

Current Biology

The Mechanism Forming the Cell Surface of Tip-Growing Rooting Cells Is Conserved among Land Plants

Highlights

- 336,000 T-DNA lines and a genome assembly were generated in *Marchantia polymorpha*
- 33 genes required for rhizoid growth were identified
- Six of the 33 genes were functionally characterized in plants for the first time
- Genes belonging to these orthogroups were active in the first land plant roots

Authors

Suvi Honkanen, Victor A.S. Jones, Giulia Morieri, ..., Denis Saint-Marcoux, Helen Prescott, Liam Dolan

Correspondence

liam.dolan@plants.ox.ac.uk

In Brief

Honkanen et al. identify 33 genes required for the growth of rhizoid rooting cells in the liverwort *Marchantia polymorpha* in a screen of 336,000 T-DNA-mutagenized lines and using a de novo genome assembly. Related genes were active during the development of the first plant rooting structures sometime before 460 million years ago.



The Mechanism Forming the Cell Surface of Tip-Growing Rooting Cells Is Conserved among Land Plants

Suvi Honkanen,^{1,2} Victor A.S. Jones,^{1,2} Giulia Morieri,¹ Clement Champion,¹ Alexander J. Hetherington,¹ Steve Kelly,¹ Hélène Proust,¹ Denis Saint-Marcoux,¹ Helen Prescott,¹ and Liam Dolan^{1,3,*}

¹Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK

²Co-first author

³Lead Contact

*Correspondence: liam.dolan@plants.ox.ac.uk

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SUMMARY

To discover mechanisms that controlled the growth of the rooting system in the earliest land plants, we identified genes that control the development of rhizoids in the liverwort *Marchantia polymorpha*. 336,000 T-DNA transformed lines were screened for mutants with defects in rhizoid growth, and a de novo genome assembly was generated to identify the mutant genes. We report the identification of 33 genes required for rhizoid growth, of which 6 had not previously been functionally characterized in green plants. We demonstrate that members of the same orthogroup are active in cell wall synthesis, cell wall integrity sensing, and vesicle trafficking during *M. polymorpha* rhizoid and *Arabidopsis thaliana* root hair growth. This indicates that the mechanism for constructing the cell surface of tip-growing rooting cells is conserved among land plants and was active in the earliest land plants that existed some-time more than 470 million years ago [1, 2].

RESULTS AND DISCUSSION

The first land plant rooting structures comprised systems of tip-growing filamentous cells (rhizoids). Comparing the genetic mechanism that controls the development of filamentous rooting cells (rhizoids and root hairs) among different groups of land plants allows us to reconstruct the mechanism that controlled the development of these first land plant rooting systems. To identify genes required for the growth of rhizoids in one of the earliest diverging taxa of land plants, we generated a mutant population of 336,000 lines by transforming germinating *Marchantia polymorpha* spores with the pCambia1300 T-DNA vector and screened for plants with defective rhizoid morphology (DRM). 301 DRM mutants were isolated (Table S1); 165 mutants were crossed to wild-type and the mutant phenotype was inherited in the F1 generation, whereas 136 were not successfully crossed (Table S1). The approximate 1:1 segregation of wild-type to DRM mutant rhizoid phenotypes in the F1 generation for each of the 165 inherited mutants indicated that the DRM

phenotypes were caused by single nuclear mutations (Table S2). The DRM mutant rhizoid phenotype co-segregated with the hygromycin resistance encoded by the hygromycin phosphotransferase gene on the T-DNA in 62 of the 165 inherited mutant lines (Table S2). This is consistent with the hypothesis that the insertion of a T-DNA carrying a functional hygromycin resistance gene caused the mutation that resulted in defective rhizoid growth in 37% of the inherited mutants (Table S2).

To identify T-DNA insertion sites, we first generated a draft assembly of the *M. polymorpha* genome. Because the plants used in the mutant screen grew from spores generated in a cross between wild-type male (Takaragaik-1 [Tak-1]) and female (Tak-2) accessions, DNA was isolated from Tak-1 and Tak-2 plants, pooled, and sequenced. Illumina HiSeq technology was used to generate 84,554,420 short-insert paired-end reads and 32,963,957 long-insert paired-end reads. The draft genome comprised 4,137 scaffolds with a total scaffold length of 206 Mb (Data S1), scaffold N50 length of 376 kb, and estimated coverage of 64× (Data S1). To identify protein-coding genes in this draft genome, we sequenced, assembled, and mapped an *M. polymorpha* gametophyte transcriptome onto the genome assembly. The transcriptome was generated using pooled RNA isolated from mature dorsal thallus epidermis (excluding midrib region and gemma cups), the meristematic zone, rhizoids, and 0- and 1-day-old gemmae. RNA was sequenced using Illumina HiSeq in 183,475,609 short-insert paired-end reads and assembled into contigs (Data S1); 29,453 gametophyte-expressed contigs were mapped to the genome assembly. The whole-genome shotgun assembly (DDBJ: LVLJ00000000) and transcriptome shotgun assembly (ENA: GEFO00000000 and GenBank: GEFO01000000) have been deposited at the DNA Data Bank of Japan, European Nucleotide Archive, and GenBank.

