Molecular Analysis of Cellulose Biosynthesis in Arabidopsis

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Cellulose, an abundant, crystalline polysaccharide, is central to plant morphogenesis and to many industries. Chemical and ultrastructural analyses together with map-based cloning indicate that the RSW1 locus of Arabidopsis encodes the catalytic subunit of cellulose synthase. The cloned gene complements the rsw1 mutant whose temperature-sensitive allele is changed in one amino acid. The mutant allele causes a specific reduction in cellulose synthesis, accumulation of noncrystalline β-1,4-glucan, disassembly of cellulose synthase, and widespread morphological abnormalities. Microfibril crystallization may require proper assembly of the RSW1 gene product into synthase complexes whereas glucan biosynthesis per se does not.

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29. For measurement of iron uptake, cells were washed with saline-EDTA and incubated with Cp for 30 min in RPMI medium; the amount of 57Fe released into the conditioned me-
dium was measured by liquid scintillation counting.
30. For measurement of iron uptake, cells were washed with phosphate-buffered saline and then with iron-
tree RPMI medium. Cells were incubated with puri-
fied human Cp (Cabiobiocem, San Diego, CA) in the presence of 0.5 μM 57Fe-NTA and 1 mM ascor-
ic acid in RPMI medium for 15 min at 25°C. The cultures were washed with saline-EDTA, and the
cells were harvested with NaOH; after neutraliza-
tion, cellular 57Fe was measured by liquid scintilla-
tion counting.
32. We are grateful to J. Giltn (Washington University) for the full-length Cp cDNA and for helpful dis-
cussions.
33. The purified reaction products were hybridized to linearized plasmid DNAs [Cp, 15 μg of pcDNA3-Cp; vector control, 15 μg of pcDNA3; and 1 μg of pcDNA3–glyceroldehyde phosphate de-
hydrogenase (SAPDH)] immobilized onto a nylon membrane.
34. We are grateful to J. Giltn (Washington University) for the full-length Cp cDNA and for helpful discus-
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and residue numbers are based on the supplementary material (14-exon RSW1). The T1 seeds of rsw1 grown at 31°C (bottom). Coincident peaks show that the rsw1 glucan is 1,4-linked. Roots frozen in nitrogen slush without cryoprotection were freeze-fractured with the use of double replica holders in a Balzers BAF 400T (18). Normal microfibrils in rsw1 (18°C). (C) Rosettes (P face) of the mutant are indistinguishable from the wild type at 18°C, but (D) are rare and sometimes irregular in rsw1 seedlings within 3 hours of transfer to 31°C. Particles in rows (arrows) can curve or cluster (box) under longer (18-hour) exposures. Scale bars, 50 nm.

Fig. 1. (A) Gas chromatography of alditol acetates of methylated sugars from laminarin (top) and cellulose (middle) standards and from the glucan purified from the ammonium oxalate fraction from shoots of rsw1 grown at 31°C (bottom). Coincident peaks show that the rsw1 glucan is 1,4-linked. (B) through (E) Roots frozen in nitrogen slush without cryoprotection were freeze-fractured with the use of double replica holders in a Balzers BAF 400T (18). (B) Normal microfibrils in rsw1 (18°C). (C) Rosettes (P face) of the mutant are indistinguishable from the wild type at 18°C, but (D) are rare and sometimes irregular in rsw1 seedlings within 3 hours of transfer to 31°C. (E) Particles in rows (arrows) can curve or cluster (box) under longer (18-hour) exposures. Scale bars, 50 nm.

Fig. 2. (A) Part of contig IV from Arabidopsis chromosome 4 (19) refined with the use of primers based on partially sequenced left (L) and right (R) ends to establish YAC overlap by PCR (vertical lines), by converting g3100 to a cleaved amplified polymorphic sequence (CAPS) marker and by detecting new Co/Ler polymorphisms in three YAC ends (CAPS marker for yUP5C8RE; RFLP markers for EG6C4LE and yUP17GLE; white boxes on the genetic map). (B) Sequences hybridizing to EST T20782 lie centrally in cosmid 23H12 and within pRSW1 [cloned into pBIN19 (20)]. The 14-exon RSW1 gene produces a predicted protein product including transmembrane helices (hatched) and the D,D,D,OVLFW (6) signature. Conserved regions (black), variable regions (lighter), and residue numbers are based on the supplementary material (17). (C) Complementation of rsw1. T1 seed of rsw1 plants transformed (21) with cosmid 23H12 were selected for kanamycin resistance (21°C over 10 to 12 days). Two days at 31°C after 5 days at 21°C caused swelling of rsw1 (left) but not of T2 (center) or wild-type (right) seedlings. (D) Wild-type (inset) and rsw1 seedlings grown for 10 days at 31°C and viewed by cryoscanning electron microscopy. Epidermal cells in all organs of rsw1 plants are misshapen.

