The *roothairless1* Gene of Maize Encodes a Homolog of *sec3*, Which Is Involved in Polar Exocytosis[1]

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The *roothairless1* (*rth1*) mutant is impaired in root hair elongation and exhibits other growth abnormalities. Unicellular root hairs elongate via localized tip growth, a process mediated by polar exocytosis of secretory vesicles. We report here the cloning of the *rth1* gene that encodes a *sec3* homolog. In yeast (*Saccharomyces cerevisiae*) and mammals, *sec3* is a subunit of the exocyst complex, which tethers exocytotic vesicles prior to their fusion. The cloning of the *rth1* gene associates the homologs of exocyst subunits to an exocytic process in plant development and supports the hypothesis that exocyst-like proteins are involved in plant exocytosis. Proteomic analyses identified four proteins that accumulate to different levels in wild-type and *rth1* primary roots. The preferential accumulation in the *rth1* mutant proteome of a negative regulator of the cell cycle (a prohibitin) may at least partially explain the delayed development and flowering of the *rth1* mutant.

The root epidermis of angiosperms is composed of two cell types: trichoblasts, which develop into cells bearing long cylindrical root hairs, and atrichoblasts, which do not develop root hairs (Larkin et al., 2003). In cultivated crops, root hairs contribute up to 77% of the total root surface (Parker et al., 2000) and are instrumental in water and nutrient acquisition, anchorage, and microbe interactions (Gilroy and Jones, 2000).

Unicellular root hairs are one of only a few cell types in higher plants that use localized tip growth for their expansion (Kropf et al., 1998). During root hair development the outward-facing cell wall that will give rise to the emerging root hair grows by deposition of cell wall polymers via polar exocytosis. Exocytosis is a fundamental membrane-trafficking event that mediates the incorporation of proteins and lipids into the newly formed plasma membrane and is thus responsible for cell growth and polarity (Hsu et al., 1999). In yeast (*Saccharomyces cerevisiae*) and mammals, a multiprotein complex, the exocyst, consisting of eight distinct subunits (SEC3, SEC5, SEC6, SEC8, SEC10, SEC15, EXO70, and EXO84), is essential for polarized exocytosis (Hsu et al., 1999). The exocyst complex localizes at the site of active exocytosis and is critical for the specification of the site of vesicle docking and fusion where it mediates the targeting and tethering of post-Golgi secretory vesicles prior to the steps mediated by SNARE and associated proteins (Elias et al., 2003). In yeast *sec3* mutants, cells deficient in individual exocyst subunits accumulate secretory vesicles in the cytoplasm, presumably because the vesicles are not able to dock and fuse with the plasma membrane (Finger and Novick, 1997). Regulation of the exocyst complex is mediated via Rab, Rho, and Ral GTPases (Lipschutz and Mostov, 2002). In yeast, the SEC3 subunit is a spatial landmark for polarized secretion independent of a functional secretory pathway (Finger et al., 1998). This led to the model that SEC3 may be the component of the complex most proximal to the target membrane and thus the only component of the complex that is in direct contact with the plasma membrane (Finger et al., 1998). In mammals, the EXO70 protein is responsible for the exocyst assembly at the plasma membrane (Matern et al., 2001).

In maize (*Zea mays*), three root hair mutants, *roothairless1, 2*, and *3* (*rth1, rth2, rth3*), have been identified that are affected in root hair elongation but exhibit normal root hair initiation (Wen and Schnable, 1994). Here, we describe the cloning and characterization of the *rth1* gene, which encodes a homolog of the SEC3 exocyst subunit. This, together with Arabidopsis (*Arabidopsis thaliana*) *sec8* (Cole et al., 2005), demonstrates for the first time the involvement of exocyst subunits in exocytotic developmental processes in plants, i.e. the elongation of root hairs and the growth of pollen tubes, respectively.
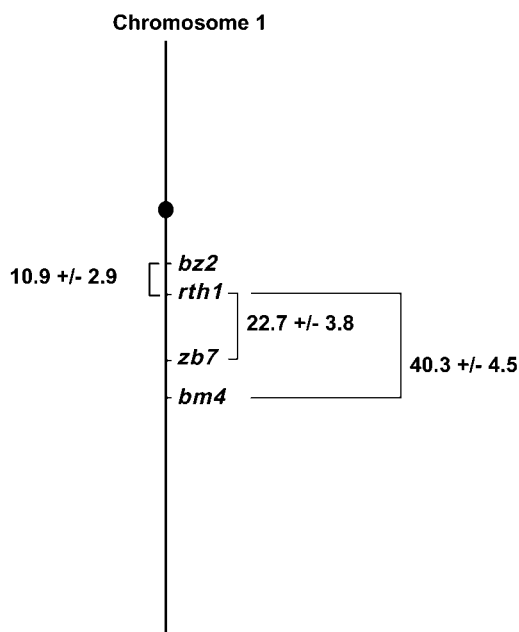
RESULTS