The genomic locations of 57 of the 62 T-DNAs linked to DRM mutations were identified by thermal asymmetric interlaced (TAIL) PCR (Data S2). The T-DNA insertion sites of the 57 DRM mutants were distributed among 31 different genes (Figure S1). TAIL PCR was also carried out on DRM mutants that were sterile and could not be crossed, and this resulted in identification of the three alleles of *MpCSLD1* and two alleles of *MpSCD*. Therefore, in total, 33 genes were identified in the mutant screen. Additional mutant alleles in eight of the 33 genes—*Mpalba-3*, *Mpemb2756-2*, *Mpexl-1*, *Mppi4ka1-5*, *Mpsti-2*, *Mpsri1-1*,

Table 1. Genes Required for Rhizoid Growth

Gene	Predicted Function of Encoded Protein	Closest <i>Arabidopsis</i> Homolog	Mutant Phenotype	No. of Mutant Alleles	In <i>Arabidopsis</i>	
					Expression Enriched in Root Hairs	Role in Root Hair Development
Cell Wall Biosynthesis and Integrity Sensing						
<i>MpCSLD1</i>	cellulose synthase-like class D protein	AT3G03050	very short rhizoids	3	yes	yes
<i>MpCSLD2</i>	cellulose synthase-like class D protein	AT3G03050	short rhizoids	5	yes	yes
<i>MpPTI</i>	PTI-like serine/threonine kinase	AT2G30740	short rhizoids	2	yes	yes
<i>MpXUT1</i>	xyloglucan-specific galacturonosyltransferase	AT5G41250	very short rhizoids	3	yes	yes
<i>MpGMP</i>	GDP-mannose pyrophosphorylase	AT2G39770	very short rhizoids	1		embryo lethal
<i>MpRHM</i>	rhamnose biosynthesis	AT1G78570	short rhizoids	1	yes	yes
<i>MpTHE</i>	CrRLK1L family receptor-like kinase	AT5G54380	very short rhizoids	1	yes	yes
Vesicle Transport and Cytoskeleton						
<i>MpPI4Ka1</i>	1-phosphatidylinositol 4-kinase alpha	AT1G49340	very short rhizoids	6		
<i>MpSCD</i>	Rab guanine nucleotide exchange factor	AT1G49040	short rhizoids	2	yes	yes
<i>MpSPI</i>	WD-40 repeat protein	AT1G03060	short rhizoids	3	yes	yes
<i>MpSRI1</i>	Rab guanine nucleotide exchange factor, similar to <i>S. cerevisiae</i> RIC1	AT3G61480	short rhizoids	3	yes	
<i>MpWDL</i>	microtubule-binding protein/TPX2 domain-containing protein	AT2G35880	curly rhizoids	3	yes	
<i>MpXI</i>	class XI myosin	AT3G12130	short rhizoids	5	yes	yes
<i>MpAP5M</i>	AP-5 complex subunit mu	AT2G20790	short rhizoids	1	yes	
<i>MpREN</i>	pleckstrin homology domain/RhoGAP domain-containing protein	AT5G12150	curly rhizoids	1	yes	
<i>MpSRI2</i>	calcium-binding EF-hand family protein, similar to <i>S. cerevisiae</i> PAN1	AT1G21630	very short rhizoids	1	yes	
<i>MpZWI</i>	calmodulin-binding/microtubule motor	AT5G65930	short rhizoids	1	yes	
Others/Unknown Function						
<i>MpALBA</i>	alba-like DNA/RNA-binding protein	AT1G76010	short rhizoids	5		
<i>MpEMB2756</i>	DUF616-containing protein, ceramidase	AT1G34550	short/few rhizoids	2		
<i>MpEXL1</i>	EXORDIUM-like	AT4G08950	short rhizoids	2		
<i>MpFBA1</i>	fructose-bisphosphate aldolase	AT4G38970	short rhizoids	4		
<i>MpGATA1</i>	class A GATA zinc-finger transcription factor	AT5G25830	short rhizoids	2	yes	
<i>MpIRE</i>	AGC kinase	AT5G62310	very short rhizoids	1 ^a	yes	yes
<i>MpSRI3</i>	unknown protein, ceramide metabolic process	AT5G42660	short rhizoids	2		
<i>MpTMT</i>	tonoplast monosaccharide transporter	AT3G51490	short rhizoids	2	yes	
<i>MpACLB-2</i>	ATP citrate lyase subunit B	AT5G49460	short rhizoids	1		
<i>MpCPR</i>	regulator of expression of pathogenesis-related (PR) genes	AT5G64930	short rhizoids	1		

(Continued on next page)

Table 1. Continued

Gene	Predicted Function of Encoded Protein	Closest <i>Arabidopsis</i> Homolog	Mutant Phenotype	No. of Mutant Alleles	In <i>Arabidopsis</i>	
					Expression Enriched in Root Hairs	Role in Root Hair Development
<i>MpGDPD</i>	glycerophosphodiester phosphodiesterase	AT3G02040	short rhizoids	1	yes	yes
<i>MpGDPDL</i>	glycerophosphodiester phosphodiesterase-like	AT3G20520	few rhizoids	1	yes	yes
<i>MpPRPL</i>	plastid ribosomal protein large subunit	AT1G07320	very short rhizoids	1		
<i>MpSQE</i>	squalene monooxygenase	AT1G58440	short rhizoids	1		yes
<i>MpSRI4</i>	unknown protein, similar to <i>S. cerevisiae</i> EFR3	AT2G41830	short rhizoids	1		
<i>MpTZP1</i>	zinc knuckle (CCHC-type) family protein	AT5G49400	short rhizoids	1		

See Data S3 for full gene names. See also Figures S1–S3 and Tables S1 and S2.
^aThe *Mpire* mutation was complemented by a transgene expressing the wild type *Mpire* gene.

