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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10. ———, ibid. 244, 9518 (1971).
17. C. Askwith et al., Cell 76, 403 (1994).
30. For measurement of iron release, cells were preincubated with 100 μM Fe-NTA for 4 hours. The cells were washed with saline-EDTA and incubated with Cp for 30 min in RPMI medium; the amount of Fe released into the conditioned medium was measured by liquid scintillation counting.
31. For measurement of iron uptake, cells were washed with phosphate-buffered saline and then with iron-free RPMI medium. Cells were incubated with purified human Cp (Calbiochem, San Diego, CA) in the presence of 0.5 μM Fe-NTA and 1 mM ascorbic 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 neutralization, cellular 55Fe was measured by liquid scintillation counting.
33. Isolated nuclei from 5 × 10⁷ HepG2 cells were incubated with 100 μM Fe-NTA for 4 hours. The cells were washed with saline-EDTA and incubated with Cp for 30 min in RPMI medium; the amount of Fe released into the conditioned medium was measured by liquid scintillation counting.
34. We are grateful to J. Gitlin (Washington University) for the full-length Cp cDNA and for helpful discussions. This work was supported by NIH grants HL29582 and HL52692 (P.L.F.) and by a Fellowship Award from the American Heart Association, Northeast Ohio Affiliate (C.K.M.).
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and residue numbers are based on the supplementary material (17) days at 31°C and viewed by cryoscanning electron microscopy. Epidermal cells in all organs of rsw1 not of T2 (center) or wild-type (right) seedlings. (D) RSW1 14-exon to EST T20782 lie centrally in cosmid 23H12 and within pRSW1 [cloned into pBIN19 (20) (hatched) and the D,D,D,QVLRW (rsw1 (21°C over 10 to 12 days). Two days at 31°C after 5 days at 21°C caused swelling of rsw1 T1 seeds of irregluar in face) of the mutant are indistinguishable from the wild type at 18°C, but (D) are rare and sometimes double replica holders in a Balzers BAF 400T (E) through the genetic map). (3) Detecting new Co/Ler polymorphisms in three YAC ends (CAPS marker for yUP5C8RE; RFLP lines), by converting g8300 to a cleaved amplified polymorphic sequence (CAPS) marker and by based on partially sequenced left (L) and right (R) ends to establish YAC overlap by PCR (vertical restriction digestion shows is (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.

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,QXRXW signature (8) characterizing a heterogeneous group of processive B-glycosyltransferases 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
inhibits cellulose synthesis and promotes accumulation of a noncrystalline β,1,4-glucan; (ii) rsw1 disassembles plasma membrane rosette-like structures, a plausible mechanism for reducing cellulose and placing the RSW1 product in the rosettes or interacting with them; (iii) the D.D, D.QXRW signature identifies the RSW1 gene product as a pro- cessive glycosyl transferase (9) in family 2 of inverting nucleotide-diphospho-sugar glycosyltransferases (10) and with demonstrated uridine 5′-diphosphate–glucose binding activity in the highly similar cotton cellulose synthase gene (3); and (iv) the wild-type allele corrects the mutant’s radial swelling that results from reduced cellulose synthesis.

The deduced 1228 amino acids versus 1081 in the cDNA sequences (4) for Ath-A and Ath-B. The noncrystalline RSW1-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.

Fig. 3. Sequence of the predicted RSW1 gene product. The D.D, D.QXRW signature is bold, conserved Cys residues are underlined, and Ala (substituted with Val in rsw1) is bold and underlined.