Phenotype of rth1

The *rth1-1* mutant was previously isolated from *Mutator* (*Mu*) transposon stocks (Wen and Schnable, 1994). Plants homozygous for this mutant initiate normal root hair primordia that fail to elongate properly. The mutant *rth1-1* also exhibits pleiotropic phenotypes that are at least similar to the symptoms of severe nutrient deficiencies (Wen and Schnable, 1994). The development of pollen tubes is not functionally affected by the *rth1-1* mutation as demonstrated by the normal transmission rate of the *rth1-1* allele through the pollen produced by plants with the genotype: *rth1-1/Rth1* (data not shown).

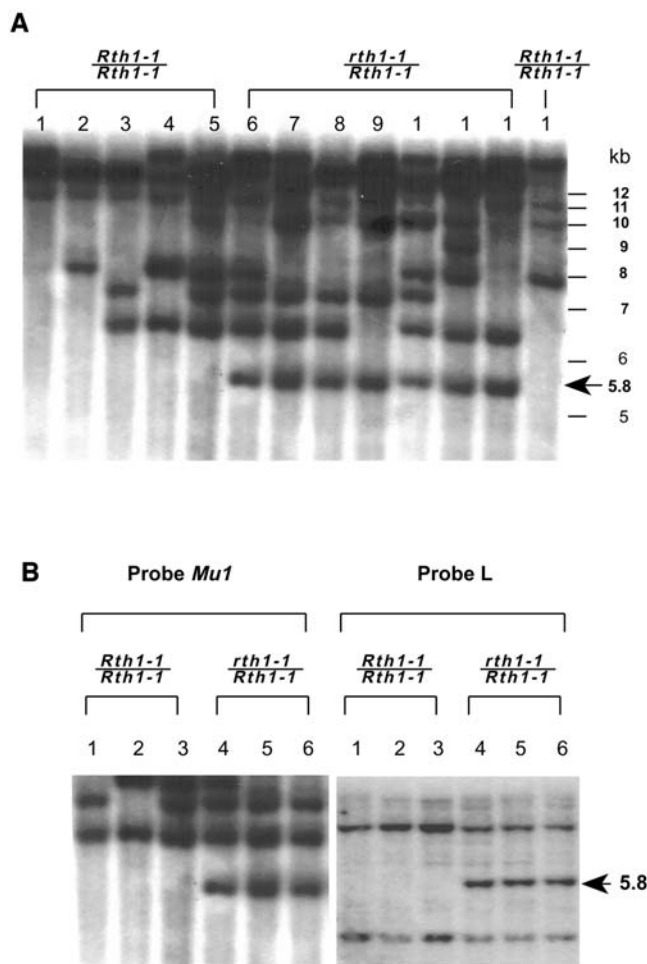### Genetic Mapping of the rth1 Locus

Initial mapping of the *rth1* locus using B-A translocation stocks placed the gene on chromosome 1L (Wen and Schnable, 1994). As described in "Materials and Methods," the *rth1* locus was mapped between the phenotypic markers *bz2* (10.9 ± 2.9 cM) and *zb7* (22.7 ± 3. 8; Fig. 1).

Cloning and Sequencing of the rth1 Gene

DNA gel-blot analyses were conducted with *Mu*-specific hybridization probes to identify *Mu*-containing DNA fragments that cosegregated with the *Mu*-induced mutant *rth1-1* allele. To facilitate this process, the *rth1-1* allele was first backcrossed to non-*Mu* stocks for three generations to reduce the number of *Mu* transposons in the genome. Cosegregation analyses revealed a 5.8-kb *Mu1*-containing *Eco*RI fragment (Fig. 2A) that