Mpsri3-2, and *Mpxut-3*—were identified by sequencing DNA flanking T-DNA insertions in sterile DRM mutants. The *Mpire* mutation was complemented with a transgene expressing the wild-type *Mpire*-coding sequence (Figure S3). Phylogenetic analysis was conducted to assign putative functions and identify related genes in *Arabidopsis thaliana* (Table 1). Trees were constructed with maximum-likelihood statistics using protein sequences

predicted from the *M. polymorpha* transcriptome assembly and published *A. thaliana* genome (Figure S2; Data S3 and S4). In total, we identified between one and five alleles in 33 genes; multiple independent mutant alleles were identified for 17 genes, and single alleles were identified for 16 genes.
Of the 33 characterized DRM genes, five—*MpCELLULOSE SYNTHASE-LIKE CLASS D 1* (*MpCSLD1*), *MpCSLD2*,

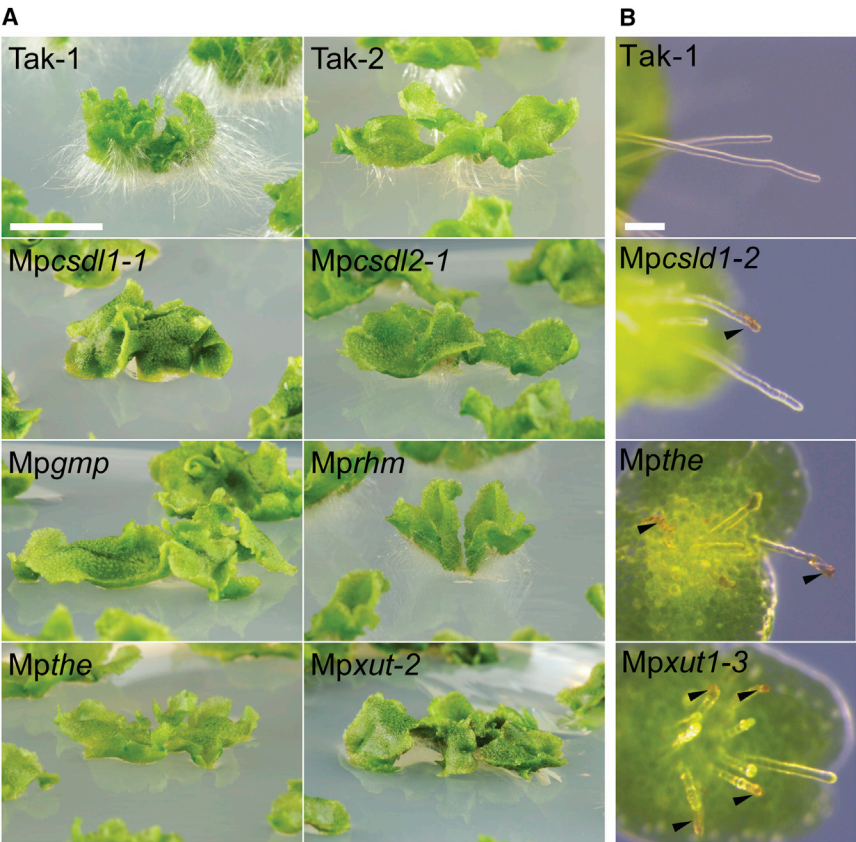


Figure 1. Phenotypes of Mutants with Defects in Cell Wall Biosynthesis and Cell Wall Integrity Sensing
Genes encoding proteins involved in cell wall biosynthesis and integrity sensing are required for rhizoid elongation.
(A) *Mpcsd1*, *Mpcsd2*, *Mpgmp*, *Mprhm*, *Mpthe*, and *Mpxut1* mutants develop shorter rhizoids than wild-type (Tak-1 and Tak-2); 21-day-old gemmalings. Scale bar, 5 mm.
(B) Defects in cell wall synthesis result in the rupture of the rhizoid tip in *Mpcsd1* and *Mpxut1* mutants. *MpTHE* is required for cell wall integrity sensing in elongating rhizoids, because *Mpthe* rhizoids rupture at their tip. Arrowheads mark the site of brown staining at rhizoid tips indicative of cell wall rupture; 2-day-old gemmalings. Scale bar, 100 μm.
See also Figure S4.

