
measurements of root hairs

For all measurements, a minimum of 100 root hairs from a minimum of five roots was measured. Root hairs were measured from captured images using ImageJ (http://rsb.info.nih.gov/ij/).

Multiple Alignments and Phylogenies

Multiple alignments were performed using Dialign (http://bibiserv.techfak.uni-bielefeld.de/dialign/). Aligned sequences were bootstrapped (1000 repetitions) using Phylo_win (Galtier et al., 1996), and phylogenetic trees were produced using Treeview (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). All programs were used in default settings unless stated otherwise.

RNA Gel Blotting

A 5-μg sample of total RNA was blotted and probed with digoxigenin-labeled fragments of TIP1 and Actin2 cDNA. Probe production, hybridization, and detection were according to the manufacturer’s instructions (Roche).

Complementation of akr1Δ Defects with TIP1 cDNA

AKR1 (BY4741; MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0) and akr1Δ (BY4741; MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YDR264c::kanMX4) haploid yeast (Saccharomyces cerevisiae) were obtained from EUROScarf (http://www.uni-frankfurt.de/re/b/15/mikro/euroscarf/). TIP1 cDNA with a C-terminal 6His FLAG epitope tag in pENTR/SD/D-TOPO was recombined into the high-copy-number 2μ origin pHYEST DEST-52 Gateway vector (Invitrogen) under the GAL1 promoter. TIP1 C401A was created by site-directed mutagenesis using the Quikchange II kit (Stratagene) on the epitope-tagged TIP1 cDNA described above in pENTR/SD/D-TOPO before being recombined into pHYEST-DEST-52. Akr1 controls were introduced in pFB575, a 2-μ autonomous replication sequence origin of replication, high-copy-number plasmid (Kao et al., 1996), to be comparable to the TIP1-containing vector. Transformants were produced using lithium acetate/polyethylene glycol–mediated transformation (Gietz and Woods, 2002) and plated onto appropriate nutrient selective medium (Anachem). Single colonies from each transformation were streaked onto selective glucose medium (Anachem) twice before being grown in selective galactose medium (Anachem). Single colonies from each transformation were streaked onto selective glucose medium (Anachem) twice before single colonies of equal size were picked and streaked onto complete selective galactose medium and grown at 25 and 30°C for 48 h to visualize colonies. Three replicates were performed per genotype using independent transformants, and each was repeated in triplicate for each temperature. For the observation of cell morphology, cultures of each genotype were grown in selective liquid medium at 25 and 30°C until mid-log phase. Cell morphology was observed using a Leica DM IRE2 inverted light microscope. Galactose-inducible YCK2::GFP was created by cloning a BamHI-Sal fragment from pJB1 (Babu et al., 2004) into pESC LEU (Stratagene). YCK2::GFP localization was determined after 3 h of galactose induction of TIP1 and YCK2::GFP expression after growth in selective rafinose medium to mid-log phase. Cells were imaged with a Leica DM IRE2 inverted fluorescence deconvolution microscope and Leica FW4000 and Deblur software. To check that the effects being observed after TIP1 was introduced to akr1Δ yeast were attributable to TIP1 and not to other effects, the akr1Δ TIP1 yeast was streaked three times on complete selective medium containing 5-fluororotic acid and assayed for restoration of the phenotype conferred by akr1Δ. The 5-fluororotic acid selects against the URA3 gene and allows the selection of colonies cured of the TIP1-containing plasmid.

2-Bromopalmitate Treatment of Root Hairs

Wild-type and tip1-2 plants were grown for 3 d after germination in constant light at 18°C before treatment with 2-bromopalmitate (2-bromohexadecanoic acid; Aldrich), which was dissolved in methanol to a concentration of 0.1 M and then diluted in sterile water to the desired concentration. A 10-μL aliquot of 2-bromopalmitate solution was applied to the medium just in front of each growing root tip. Plants were grown for 6 h at 18°C under constant light before being photographed. Plants were also observed after growing overnight to determine whether hair growth was suppressed permanently in treated areas and that hair growth returned to normal after leaving the treated area.

In Vivo [3H]Palmitic Acid Labeling

Yeast strains used were as for complementing the akr1Δ mutant. Yeast strains were grown in 50 mL of selective glucose medium to mid-log phase at 25°C. Cells were harvested and diluted to OD600 = 0.1 in 50 mL of selective galactose medium and allowed to grow for 12 h. An aliquot of 2 × 109 cells was removed to 20 mL of fresh selective galactose medium supplemented with 1 μg/mL cerulenin and 1 mCi of [3H]palmitic acid (DuPont–New England Nuclear). Cells were labeled for 3 h before harvesting. An aliquot of 1 × 109 cells was lysed by ice using glass beads in 200 μL of Celytic-Y (Sigma-Aldrich). The lysate was removed to a fresh tube, and the beads were washed twice with 400 μL of Celytic-Y. The washes were added to the lysate, spun briefly to pellet debris, and applied to 10 μL of M2 anti-FLAG agarose (Sigma-Aldrich). The lysate was allowed to bind to 1 h at 4°C and washed three times with Tris-buffered saline before being eluted into 20 μL of glycine, pH 3.5, for 15 min at room temperature. After 10 μL of SDS sample buffer (no β-mercaptoethanol or DTT) was added, each sample was heated at 80°C for 3 min and loaded immediately on a 10% SDS-PAGE gel. Membranes for protein gel blotting were blocked with 5% nonfat milk powder, and TIP1 was detected using an M2 anti-FLAG horseradish peroxidase conjugate (Sigma-Aldrich). Gels for fluorography were fixed, treated with Amplify (Amersham), and dried before being exposed to Hyperfilm MP (Amersham) for 2 to 6 d.

Accession Numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession number AY965346 and with the curators of TAIR (http://www.arabidopsis.org) as an update to locus At5g20350.

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