Figure 2. Identification of a fragment cosegregating with the *rth1-1* mutation in DNA gel-blot experiments. A, A 5.8-kb genomic *Eco*RI fragment cosegregates with the presence of *rth1-1* mutant alleles. *Rth1/Rth1*, homozygous wild-type plants; *rth1-1/Rth1*, heterozygous plants. B, A 5.8-kb *Eco*RI fragment cosegregated with the presence of *rth1-1* mutant alleles when using *Mu1* or probe L (Fig. 3) hybridization probes. The *Mu* transposon flanking probe L was isolated in a screening of a size-selected *Eco*RI λ phage library generated from a homozygous *rth1-1/rth1-1* mutant plants and hybridized with a *Mu1* probe.

cosegregated absolutely with the *rth1-1* allele among progeny (97 *rth1-1/Rth1* and 80 *Rth1/Rth1*) of the cross: *Rth1/Rth1* × *rth1-1/Rth1*. To clone this 5.8-kb *Eco*RI fragment, a λ library prepared with size-selected (5.5–6 kb) *Eco*RI-digested DNA from the *rth1-1/rth1-1* mutant allele was generated (see "Materials and Methods"). The insert of a phage that hybridized to *Mu1* was subcloned as pRth1 (see "Materials and Methods"). When used as a hybridization probe against the original cosegregation blot (Fig. 2B), probe L, which flanks the *Mu1* element in pRth1, detected the same 5.8-kb *Eco*RI band as the initial DNA gel-blotting experiment. Subsequently, probe L (Fig. 3) was used (see "Materials and Methods") to isolate an apparently full-length cDNA clone, pYJ5, and two genomic clones, φB73GS3 and φB73GP1. Sequencing of φB73GS3 and φB73GP1 revealed that together they

Figure 1. Relative position of the *rth1* locus on chromosome 1L compared to the phenotypic markers *bz2*, *zb7*, and *bm4*.

contain the entire transcript defined by the cDNA clone, pYJ5. An alignment of pYJ5 with φB73GS3 and φB73GP1 established that the *rth1* gene contains 25 exons (Fig. 3). A *hopscotch*-like retrotransposon (White et al., 1994) was detected within intron 19 of *rth1-1* (Fig. 3).

An Independently Generated Allele of *rth1* Confirms the Identity of the Cloned Sequence

Analyses of independent mutant alleles allow for the confirmation that a mutant phenotype was generated by a *Mu* insertion in a particular gene. An additional mutant allele of *rth1*, *rth1-2*, was obtained by screening the Pioneer Hi-Bred (Johnston, IA) TUSC (trait utility system of corn) population (Bensen et al., 1995). Subsequent analyses revealed that a *Mu7* transposon had inserted into intron number 18 of the *rth1-2* allele (Fig. 3). This insertion, however, has no effect on root hair development (data not shown). A forward genetic screen (see "Materials and Methods") identified a deletion derivative of *rth1-2*, *rth1-2d*, that does condition a mutant root hair phenotype. This deletion derivative arose via deletion of exon number 19 (Fig. 3). As such, the isolation of this allele confirms that the cloned gene is indeed *rth1*.

Searches of the Maize Assembled Genomic Islands (MAGI) Web site (www.plantgenomics.iastate.edu/maize) revealed that there is at least one homolog of *rth1* in the maize genome.

The RTH1 Protein Is a SEC3 Homolog

The 2,936-bp *rth1* transcript contains an open reading frame that encodes an 889-amino acid 100.3-kD protein (as determined with the Expasy pI/Mw tool: http://www.expasy.org/tools/pi_tool.html). Using the BLASTX algorithm (Altschul et al., 1997), the deduced amino acid sequence failed to detect significant similarity to plant proteins with known functions but did identify *rth1* homologs in the Arabidopsis (At1g47550, At1g47560) and rice (*Oryza sativa*; AC091246, AY224571) genomes. In addition, BLASTX analysis revealed a high degree of sequence similarity (an E-value of 0 [810 bits] and approximately 40% identity over 800 residues) between RTH1 and SEC3 (KOG2148.1).

## Expression of *rth1* in Roots and Leaves

RNA-blot analyses were performed with RNA samples isolated from leaves and roots of 2-week-old seedlings. RNA samples from both wild-type (B73) and *rth1-1* mutant seedlings were analyzed. A 2.0-kb *Eco*RI fragment from the full-length cDNA (pYJ5) was used as a hybridization probe to detect *rth1* transcripts. A 3-kb transcript that corresponded in size with the isolated full-length cDNA was detected in both roots and in leaves. In *rth1-1* mutant roots and leaves, an additional 4.4-kb *rth1-1*-hybridizing transcript was detected that was not detectable in wild-type roots and leaves. The 3-kb wild-type transcript accumulated to levels about five times higher than the 3.0- and 4.4-kb transcript in *rth1-1* roots and leaves (Fig. 4).