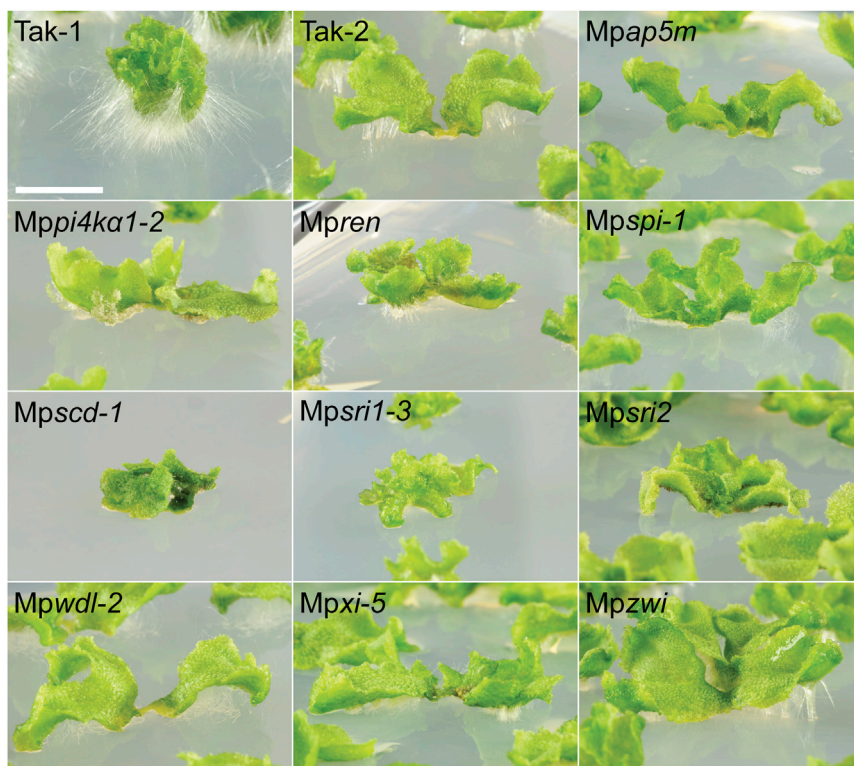


Figure 2. Phenotypes of Mutants with Defects in Cytoskeleton Function and Membrane Trafficking

Rhizoid elongation is defective in mutants with defects in cytoskeleton organization and function (Mpren, Mpscd, Mpxi, Mpzwi, Mpwdl) and membrane trafficking (Mpap5m, Mppi4ka1, Mpspi, Mpsri1, Mpsri2); 21-day-old gemmalings. Scale bar, 5 mm. See also Figure S4.

elongation (Figure 1B). To test the hypothesis that the MpTHE protein controls cell wall integrity in *M. polymorpha*, we examined rhizoid tips in Mpthe mutants for evidence of bursting. The tips of Mpthe rhizoids are brown as a result of rhizoid rupture during elongation and do not reach the same length as wild-type (Figure 1B). The defective elongation and cell integrity phenotypes of Mpthe and Atfer mutant rhizoids and root hairs, respectively, suggest that CrRLK1L proteins carry out similar functions in cell wall integrity signaling in both species and in their last common ancestor. The AtMARIS PTI kinase is a receptor-like cytoplasmic kinase (RLCK) required for

MpXYLOGLUCAN-SPECIFIC GALACTURONOSYLTRANSFERASE 1 (MpXUT1), MpGDP-MANNOSE PYROPHOSPHORYLASE (MpGMP), and MpRHAMNOSE BIOSYNTHESIS 1 (MpRHM1)—encode proteins that are predicted to function in the synthesis of cell wall polysaccharides. Consistent with the assigned functions, each of these DRM mutants—Mpcsd1, Mpcsd2, Mpxut1, Mpgmp, and Mprhm1—develops shorter rhizoids than wild-type, and Mpcsd1 and Mpxut1 mutant rhizoids also burst at their tips (Figure 1; Table 1). Closely related *A. thaliana* orthologous members—AtCSLD3, AtXUT1, and AtRHM1—are expressed in root hairs and required for root hair growth because Atcsld3, Atxut1, and Atrhm1 mutants develop short root hairs [3–7]. A role for AtGMP1 (AT2G39770) in root hair development has not yet been defined. This is most likely because loss of AtGMP1 function is lethal and mutants do not survive to the stage where root hairs develop [8]. Taken together, these data demonstrate that the same molecular mechanism for wall synthesis operates in *M. polymorpha* rhizoids and *A. thaliana* root hairs.

The sensing of cell wall integrity requires a signal transduction cascade that has been defined in *A. thaliana*. Receptor kinases in the *Catharanthus roseus* RECEPTOR KINASE 1-LIKE (CrRLK1L) subclass are required for cell wall integrity sensing in tip-growing cells [9–11]. Maximum-likelihood phylogenetic trees were constructed using CrRLK1L protein sequences from *A. thaliana* and *M. polymorpha* (Figure S2). There are 17 CrRLK1L family members in *A. thaliana* [12], which include AtTHESEUS (AtTHE) and AtFERONIA (AtFER). There is a single member of this family in *M. polymorpha* that we designated MpTHE because it is more similar to AtTHE than to any other *A. thaliana* protein in this family (Figure S2). Mpthe mutants develop short and irregularly shaped rhizoids, indicating that the MpTHE protein is required for rhizoid

cell wall integrity signaling, and acts downstream of CrRLK1L proteins in *A. thaliana* [13]. *M. polymorpha* has a single PTI protein that is sister to a group of six *A. thaliana* PTI proteins that includes MARIS. Mutants with defective MpPTI function develop short rhizoids (Figure 1A), just as mutants that lack AtMRI activity develop short root hairs in *A. thaliana*. Taken together, these data suggest that at least some of the components associated with wall integrity sensing—RLCK and CrRLK1L proteins—have been conserved since *M. polymorpha* and *A. thaliana* last shared a common ancestor.

