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Review

Cellulose synthesis: mutational analysis and genomic perspectives using *Arabidopsis thaliana*

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Abstract. Cellulose microfibrils containing crystalline β-1,4-glucan provide the major structural framework in higher-plant cell walls. Genetic analyses of *Arabidopsis thaliana* now link specific genes to plant cellulose production just as was achieved some years earlier with bacteria. Cellulose-deficient mutants have defects in several members of one family within a complex glycosyltransferase superfamily and in one member of a small family of membrane-bound endo-1,4-β-glucanases. The mutants also accumulate a readily extractable β-1,4-glucan that has short chains which, in at least one case, are lipid linked. Cellulose could be made by direct extension of the glucan chain by the glycosyltransferase or, as the mutant suggests, by an indirect route which makes lipid-linked oligosaccharides. Models discussed incorporate the known enzymes and lipo-glucan and raise the possibility that different CesA glycosyltransferases may catalyse different steps.

Key words. Cellulose synthesis; Mutants; *Arabidopsis thaliana*; Glycosyltransferase; Endo-1,4-β-glucanase; Lipo-glucan.

The plant cell wall

Cellulosic cell walls are an important and distinctive feature of higher plants. Progress in understanding different facets of cell wall biology has been uneven: relatively sophisticated views of cell wall composition [1] and ultrastructure [2] contrast with the sketchy knowledge of polysaccharide biosynthesis. Genetics has recently driven exciting progress in understanding how cellulose is made, and similar progress in understanding the synthesis of other polysaccharides can be anticipated. Moreover, when we look beyond the phase of using mutants to link genes to polysaccharide production, the mutants will provide a resource that can invigorate studies of how cell wall assembly, structure and function respond to changes in polysaccharide composition.

This review gives a perspective on the use of genetic technologies, and discusses recent progress in understanding cellulose synthesis and its links to growth and cell division. The focus is *Arabidopsis thaliana* whose genome sequence has been completed and where most recent genetic analysis has been pursued.

Cellulose fundamentals

Cellulose, the simplest of polysaccharides in primary structure (unbranched chains of β-1,4-linked glucose residues), can form several alternative structures when those chains associate by hydrogen bonds and van der Waal's forces. All naturally occurring plant cellulose is the parallel-chain cellulose I isomorph rather than the antiparallel cellulose II (or the various additional forms that arise in vitro [3]). Nascent chains are thought to become trapped in the energetically unfavourable cellulose I iso-

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morph when a single synthase site simultaneously elongates neighbouring, identically polarized β-1,4-glucan chains. VanderHart and Atalla [4, 5] identified coexisting Iα and Iβ forms in their nuclear magnetic resonance (NMR) study of native celluloses. Baker et al. [6] resolved the cellobiose repeating unit in atomic-force microscope images of native *Valonia* cellulose. They explain the surface features expected for Iα and Iβ forms and point out that Iα/Iβ differences can be viewed as the result of hydrogen-bonded cellulose sheets slipping relative to each other.

The varying sizes of cellulose microfibrils in the walls of higher plants encouraged the search for a basic building block that might be the product of a single synthase site. Frey-Wyssling [7] envisaged elementary fibrils about 3.5 nm wide aggregating into larger microfibrils, and recent NMR results identify cellulose crystallites that are ≤ 3 nm in diameter [8–11] even in walls such as those of onion where electron microscopy shows much bigger microfibrils [2, 11]. (NMR size estimates depend on assigning specific chemical shifts to cellulose chains on the surface of crystallites and other shifts to chains within the crystallite. The estimate of surface:internal chains derived from ratioing the shifts can then be used to deduce the size and shape of crystallites.)

Cell wall polysaccharide biosynthesis with a genomic perspective

There are extensive descriptions of the polysaccharides found in the walls of dicot plants such as *Arabidopsis* (often referred to as type 1 walls [1]). The *Arabidopsis* genome must encode all the enzymes and other proteins needed to make and modify those polysaccharides. Its sequence is now complete [12] but the process of matching the proteins it encodes to the synthesis of specific polysaccharides has scarcely begun. With estimates suggesting that at least 21 enzymes are required to produce the linkages of one pectic polysaccharide (RGII) alone, the size of the task is clearly substantial. Genome sequencing offers numerous candidate glycosyltransferases (although not all glycosyltransferases may yet be recognisable [12]) and their functions now need defining in terms of donor, acceptor and linkage formed.

In 1995, Saxena et al. [13] proposed criteria to recognise glycosyltransferases from sequence data. They used hydrophobic cluster analysis to identify conserved features in several glycosyltransferases which we refer to as the D,D,D,QxxRW signature. That is, three varyingly spaced aspartic acid residues followed at a variable distance by five contiguous residues (QxxRW where x is any amino acid residue). The aspartic acid residues are thought to be involved in catalysis, whereas the function of the QxxRW motif is unknown. In the same year, Nagahashi et al. [14] showed experimentally that several of those signature residues are indeed essential for yeast chitin synthase activity.