Massively Parallel Signature Sequencing Database Expression Profiles of *rth1* in Different Organs during Development

To obtain a detailed picture of the spatial and temporal expression of the *rth1* gene the massively parallel signature sequencing database (MPSS; Brenner et al., 2000) at DuPont (Wilmington, DE) was surveyed (see "Materials and Methods"). The abundance of the *rth1* transcript was quantified in 50 different samples representing a wide variety of tissues and developmental stages (Fig. 5). The *Rth1* gene was expressed in all surveyed tissues/developmental stages.

Proteome Analysis of 5-d-Old *rth1* Primary Roots

The only obvious morphological difference in the primary roots of 5-d-old wild-type and *rth1-1* seedlings is that the mutants display reduced elongation of the unicellular root hairs formed in the epidermis. The soluble proteomes of 5-d-old wild-type and *rth1-1* primary roots were compared in triplicate from
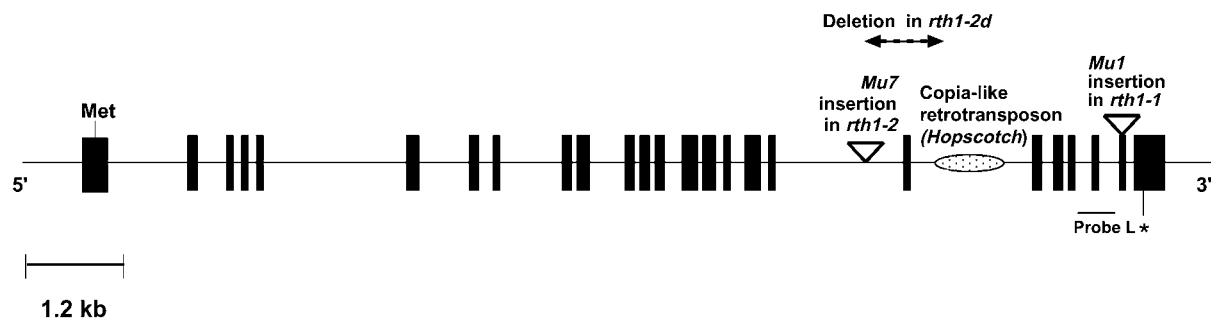
Figure 3. Genomic structure of the *rth1* gene. Exons are indicated by black boxes and introns are indicated as solid lines. Triangles indicate the insertions of *Mu* elements in *rth1-1* and *rth1-2* alleles, and the deletion in *rth1-2d* allele is illustrated as dashed line between two arrow heads above the *rth1* gene structure. Probe L used in Figure 2B is indicated as solid line. Asterisk, position of stop codon.
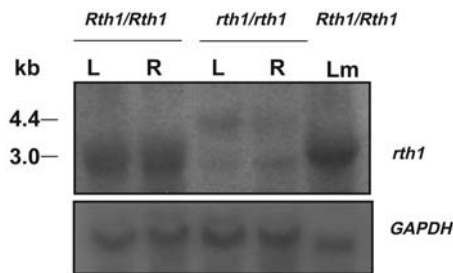
Figure 4. Expression of the *rth1* gene in homozgous wild-type (*Rth1/Rth1*) and mutant (*rth1-1/rth1-1*) leaves (L, Lm) and roots (R). Expression of the constitutively expressed GAPDH gene (X07156) was used as a control to indicate similar RNA amounts in all lanes. Lanes L and R, Total RNA; lane Lm, mRNA.

independent biological replicates on two-dimensional protein gels with a pH range of 4 to 7 to identify proteins that exhibit at least a 3-fold higher level of accumulation in one of the proteomes. After staining with Coomassie Blue, 158 proteins could be detected from the *rth1-1* and wild-type roots. One of these proteins accumulated to significantly higher levels in the wild-type primary root proteome, while three proteins accumulated to significantly higher levels in the proteome of *rth1-1* mutants (Table I). Thus, 2.5% (4/158) of the proteins detected in this study accumulated preferentially in wild type or *rth1-1*. Three of the four differentially accumulating proteins (Table I, protein nos. 1, 2, and 4) were identified via MS-fit (http://

prospector.ucsf.edu/ucsfhtml4.0/msfit.htm) database searches in the maize expressed sequence tag (EST) contig (www.maizegdb.org) and the MAGI (www.plantgenomics.iastate.edu/maize) databases using the criteria defined in Hochholdinger et al. (2004a). However, BLASTX database searches revealed a function for only one of these proteins. This was prohibitin that accumulated to a level that was 3 times higher in *rth1-1* mutants as compared to wild type.