Ten of the 33 genes identified in this screen encode proteins involved in vesicle transport or cytoskeleton function (Table 1; Figure 2) [14–23]. Close homologs of nine are highly expressed in root hairs, and three of these are required for root hair growth in *A. thaliana*. Mutations in the gene encoding the plant-specific class XI myosin, MpXI, result in the development of short rhizoids (Table 1; Figure 2), just as triple and quadruple *myosinXI* mutants develop short root hairs in *A. thaliana* [16, 17]. MpREN is a predicted ROP-GAP protein (Table 1; Figure 2) [18], and because ROPs control microfilament dynamics in *A. thaliana* [24–27] it is likely that this protein modulates microfilament dynamics during rhizoid growth. Microtubules are involved in growth direction control in tip-growing cells [28]. Consistent with this role is the observation that Mp wave dampened-like (Mpwdl) mutants developed wavy-shaped rhizoids similar to oryzalin- or Taxol-treated root hairs (Table 1; Figure 2) [28]. Mpzwi rhizoids are shorter than wild-type, indicating that the ZWICHEL-like (ZWI) kinesin motor is required for rhizoid growth (Table 1; Figure 2). Whereas homologs of MpWDL and MpZWI have not been shown to be required for root hair growth in *A. thaliana*, the expression of AtZWI and two AtWDL genes (AT2G35880 and AT4G32330) is

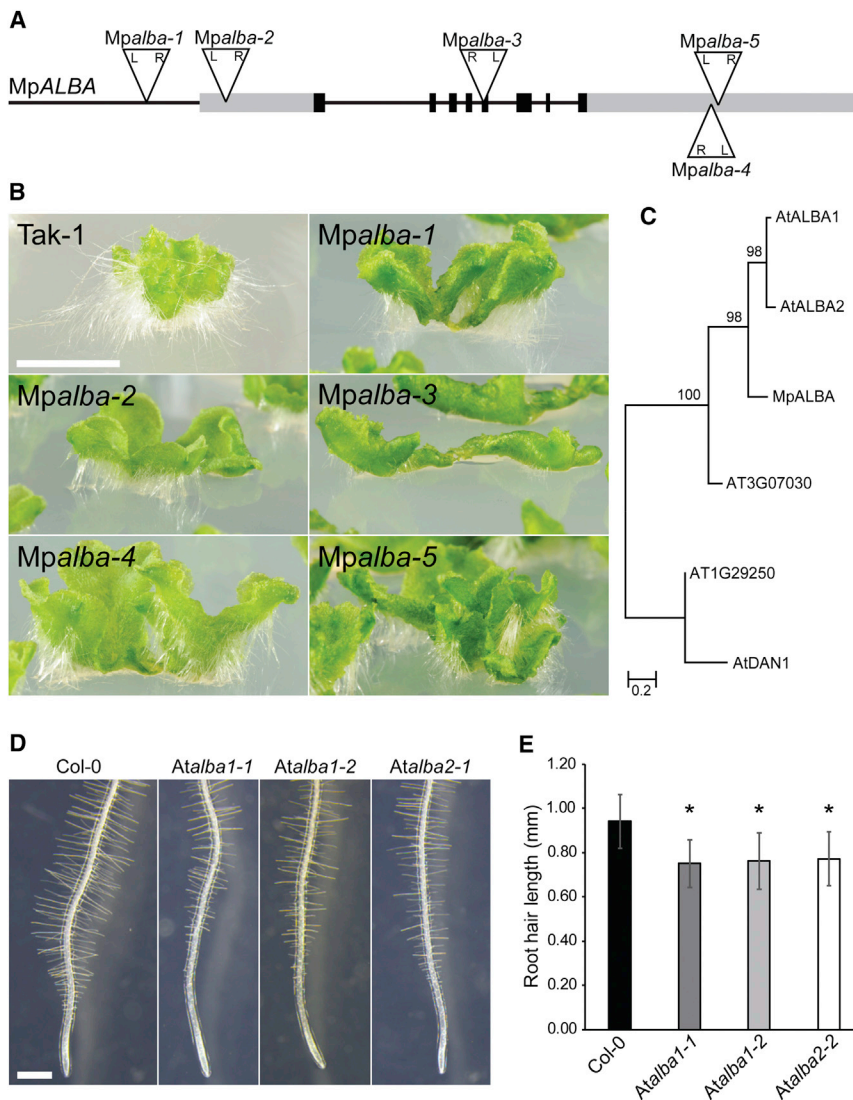


Figure 3. ALBA Proteins Are Required for Rhizoid and Root Hair Elongation in *M. polymorpha* and *A. thaliana*, Respectively

(A) Gene structure of *MpALBA*, with the T-DNA insertion sites for each allele indicated with a triangle. L and R indicate the location of the left and right borders of the T-DNA, respectively. Boxes represent exons; gray shows UTRs, and black shows coding sequences.