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3. H.-G. Nam et al., Plant Cell 9, 1599 (1997) or CAPS markers in F2 or F3 families from a cross to Landsberg (Ler). Mapping information at Arabidopsis thaliana Database (AtdB) (Stanford, CA).
4. EST T20782 became of interest when located to YACs in the rsw1 interval as a part of a mapping program (H. H. Höfte et al., Plant J. 4, 1051 (1993) and aligned in Northern Database ESTs D41866, D41766, D40691, and D41261). These 5′-end markers show weak sequence similarities to a bacterial cellulose synthase (C. Wong et al., Proc. Natl. Acad. Sci. U.S.A. 93, 12637 (1996)) or of starch (Sigma; 200 μg ml−1) was digested with mixtures of endo-cellulases (E.C. 3.2.1.24, 3.2.1.21; Sigma) or α-amylose (E.C. 3.2.1.1; Sigma) and α-glucosidase (E.C. 3.2.1.20; Sigma). The former enzyme pair was inactive against 1,3-linked laminarin and starch but released 83% of the glucose released by trifluoroacetic acid from the rsw1 glucan, whereas the latter pair released none but could release 95% of the glucose from starch.
7. EST T20782 became of interest when located to YACs in the rsw1 interval as a part of a mapping program (H. H. Höfte et al., Plant J. 4, 1051 (1993) and aligned in Northern Database ESTs D41866, D41766, D40691, and D41261). These 5′-end markers show weak sequence similarities to a bacterial cellulose synthase (C. Wong et al., Proc. Natl. Acad. Sci. U.S.A. 93, 12637 (1996)) or of starch (Sigma; 200 μg ml−1) was digested with mixtures of endo-cellulases (E.C. 3.2.1.24, 3.2.1.21; Sigma) or α-amylose (E.C. 3.2.1.1; Sigma) and α-glucosidase (E.C. 3.2.1.20; Sigma). The former enzyme pair was inactive against 1,3-linked laminarin and starch but released 83% of the glucose released by trifluoroacetic acid from the rsw1 glucan, whereas the latter pair released none but could release 95% of the glucose from starch.
8. Single-letter abbreviations for the 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 D,D,D,QXXRW signature is bold and underlined.
11. Ath-A and Ath-B were cDNA clones isolated by hybridization with the T20782 insert, and on YAC508, which spans the rsw1 locus (Fig. 2A). T20782 is part of the RSW1 genomic cosmids 23H12 (N. E. Osiewicz, F. B. Martin, F. M. Ausubel, Nucleic Acids Res. 16, 10765 (1988) and aligned in Northern Database ESTs D41866, D41766, D40691, and D41261). These 5′-end markers show weak sequence similarities to a bacterial cellulose synthase (C. Wong et al., Proc. Natl. Acad. Sci. U.S.A. 93, 12637 (1996)) or of starch (Sigma; 200 μg ml−1) was digested with mixtures of endo-cellulases (E.C. 3.2.1.24, 3.2.1.21; Sigma) or α-amylose (E.C. 3.2.1.1; Sigma) and α-glucosidase (E.C. 3.2.1.20; Sigma). The former enzyme pair was inactive against 1,3-linked laminarin and starch but released 83% of the glucose released by trifluoroacetic acid from the rsw1 glucan, whereas the latter pair released none but could release 95% of the glucose from starch.
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 of 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 mouse with targeted ablation of the gene FGF7 or keratinocyte growth factor, which resulted in defects in the positioning and angling of the hair follicles (2). More recently, a mutation in a structural protein, mouse desmoglein 3 (encoded by the gene ds3), was found to be the underlying mutation in the naturally occurring mouse phenotype, balding (5). Finally, the nude mouse phenotype, characterized by hairlessness and athymia, was found to be the result of mutations in the winged-helix nude (whn) gene, a member of the winged-helix class of transcription factors (6).

There are several forms of hereditary human hair loss, known collectively as alopecias, which may represent a dysregulation of the cycle of hair growth and remodeling (1), yet the molecular basis of the alopecias has remained largely unexplored (7). The most common form of hair loss, known as androgenetic alopecia (male pattern baldness), is believed by some to affect ~80% of the population (7). Alopecia areata is a common dermatologic disease affecting 2.5 million individuals in the United States alone, which causes round, patchy hair loss on the scalp (7). Alopecia areata can progress to involve hair loss from the entire scalp; this condition is referred to as alopecia totalis. Alopecia universalis (AU) is the term for the most extreme example of disease progression, which results in the complete absence of scalp and body hair (7). Although an autoimmune pathomechanism for alopecia areata has been