DISCUSSION

Root hair formation starts with pattern specification of the epidermal cells followed by root hair initiation, bulge formation, and, finally, root hair elongation. In the dicot model plant Arabidopsis, numerous mutants that affect each of these steps in root hair formation have been characterized (for review, see Schiefelbein, 2000). Epidermal pattern specification in Arabidopsis is well characterized on the molecular level by transcriptional networks using transcriptional feedback loops and lateral inhibition signaling pathways (Lee and Schiefelbein, 2002).

In addition, a considerable number of Arabidopsis mutants that affect root hair elongation have been identified. Carol and Dolan (2002) divided these mutants in two classes: first, mutants that are affected in the transition to tip growth and rarely form root hairs longer than 40 μm. This class comprises the mutants
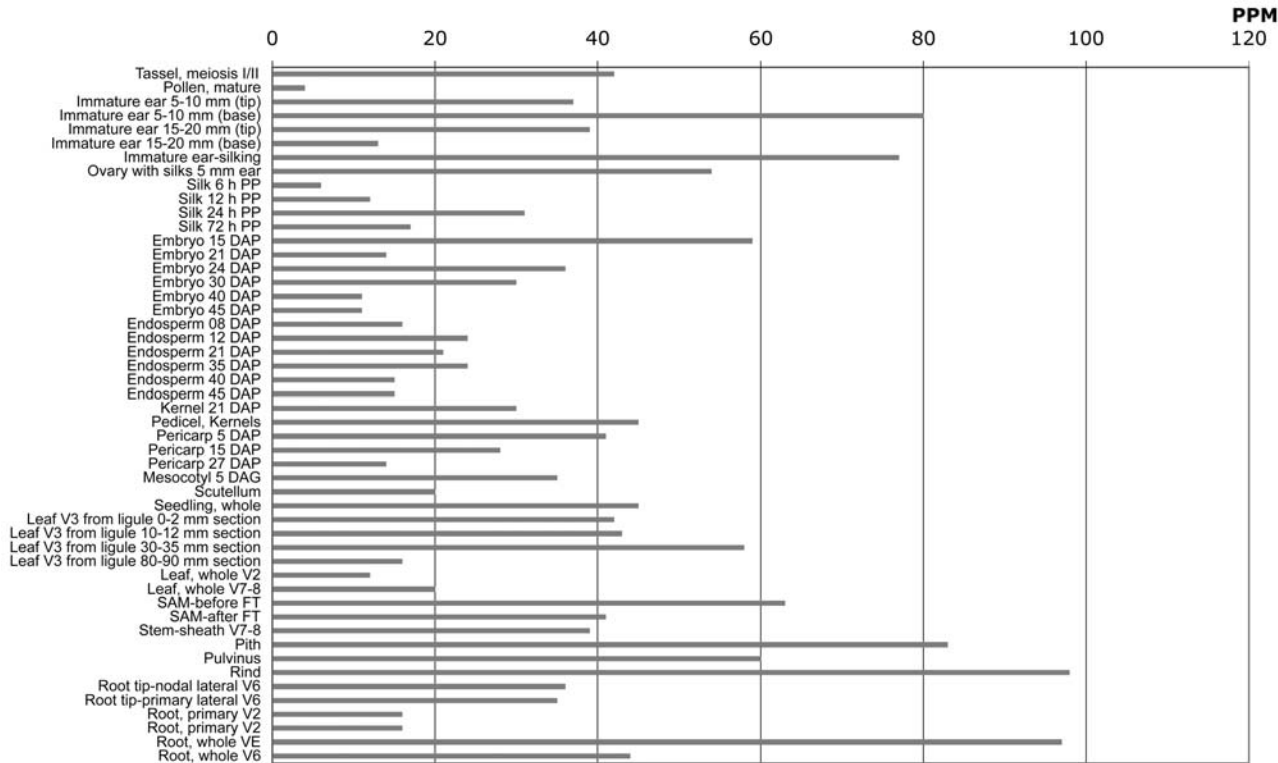
Figure 5. Relative expression levels (in ppm) of the *rth1* gene in 50 different tissues and developmental stages of maize obtained via the Lynx MPSS system.