(B) *Mpalba* mutant rhizoids are shorter than WT (*Tak-1*); 21-day-old gemmalings. Scale bar, 5 mm.

(C) Maximum-likelihood tree of ALBA proteins from *M. polymorpha* and *A. thaliana*. Nodes are marked with approximate likelihood ratio test (aLRT) values. The scale bar represents the average number of substitutions per site.

(D) *A. thaliana* T-DNA insertion mutants of AT1G76010 (*Atalba1*) or AT1G20220 (*Atalba2*) develop shorter root hairs than wild-type (*Col-0*); 5-day-old seedlings. Scale bar, 1 mm.

(E) Root hairs of plants homozygous for *Atalba1* (AT1G76010) and *Atalba2* (AT1G20220) are significantly shorter than those of WT (*Col-0*). Asterisks indicate significant difference from WT (t test, $n > 40$, $p \leq 0.001$). Error bars indicate SD.

enriched in root hairs (*Arabidopsis* eFP Browser 2.0 [29, 30]) compared to other root cells, suggesting that they are active during root hair growth.

Three genes were identified that encode proteins predicted to be involved in endocytosis but that have not been functionally characterized in green plants to date. Their predicted function is based on the roles of similar proteins in yeast and mammals (Table 1; Figure 2). *MpSRI2* (*Mp SHORT RHIZOIDS2*) encodes an EF-hand-containing protein that is similar to *S. cerevisiae* PAN1. PAN1 is required for association of the ARP-actin polymerization complex with clathrin-coated vesicles during endocytosis in yeast [19]. *Mp SHORT RHIZOIDS1* (*MpSRI1*) encodes a protein similar to *S. cerevisiae* RIC1, which is a guanine exchange factor involved in activating Ypt6p GTPase and required for trafficking from early endosomes to the Golgi late in the endocytosis pathway [23]. *MpAP5M* is predicted to encode the subunit mu of the adaptor protein 5 (AP5) complex. AP5 is a tetrameric protein complex that coats vesicles acting as a cargo adaptor complex and is likely to be involved in endocytosis, but its precise function remains to be defined in any organism [20].

This hypothetical role is the observation that rhizoid development is also defective in mutants in which the PI4 kinase alpha is defective (Table 1). ALBA proteins are nucleic-acid-binding proteins that form chromatin in archaea and bind RNA in a number of animal parasites [34–36]. Not only is *MpALBA* required for rhizoid development because *Mpalba* mutants develop short rhizoids but we discovered that loss-of-function *alba* mutants in *A. thaliana* develop shorter root hairs than wild-type (Table 1; Figure 3). This indicates that ALBA proteins are required for tip growth in both *M. polymorpha* and *A. thaliana*, and therefore are likely to be required for tip growth in rhizoids or root hairs throughout the land plants.

These data demonstrate that genes in the same orthogroups control the synthesis of new cell surface in liverwort rhizoids and angiosperm root hairs. This conservation suggests that this mechanism acted during the growth of the first land plant rooting structures at or soon after the colonization of the land by streptophytes. These data also indicate that some of these genes—such as *THE* and *PTI*—were co-opted during the evolution of pollen tubes, one of a suite of traits that evolved during the

evolution of the seed plant life cycle. Some genes previously shown to be involved in root hair growth have not been identified in this screen. This may be because the screen was not carried out to saturation and other rhizoid development genes remain to be discovered. Furthermore, many *Arabidopsis* gene families or subfamilies that contain genes implicated in root hair growth were present as a single-copy gene in *M. polymorpha*. Therefore, the *M. polymorpha* homologs of some genes involved in root hair growth are likely to have more general developmental roles than their *Arabidopsis* counterparts, and consequently result in severe growth defects or lethality when mutated. Moreover, it is likely that the function of some genes involved in root hair growth diversified in the lineage leading to the tracheophytes after the divergence of the last common ancestor of liverworts and angiosperms. Such divergence of function is supported by the observation that the phenotypes of some loss-of-function mutants in genes from the same orthogroup are different in *M. polymorpha* and *A. thaliana*. These data are consistent with the hypothesis that the evolution of the land plant body and life cycle involved a core set of genes with conserved functions that were active in the earliest land plants and underwent duplication followed by neofunctionalization. These novel functions programmed the development of novel structures and contributed to increased life cycle diversity during the subsequent radiation of land plants.

ACCESSION NUMBERS

The accession numbers for the whole-genome and transcriptome shotgun assembly data reported in this paper are DDBJ: LVLJ000000000, ENA: GEFO000000000, and GenBank: GEFO010000000.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, two tables, and four datasets and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2016.09.062>.