Rosettes (terminal complexes) are the putative hexameric cellulose synthase complexes of higher plant plasma membranes (5). Freeze-fractured root cells of wild type and mutant grown at 18°C show cellulose microfibrils (Fig. 1B). Rosettes on the P face of the mutant plasma membrane at 18°C (Fig. 1C) resemble those of the wild type, but transferring the mutant to 31°C reduces rosette numbers within 30 min, with extensive loss after 3 hours (Fig. 1D) and a loss of definition to the terminal globules on the E face. Plasma membrane particles tend to align in the mutant after prolonged exposure to the restrictive temperature (Fig. 1E). Cortical microtubules that align cellulose microfibrils and Golgi bodies that synthesize other wall polysaccharides appeared unchanged.

The rsw1 mutation therefore disassembles cellulose synthase complexes, reduces cellulose accumulation, and causes β,1,4-glucan to accumulate in a noncrystalline form. It maps (6) to a region of chromosome 4 (Fig. 2A) to which a mapping program had assigned an expressed sequence tag (EST) that, it was deduced, might show weak similarities to a bacterial cellulose synthase (7). Full sequence of the EST partial cDNA indeed showed all except the first D of a D,D,D,OVLFW signature (8) characterizing a heterogeneous group of processive β-glycosyl transferases and more extended but still weak similarities to a subset (9). Correcting radial swelling by transforming rsw1 (Fig. 2C) with full-length genomic clones (Fig. 2B) identical to sequences found on a yeast artificial chromosome (YAC) covering the mapped site proves that the gene is RSW1. The 3.8-kb RSW1 transcript is widespread, as are misshapen cells in mutant plants grown at 31°C (Fig. 2D). A similarly sized transcript in the mutant is consistent with the mutant allele substituting Val for Ala549 after a C to T nucleotide change (7).

Four pieces of evidence make a compelling case that the RSW1 gene product encodes the catalytic subunit of cellulose synthase: (i) The rsw1 mutation selectively

trile. Shoots of rsw1 seedlings grown at the restrictive temperature (31°C) have less cellulose than wild-type seedlings (159 ± 19 versus 363 ± 28 nmol of glucose per milligram of plant dry weight) but more of an ammonium oxalate–extracted glucan (195 versus 58 nmol mg⁻¹), which methylation analysis (Fig. 1A) and enzyme digestion show is β,1,4–linked (4). Facilitated extraction and digestion by enzymes and trifluoroacetic acid indicate low crystallinity, the property that makes cellulose resistant to extraction and digestion. Smaller changes in Golgi-synthesized polysaccharides show that RSW1 is specifically involved in cellulose biosynthesis.
inhibits cellulose synthesis and promotes accumulation of a noncrystalline β-1,4-glucan; (ii) rsw1 disassembles plasma membrane rosettes, a plausible mechanism for reducing cellulose and placing the RSW1 product in the rosettes or interacting with them; (iii) the D,D,QQXRW signature identifies the RSW1 gene product as a processive glycosyl transferase (9) in family 2 of inverting nucleotide-diphospho-sugar glycosyltransferases (10) and with demonstrated uridine 5′-diphosphate–glucose binding ability in the highly similar cotton celA1 gene (3); and (iv) the wild-type allele corrects the mutant’s radial swelling that results from reduced cellulose synthesis.