Table I. *Proteins that accumulated significantly different between wild-type and rth1 primary roots*

Spot No.	MOWSE Score[a]	Peptides Matched (n)[b]	% EST/Protein Covered[c]	M_r Gel/M_r Predicted[d]	pI Gel/pI Pedicted[e]	EST/MAGI AC[f]	Function (BLASTX) [Species] AC[g]	Specificity wt:rth1
1	5.5e+3	4	21	14.5/12.8	5.52/11.4	MAGI_95434	Hypothetical protein [rice] NP_922344	3.8
2	1.57e+4	4	42	30.2/30.7	6.97/6.55	BQ294333	Prohibitin [maize] AAF68385	1:3.0
3	n. i.[h]			22.8	5.76			1:3.5
4	1.09e+3	4	61	22.7/n.p.[i]	6.86/n.p.	AI629640	None	1:6.9

[a]Statistical probability of true positive identification of predicted proteins (cutoff value, 1e+3). [b]Number of peptides matching predicted protein sequences. [c]Percentage of predicted protein sequence covered by matched peptides. [d]Molecular mass of protein on gel/of predicted protein. [e]pI of proteins on gel/of predicted protein. [f]EST or MAGI accession of predicted protein (cutoff value, 1e-10); EST ACs according to www.maizegdb.org; MAGI ACs according to the maize genome assembly project (http://maize.ece.iastate.edu.) [g]As obtained via BLASTX GenBank database searches (cutoff value:1e-10). [h]Not identified. [i]No prediciton for proteins that did not yield a BLASTX db hit.

rhd2, *shv1*, *shv2*, *shv3*, *trh1*, and *kjk* (see Carol and Dolan, 2002, and refs. therein). The second class includes mutants that are affected in tip growth and grow longer than 40 μm, but are always shorter than wild-type root hairs. This class comprises the mutants *rhd3*, *rhd4*, *bst1*, *cen1*, *cen2*, *cen3*, *scn1*, *cow1*, and *tip1* (see Carol and Dolan, 2002, and refs. therein). Several of these genes have been cloned. None of these genes is, however, related to the exocyst complex. The *rhd2* gene, for example, encodes an NADPH oxidase required for the localized Ca^{2+} channel activity (Foreman et al., 2003), *trh1* encodes a potassium transporter (Rigas et al., 2001), *rhd3* encodes a member of a GTP-binding protein (Wang et al., 1997), and *kjk* encodes a cellulose synthase-like gene (Favery et al., 2001).

In monocot cereals, genetic analysis of root development has been initiated only recently (summarized in Hochholdinger et al., 2004b, 2004c). Three nonallelic maize mutants (*rth1*, *rth2*, and *rth3*; Wen and Schnable, 1994) that block root hair elongation and one rice mutant (*rh2*; Ichii et al., 2000) that blocks root hair initiation have been described. We report here the cloning and initial molecular characterization of the *rth1* gene, which is instrumental in the exocytotic tip growth of root hairs.

The RTH1 protein exhibits a high degree of similarity to SEC3 proteins; 95% of the predicted RTH1 protein aligns with the SEC3 consensus sequence (KOG2148.1) generated via the comparison of all available SEC3 proteins with an E-value of 0.0 and a score of 810 bits. The SEC3 protein in yeast and mammals is part of a multiprotein complex composed of eight distinct subunits designated exocyst. The exocyst complex is crucial for the precise specification of the site of vesicle docking and fusion during exocytosis (Elias et al., 2003). The SEC3 protein in yeast is the direct link between the exocyst complex with the plasma membrane and has been proposed as a landmark for defining polarized domains of the plasma membrane (Lipschutz and Mostov, 2002).

A growing body of evidence suggests the existence of exocyst-like complexes in plants. Database searches have identified in the Arabidopsis and rice genomes homologs of all the exocyst subunit genes (Elias et al.,

2003). The presence of an exocyst complex in Arabidopsis was demonstrated via immunoprecipitation experiments using SEC5 and SEC6 antibodies. These results suggested that these two subunits belong to the same high-M_r complex, which can be localized to the tip of growing pollen tubes (Zarsky et al., 2004; V. Zarsky, personal communication). Electron microscopic images of cell plate-forming vesicles in shoot and root apices of Arabidopsis revealed L- and Y-shaped linker complexes, which are believed to tether Golgi-derived vesicles prior to their fusion. These linker complexes strongly reassemble the images of purified exocyst complexes from mammals and yeast both in morphology and length (Segui-Simarro et al., 2004). It is proposed that the L-shaped complexes contain exocyst subunits other than SEC3, while conditions that allow binding of SEC3 to the target membrane promote the formation of the Y-shaped complexes and thus the functional tethering complexes.