AUTHOR CONTRIBUTIONS

S.H. screened 150,000 T-DNA lines and identified 38 tagged mutants; V.A.S.J. screened 105,000 lines and identified 8 tagged mutants; G.M. screened 81,000 lines and identified 15 tagged mutants; G.M. and H. Proust isolated DNA for genome sequencing and RNA for RNA sequencing; C.C. and A.J.H. assembled the genome under the guidance of S.K.; D.S.-M. helped with the coding; A.J.H. constructed the transcriptome under the guidance of S.K.; C.C. worked with G.M. to determine co-segregation of 4 mutants and carried out TAIL PCR; H. Prescott established all *M. polymorpha* growth and transformation protocols; S.H., V.A.S.J., and L.D. wrote the paper with much input from G.M. and comments from other authors; genes were grouped according to the classification established by S.H.; and L.D. conceived and designed the project.

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REFERENCES

1. Kenrick, P., and Crane, P.R. (1997). The origin and early evolution of plants on land. *Nature* **389**, 33–39.
2. Wellman, C.H., and Gray, J. (2000). The microfossil record of early land plants. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **355**, 717–731.
3. Peña, M.J., Kong, Y., York, W.S., and O'Neill, M.A. (2012). A galacturonic acid-containing xyloglucan is involved in *Arabidopsis* root hair tip growth. *Plant Cell* **24**, 4511–4524.
4. Diet, A., Link, B., Seifert, G.J., Schellenberg, B., Wagner, U., Pauly, M., Reiter, W.-D., and Ringli, C. (2006). The *Arabidopsis* root hair cell wall formation mutant *lr1* is suppressed by mutations in the *RHM1* gene encoding a UDP-L-rhamnose synthase. *Plant Cell* **18**, 1630–1641.
5. Ringli, C., Bigler, L., Kuhn, B.M., Leiber, R.M., Diet, A., Santelia, D., Frey, B., Pollmann, S., and Klein, M. (2008). The modified flavonol glycosylation profile in the *Arabidopsis* *rol1* mutants results in alterations in plant growth and cell shape formation. *Plant Cell* **20**, 1470–1481.
6. Wang, X., Cnops, G., Vanderhaeghen, R., De Block, S., Van Montagu, M., and Van Lijsebettens, M. (2001). *AtCSLD3*, a cellulose synthase-like gene important for root hair growth in *Arabidopsis*. *Plant Physiol.* **126**, 575–586.
7. Favery, B., Ryan, E., Foreman, J., Linstead, P., Boudonck, K., Steer, M., Shaw, P., and Dolan, L. (2001). *KOJAK* encodes a cellulose synthase-like protein required for root hair cell morphogenesis in *Arabidopsis*. *Genes Dev.* **15**, 79–89.
8. Lukowitz, W., Nickle, T.C., Meinke, D.W., Last, R.L., Conklin, P.L., and Somerville, C.R. (2001). *Arabidopsis* *cyt1* mutants are deficient in a mannose-1-phosphate guanylyltransferase and point to a requirement of N-linked glycosylation for cellulose biosynthesis. *Proc. Natl. Acad. Sci. USA* **98**, 2262–2267.
9. Boisson-Dernier, A., Roy, S., Kritsas, K., Grobei, M.A., Jaciubek, M., Schroeder, J.I., and Grossniklaus, U. (2009). Disruption of the pollen-expressed *FERONIA* homologs *ANXUR1* and *ANXUR2* triggers pollen tube discharge. *Development* **136**, 3279–3288.
10. Miyazaki, S., Murata, T., Sakurai-Ozato, N., Kubo, M., Demura, T., Fukuda, H., and Hasebe, M. (2009). *ANXUR1* and 2, sister genes to *FERONIA/SIRENE*, are male factors for coordinated fertilization. *Curr. Biol.* **19**, 1327–1331.
11. Duan, Q., Kita, D., Li, C., Cheung, A.Y., and Wu, H.-M. (2010). *FERONIA* receptor-like kinase regulates RHO GTPase signaling of root hair development. *Proc. Natl. Acad. Sci. USA* **107**, 17821–17826.
12. Cheung, A.Y., and Wu, H.-M. (2011). THESEUS 1, *FERONIA* and relatives: a family of cell wall-sensing receptor kinases? *Curr. Opin. Plant Biol.* **14**, 632–641.
13. Boisson-Dernier, A., Franck, C.M., Lituev, D.S., and Grossniklaus, U. (2015). Receptor-like cytoplasmic kinase MARIS functions downstream of CrRLK1L-dependent signaling during tip growth. *Proc. Natl. Acad. Sci. USA* **112**, 12211–12216.
14. Falbel, T.G., Koch, L.M., Nadeau, J.A., Segui-Simarro, J.M., Sack, F.D., and Bednarek, S.Y. (2003). SCD1 is required for cytokinesis and polarized cell expansion in *Arabidopsis thaliana*. *Development* **130**, 4011–4024.
15. Siedler, R., Jakoby, M., Marin, B., Galiana-Jaime, E., and Hülkamp, M. (2009). The cell morphogenesis gene *SPRRIG* in *Arabidopsis* encodes a WD/BEACH domain protein. *Plant J.* **59**, 612–621.