The deduced 122-kD RSW1 product (Fig. 3) closely resembles the products of Ath-A and Ath-B [two full-length Arabidopsis CDAs (11)], of the cotton celA1 genes proposed as cellulose synthase catalytic subunits (3), and of rice ESTs D48636 (3) and D39394 (11). Architecture is conserved (Fig. 2B). Six predicted membrane-spanning regions lie close to the COOH terminus, and two others separate an extended NH2-terminal region from a small, proline-rich cytoplasmic tail. The NH2 terminus functions as a prokaryotic glycosyl transferases (3, 7, 9, 12). Strikingly variable regions extend intern, highly conserved regions, which are particularly prominent in the central domain (11). The NH2-terminal regions are heterogeneous except for a cysteine-rich domain that may cause protein-protein binding (13). The predicted products of five Arabidopsis genomic sequences (14) diverge further from RSW1, Ath-A, and Ath-B: they are smaller (710 to 828 amino acids versus 1081 in RSW1), lack an extended NH2 terminus, vary in the number and position of predicted transmembrane helices, retain extensive sequence similarities in the central domain but have major insertions and deletions, and differ in their D,D spacings and QQXRW motifs. Arabidopsis EST fragments recently proposed as cellulose synthases (15) show little sequence similarity to RSW1. All belong to the large class of Arabidopsis Csi genes (cellulose-synthe-like) (16), but weak similarities do not prove a function in cellulose synthesis given the widely different polymers produced by enzymes sharing weak related sequences (10). In our view, only Ath-A and Ath-B of the full-length Arabidopsis genes sufficiently resemble the functionally characterized RSW1 to be prime candidates for additional cellulose synthases.

In conclusion, chemical and ultrastructural changes in the cellulose-deficient mutant combine with gene cloning, complementation of the mutant, and sequence analyses to show that the RSW1 locus encodes the catalytic subunit of cellulose synthase. The noncrystalline β-1,4-glucan in the shoot of the rsw1 mutant suggests that the mutant allele interrupts assembly of glucan chains into microfibrils. We hypothesize that at the restrictive temperature, mutant synthase complexes disassemble to monomers (or smaller oligomers) undetectable by freeze etching. The monomers continue producing β-1,4-glucan, but the disordered chains fail to crystallize in an acid-resistant form. Crystallization—with consequences for wall mechanics that are central to morphogenesis and industrial fiber usage—therefore requires assembled rosettes.

REFERENCES AND NOTES

4. Shoots from wild-type Columbia (Co) and backcrossed rsw1 grown on agar for 7 days at 21°C or for 2 days at 21°C and 5 days at 31°C (2) were rinsed and stored in liquid nitrogen. Carbohydrate fractionation was essentially as described by J. R. Scopp, C. Waldron, I. M. Marrina, Pestic. Biochem. Physiol. 39, 93 (1991). Glucan was extracted with ammonium oxalate from three separate batches of rsw1 purified in the supernatant after precipitation of peaks. Since overnight with 2% cetyltrimethylammonium bromide. Glucose in trifluoroacetic acid hydrolysates was quantified against inositol internal standard by gas chromatography electron-impact–mass spectrometry (Fisons MD800) of alditol acetates as cellulose synthase [H. C. Wong et al. (World Scientific, Singapore, 1991)]. Glucan from starch (Sigma; 200 mg · ml−1) or starch (Sigma; 200 mg · ml−1) was digested with mixtures of endo-cellulase (E.C. 3.2.1.4; Megazyme, Australia) and β-glucosidase (E.C. 3.2.1.21; Sigma) or α-amylase (E.C. 3.2.1.1; Sigma) and β-glucosidase (E.C. 3.1.2.1; Sigma). The former enzyme mixture was inactive against 1,3-linked laminarin and starch but released 83% of the glucose released by trifuloroacetic acid from the rsw1 glucan, whereas the latter pair was only partially effective but could release 95% of the glucose from starch.


7. EST T20782 became of interest when located to YACs in the rsw1 interval as part of a mapping program [H. H. Höfte et al., Plant J. 4, 1051 (1993) and aligned in a contig to rice ESTs (D41966, D41766, D40891, and D41261)]. These ESTs and their 5′ ends show weak sequence similarities to a bacterial cellulose synthase [H. C. Wong et al., Proc. Natl. Acad. Sci. U.S.A. 90, 3111 (1993)], which hybridizes with the T20782 insert, and on YAC5C8, which spans the rsw1 locus (Fig. 2A). T20782 is part of the RSW1 transcript derived from exons 8 through 14 and 12 base pairs from exon 7. Exons 1 to 8 were sequenced from cDNA polymerase chain reaction (PCR) products amplified from Cw double-stranded cDNA with primers upstream of the RSW1 start site and a primer from within the EST. We determined the rsw1 mutation by sequencing two PCR fragments spanning the full rsw1 cDNA and rsw1 genomic PCR fragments corresponding to the region.