Although it is becoming clear that plants do contain exocyst-like complexes, their functions have not yet been determined. Because *rth1* mutants exhibit perturbed root hair elongation, our findings that RTH1 is a SEC3 homolog indicates that in plants exocyst components are required for normal exocytosis in the tip of root hairs. Interestingly, pollen tube growth, which also occurs via tip growth, does not appear to be affected by the *rth1* mutant. This finding is consistent with our observation that, like the Arabidopsis and rice genomes (Elias et al., 2003), the maize genome contains at least two *rth1/sec3* homologs, which may be differentially expressed.

MPSS analyses revealed that *Rth1* transcripts accumulate in all tissues analyzed. This is consistent with the pleiotropic phenotypes associated with the *rth1-1* mutant (Wen and Schnable, 1994). In the mutant, two *rth1-1* transcripts with a size of 3.0 and 4.4 kb were detected. The 4.4-kb fragment is consistent with a 1.4-kb *Mu1* insertion in the 3.0-kb *rth1-1* transcript.

When grown under field conditions, *rth1-1* mutant plants are stunted, produce purplish leaves, do not form ears, only rarely produce tassels, and even more rarely shed pollen (Wen and Schnable, 1994). The

finding that *rth1-1* mutants are more vigorous when grown under hydroponic conditions (some even shed pollen; Wen and Schnable, 1994) suggests that these pleiotropic mutant phenotypes may be secondary effects of the mutation due to limited access to water and/or nutrients. On the other hand, proteomic analyses identified a prohibitin that accumulated to levels that were three times higher in *rth1-1* seedling roots than in wild-type roots. Prohibitins comprise a protein superfamily that negatively controls the cell proliferation in maize (Nadimpalli et al., 2000). Hence, the overexpression of this prohibitin could at least partially explain the delayed development and flowering of the *rth1-1* mutant and why the pleiotropic *rth1-1* mutant phenotypes are not fully normalized under hydroponic conditions (Wen and Schnable, 1994).

In summary, the cloning of the maize *rth1* gene, along with the results of Cole et al. (2005), provide examples of morphogenetic mutants in plants that are impaired in predicted components of the exocyst complex. Because *rth1* fails to elongate already-initiated root hairs and Arabidopsis *sec8* is impaired in pollen tube elongation (Cole et al., 2005). Both mutants support the hypothesis that homologs of yeast and mammal exocyst subunits are involved in exocytosis in plants.

MATERIALS AND METHODS

Isolation of New *rth1* Alleles and Maintenance of the Mutant Stocks

The root hair elongation mutant *rth1-1* was maintained by backcrossing heterozygous plants to the inbred lines B73 over numerous generations as described previously (Wen and Schnable, 1994). A *Mu* transposon-specific PCR oligonucleotide and *rth1* gene-specific oligonucleotides were used in a reverse genetic screen of the Pioneer Hi-Bred TUSC population (Bensen et al., 1995) to identify *Mu* insertion alleles of *rth1*. This led to the identification of an additional allele, *rth1-2*.

A deletion derivative of *rth1-2*, *rth1-2d*, was identified via a forward genetic screen. Heterozygous *rth1-2/Rth1* plants that carried an active *Mu* transposon system were crossed to *rth1-1/Rth1* plants (*rth1-1/Rth1 × rth1-2/Rth1 Mu*). A phenotypic screen of the resulting F_1-progeny resulted in the isolation of the *rth1-2d* allele.

Genetic Mapping of the *rth1* Gene

After the initial B-A translocation mapping of *rth1* to the long arm of chromosome 1 (Wen and Schnable, 1994), a higher resolution mapping experiment was conducted using phenotypic markers. A stock homozygous for three visible markers *bronze kernel 2* (*bz2*), *zebra leaf 7* (*zb7*), and *brown midrib 4* (*bm4*) located on the long arm of chromosome 1 was crossed to heterozygous *rth1-1 Bz2 Zb7 Bm4/Rth1 Bz2 Zb7 Bm4 × Rth1 bz2 zb7 bm4/Rth1 bz2 zb7 bm4*). The resulting progenies were selfed and backcrossed to the triple mutant *bz2 zb7 bm4* parent line. The backcrosses from the plants with self-pollinated progenies segregating for the *rth1-1* mutant phenotype (*Rth1 bz2 zb7 bm4/Rth1 bz2 zb7 bm4 × rth1-1 Bz2 Zb7 Bm4 /Rth1 bz2 zb7 bm4*) were further analyzed. Based on the phenotypes of these backcross progenies genetic distances were calculated between the visible markers and *rth1*.