16. Peremyslov, V.V., Prokhnevsky, A.I., Avisar, D., and Dolja, V.V. (2008). Two class XI myosins function in organelle trafficking and root hair development in *Arabidopsis*. *Plant Physiol.* **146**, 1109–1116.
17. Peremyslov, V.V., Prokhnevsky, A.I., and Dolja, V.V. (2010). Class XI myosins are required for development, cell expansion, and F-actin organization in *Arabidopsis*. *Plant Cell* **22**, 1883–1897.
18. Hwang, J.-U., Vernoud, V., Szumlanski, A., Nielsen, E., and Yang, Z. (2008). A tip-localized RhoGAP controls cell polarity by globally inhibiting Rho GTPase at the cell apex. *Curr. Biol.* **18**, 1907–1916.
19. Bradford, M.K., Whitworth, K., and Wendland, B. (2015). *Pan1* regulates transitions between stages of clathrin-mediated endocytosis. *Mol. Biol. Cell* **26**, 1371–1385.
20. Hirst, J., Barlow, L.D., Francisco, G.C., Sahlender, D.A., Seaman, M.N.J., Dacks, J.B., and Robinson, M.S. (2011). The fifth adaptor protein complex. *PLoS Biol.* **9**, e1001170.
21. Perrin, R.M., Wang, Y., Yuen, C.Y.L., Will, J., and Masson, P.H. (2007). WVD2 is a novel microtubule-associated protein in *Arabidopsis thaliana*. *Plant J.* **49**, 961–971.
22. Oppenheimer, D.G., Pollock, M.A., Vacik, J., Szymanski, D.B., Ericson, B., Feldmann, K., and Marks, M.D. (1997). Essential role of a kinesin-like protein in *Arabidopsis* trichome morphogenesis. *Proc. Natl. Acad. Sci. USA* **94**, 6261–6266.
23. Siniosoglou, S., Peak-Chew, S.Y., and Pelham, H.R. (2000). Ric1p and Rgp1p form a complex that catalyses nucleotide exchange on Ypt6p. *EMBO J.* **19**, 4885–4894.
24. Li, H., Lin, Y., Heath, R.M., Zhu, M.X., and Yang, Z. (1999). Control of pollen tube tip growth by a Rop GTPase-dependent pathway that leads to tip-localized calcium influx. *Plant Cell* **11**, 1731–1742.
25. Fu, Y., Wu, G., and Yang, Z. (2001). Rop GTPase-dependent dynamics of tip-localized F-actin controls tip growth in pollen tubes. *J. Cell Biol.* **152**, 1019–1032.
26. Gu, Y., Fu, Y., Dowd, P., Li, S., Vernoud, V., Gilroy, S., and Yang, Z. (2005). A Rho family GTPase controls actin dynamics and tip growth via two counteracting downstream pathways in pollen tubes. *J. Cell Biol.* **169**, 127–138.
27. Fu, Y., Gu, Y., Zheng, Z., Wasteneys, G., and Yang, Z. (2005). *Arabidopsis* interdigitating cell growth requires two antagonistic pathways with opposing action on cell morphogenesis. *Cell* **120**, 687–700.
28. Bibikova, T.N., Blancaflor, E.B., and Gilroy, S. (1999). Microtubules regulate tip growth and orientation in root hairs of *Arabidopsis thaliana*. *Plant J.* **17**, 657–665.
29. Schmid, M., Davison, T.S., Henz, S.R., Pape, U.J., Demar, M., Vingron, M., Schölkopf, B., Weigel, D., and Lohmann, J.U. (2005). A gene expression map of *Arabidopsis thaliana* development. *Nat. Genet.* **37**, 501–506.
30. Winter, D., Vinegar, B., Nahal, H., Ammar, R., Wilson, G.V., and Provart, N.J. (2007). An “Electronic Fluorescent Pictograph” browser for exploring and analyzing large-scale biological data sets. *PLoS ONE* **2**, e718.
31. Tzafrir, I., Pena-Muralla, R., Dickerman, A., Berg, M., Rogers, R., Hutchens, S., Sweeney, T.C., McElver, J., Aux, G., Patton, D., and Meinke, D. (2004). Identification of genes required for embryo development in *Arabidopsis*. *Plant Physiol.* **135**, 1206–1220.
32. Lloyd, J., and Meinke, D. (2012). A comprehensive dataset of genes with a loss-of-function mutant phenotype in *Arabidopsis*. *Plant Physiol.* **158**, 1115–1129.
33. Baird, D., Stefan, C., Audhya, A., Weys, S., and Emr, S.D. (2008). Assembly of the PtdIns 4-kinase Stt4 complex at the plasma membrane requires Ypp1 and Efr3. *J. Cell Biol.* **183**, 1061–1074.
34. Aravind, L., Iyer, L.M., and Anantharaman, V. (2003). The two faces of Alba: the evolutionary connection between proteins participating in chromatin structure and RNA metabolism. *Genome Biol.* **4**, R64.
35. Guo, R., Xue, H., and Huang, L. (2003). Ssh10b, a conserved thermophilic archaeal protein, binds RNA in vivo. *Mol. Microbiol.* **50**, 1605–1615.
36. Marsh, V.L., Peak-Chew, S.Y., and Bell, S.D. (2005). Sir2 and the acetyltransferase, Pat, regulate the archaeal chromatin protein, Alba. *J. Biol. Chem.* **280**, 21122–21128.