8. Single-letter abbreviations for amino acid residues are as follows: D, Asp; L, Leu; Q, Gln; R, Arg; V, Val; and W. Trp. X stands for any single residue; the comma stands for intervening sequences of residues of arbitrary length.


11. At-A and Ath-B were CDA clones isolated by hy-
Alopecia Universalis Associated with a Mutation in the Human hairless Gene

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There are several forms of hereditary human hair loss, known collectively as alopecias, the molecular bases of which are entirely unknown. A kindred with a rare, recessively inherited type of alopecia universalis was used to search for a locus by homozygosity mapping, and linkage was established in a 6-centimorgan interval on chromosome 8p12 (the logarithm of the odds favoring linkage score was 6.19). The human homolog of a murine gene, hairless, was localized in this interval by radiation hybrid mapping, and a missense mutation was found in affected individuals. Human hairless encodes a putative single zinc finger transcription factor protein with restricted expression in the brain and skin.

The human hair follicle is a dynamic structure that generates hair through a complex and exquisitely regulated cycle of growth and remodeling (1). Despite the extensive descriptive understanding of the hair cycle, currently, very little is known about the molecular control of the signals that regulate progression through the hair cycle, although it is clear that at least some potentially influential regulatory molecules may play a role (1). For example, a knock-out mouse with targeted ablation of the gene encoding the fibroblast growth factor 5 (FGF5) provided evidence that FGF5 is an inhibitor of hair elongation, and the mouse had an increase in hair length due to an increase in the time that follicles remain in anagen. The FGF5 gene was also deleted in a putative single zinc finger transcription factor protein with restricted expression in the brain and skin.

The first 90 residues of the predicted gene products of RSW1, Ath-A, Ath-B, and rce D39394, from Arabidopsis Biological Resource Center (Columbus, OH); rice EST D39394, from the MAFF DNA Bank (Tsukuba, Japan). The sequences were analyzed at the Australian National Genome Information Service with the use of Wisconsin GCG software (J. Devereux, P. Haeberli, R. Smithies, Nucleic Acids Res. 12, 387 (1984)). Sequences for RSW1, Ath-A, Ath-B, and rice D39394 have been deposited at GenBank (AF207172, AF207173, AF207174, and AF300552, respectively); comparisons are provided as supplementary material (17).


13. The first 90 residues of the predicted gene products of RSW1, Ath-A, Ath-B, rice EST D39394, and cot- ton cellA1 resemble three putative plant basic leucine zipper transcription factors (X97904, L28003, and L28004) with conserved Cys spacing (underlined, Fig. 3). Similar motifs are implicated in protein-protein or protein-lipid interaction [P. S. Fremeron, Ann. N.Y. Acad. Sci. 864, 174 (1993)], and cellA1 was reported to bind Zn2+ (Y. Kawagoe and D. P. Delmer, Plant Physiol. 114 (suppl.), 85 (1997)).

14. Five protein sequences (accession numbers 2262116, 2262115, 2262214, 2244887, and 2244890) were identified by Gapped BLASTX searches of GenBank with the use of RSW1 and were analyzed as in (17).


17. Supplementary material available at www.sciencemag.org/feature/data/974564.shl


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bromidization from a cDNA library of 21-day-old Co plants using a PCR fragment spanning the 5’ part of RSW1 transcript. Ath-A is on YAC CIC9H6, chromosome 4. The EST T20782 clone was from Arabidop- sisis Biological Resource Center (Columbus, OH); rice EST D39394, from the MAFF DNA Bank (Tsukuba, Japan). The sequences were analyzed at the Australian National Genome Information Service with the use of Wisconsin GCG software (J. Devereux, P. Haeberli, O. Smithies, Nucleic Acids Res. 12, 387 (1984)). Sequences for RSW1, Ath-A, Ath-B, and rice D39394 have been deposited at GenBank (AF207172, AF207173, AF207174, and AF300552, respectively); comparisons are provided as supplementary material (17).


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