Isolation and Analysis of Nucleic Acids

Genomic DNA was extracted from 4-week-old freeze-dried leaves via a cetyl-trimethyl-ammonium bromide protocol (Saghai-Maroof et al., 1984). Total RNA was isolated from 2-week-old leaves and roots using a phenol/SDS

protocol (Ausubel et al., 1994). An oligo(dT)-cellulose column (Molecular Research Center, Cincinnati) was used to purify mRNA from this RNA. DNA and RNA gel-blot experiments were performed with 10 μg of genomic DNA and total RNA or 0.1 mg mRNA per sample. Electrophoresis and transfer to nylon membranes of nucleic acids, crosslinking, probe labeling, prehybridization, hybridization, and posthybridization washes of DNA and RNA gel blots were conducted as previously described (Stinard et al., 1993).

DNA Gel-Blot Experiments to Clone the *rth1* Gene

After the identification of a DNA fragment that cosegregated with the mutant phenotype in DNA gel-blotting experiments with a *Mu*-specific probe, a size-selected library containing 5.5 to 6.0 kb *Eco*RI-digested genomic DNA fragments cloned into λNM1149 (Murray, 1983) was constructed from *rth1-1/ rth1-1* mutant seedlings. This library was screened with a radioactively labeled *Mu1*-specific probe, a 0.9-kb *Mlu*I fragment of the plasmid pMJ9 (Barker et al., 1984) according to Sambrook et al. (1989). The insert of a phage (λ no.1) that hybridized to *Mu1* was subcloned into the *Eco*RI site of pBSIIKS+ (Stratagene, La Jolla, CA) as pRth1. pRth1 was digested with *Bam*HI and *Bgl*II and self ligated to form pLR. The DNA fragment flanking the 3′-end of *Mu1* element in pLR (probe L) was obtained via PCR amplification using a *Mu1*-specific primer in combination with a vector-specific primer (universal primer). Probe L was used to screen cDNA libraries prepared from B73 seedlings (λZAPII cDNA library, A. Barkan, unpublished data) and identified a 2.2-kb *rth1* cDNA, pa2. The 5′-end a 1.3-kb fragment of pa2 was used as a probe to screen a HybriZAP B73 seedling cDNA library (Y. Xia, B. Nikolau, and P.S. Schnable, unpublished data), which resulted in the isolation of a full-length *rth1* cDNA clone (pYJ5). To uncover the genomic organization of *rth1* two overlapping genomic phage clones, φB73GS3 and φB73GP1 that in combination included the full-length cDNA of the *rth1* cDNA were sequenced using the Tn1000-based sequencing system (Morgan et al., 1996).

Survey of MPSS

The MPSS technology allows for the quantification of 17-bp sequences in populations of $2 × 10^5$ to $2 × 10^6$ cDNAs. These 17-bp signature sequences almost always correspond to unique cDNAs thus allowing the quantification of the abundance of a particular cDNA in a sample representing a particular organ and developmental stage (Christensen et al., 2003).

Proteomic Analyses of Protein Profiles of *rth1*

Soluble proteins of wild-type and *rth1-1* roots collected from segregating families backcrossed to B73 for 12 generations were isolated from 5-d-old primary roots as described previously (Hochholdinger et al., 2004a). Two-dimensional separation of the proteins on pH 4 to 7 IPGphor strips (Amersham Biosciences Europe, Freiburg, Germany) followed by 12% polyacrylamide SDS gel electrophoresis, Coomassie staining of the proteins, PDQuest data analysis, excision of differential protein spots, washing, trypsin digestion, matrix-assisted laser-desorption ionization time of flight analyses and database identification of the proteins was performed as described before (Hochholdinger et al., 2004a). Each experiment was performed in triplicate with independent biological replicates, each of which consisted of a pool of primary roots from approximately 20 seedlings.

Upon request, all novel materials described in this publication will be made available in a timely manner for noncommercial research purposes, subject to the requisite permission from any third-party owners of all or parts of the material. Obtaining any permissions will be the responsibility of the requestor. A Material Transfer Agreement governs the distribution of the *rth1-2* mutant; inquiries should be directed to Dr. Wesley Bruce.

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