Saxena et al. [13] could not at that time draw on plants for proven examples of such glycosyltransferases or even for candidate genes. Now, however, more than 40 such sequences are known from the *Arabidopsis* genome and have been assigned to seven families within a glycosyltransferase superfamily [15]. Likewise, a large gene family exists in maize [16] and numerous related sequences from other plant species are being revealed by the partial sequences from expressed sequence tags and by genomic sequencing. Although D,D,D,QxxRW signature proteins form the largest group of recognised glycosyltransferases, other sequences in the *Arabidopsis* database show similarities to further glycosyltransferases. A few examples include genes encoding proteins that resemble the FKS1 β-1,3-glucan synthase of *Saccharomyces cerevisiae*, a similarity first noted by Cui et al. for the cotton CFL1 gene (GenBank AAD25952; see http://cellwall.stanford.edu/gsl/species/arabidopsis_thaliana/index.shtml), proteins with motifs similar to those seen in various bacterial glycosyltransferases [17] and a protein (GenBank T01593) showing sequence similarity to *alg6*, a glucosyltransferase involved in glycoprotein processing in budding yeast [18].

Cross-referencing genes and polysaccharides

Now that genome sequencing has rendered obsolete gene discovery per se, linking particular genes to the production of particular polysaccharides comes to limit our understanding of cell wall polysaccharide production. Glycosyltransferases will be one important class of enzyme for polysaccharide synthesis but, because they are only one protein class required for bacterial cellulose production (see below), we should expect that the search for participants in plant cellulose synthesis will extend beyond glycosyltransferases. Exactly which glycosyltransferase undertakes which reaction and the identities of any other types of protein involved are not obvious from current database searches. A particular gene can be linked to a particular polysaccharide by starting with in vitro preparations that catalyse a particular reaction, with mutant plants that are deficient in a particular polysaccharide or with a candidate glycosyltransferase gene itself and working to define its function. These are the approaches of enzymology, classical genetics and reverse genetics respectively.

Protein purification leading to gene cloning has successfully characterised some structural proteins of cell walls (e. g. extensins, proline-rich proteins) and some enzymes modifying cell wall polysaccharides (e. g. xyloglucan endotransglycosylase, endo-1,4-β-glucanases). Until very

recently, however, no genes encoding glycosyltransferases or other enzymes involved in polysaccharide synthesis had been identified by starting from an enzyme preparation. Perrin et al. [19] recently changed this gloomy prognosis when they purified a xyloglucan fucosyltransferase from pea epicotyls, cloned the homologous gene from *Arabidopsis*, immunoprecipitated specific enzyme activity from solubilized membrane proteins and showed enzyme activity in mammalian cells expressing the *Arabidopsis* gene. The notable success of Perrin et al. [19] will encourage further efforts using enzymological strategies but the extent of success with other reactions and other enzymes remains to be seen. Certainly, the full *Arabidopsis* genome sequence maximises the value of partial sequence, mass spectrometric or proteolytic cleavage information even when this comes from impure enzyme preparations. Candidates can be prioritised for relevance during an initial database search rather than after a lengthy process of cloning and sequencing.

Classical mutational analysis starts from plants showing changes in the quantity or structure of a particular polysaccharide and works back to identify the altered gene. It makes no assumptions about the sequence or the functions of the genes that are required to make a particular polysaccharide. This is often a strength, particularly in the early stages of analysis and in uncovering the participation of unexpected proteins. We can anticipate that cell wall composition will be changed by mutations in genes encoding proteins that synthesise or modify polysaccharides, that produce substrates for synthetic enzymes, that build the cellular organisation required for cell wall assembly (e.g. cytoskeleton, Golgi secretion pathway, protein-processing enzymes) or that are components in regulatory pathways. Table 1 lists mutants in which cell wall composition is changed or which almost certainly involve changes to enzymes involved in polysaccharide production or which result in incomplete or bursting cell walls.

Several strategies have successfuly identified cell wall mutants in *Arabidopsis*. Reiter et al. [22] used gas chromatography/mass spectrometry to detect mutations changing the monosaccharide composition of cell wall hydrolysates. The *mur* mutants characterised so far fail to make substrates for synthases rather than being defective in the synthases themselves. This may not be an invariant feature of this screen but, because most monosaccharides occur in several polysaccharides, large deficiencies may often reflect defects in intermediary metabolism that prevent incorporation into all polymers rather than synthase defects that would probably affect only one polysaccharide. Fourier transform infra-red spectroscopy promises more rapid preliminary detection of some changes in cell wall composition [37].

Screens for cellulose-deficient mutants using less direct selection have identified defects in synthesis itself. Primary selection has been for morphological changes such as root radial swelling [38] or reduced hypocotyl extension [25, 39], anatomical changes such as reduced trichome birefringence [31] and xylem collapse [30] and physiological traits such as resistance to the herbicide isoxaben [32, 34]. The mutants are linked to cellulose either by cell wall polysaccharide analyses or, in the case of the herbicide, by knowledge that it selectively inhibits cellulose synthesis [40]. These mutants have linked cellulose synthesis to members of the CesA family within the D,D,D,QxxRW glycosyltransferase superfamily [15] and to a membrane-bound endo-1,4-β-glucanase. These will receive more detailed coverage below.

Two opposing problems potentially limit classical mutational analysis: gene redundancy such that mutating one of several genes performing similar functions does not produce a phenotype and, at the extreme of non-redun-

Table 1. Mutants of *Arabidopsis thaliana* that change cell wall composition, result in incomplete cell walls or cell walls that burst.

Change	Mutant	Other phenotype	Impaired gene product or function	References
Fuc ↓	*mur1*	slightly dwarfed, brittle	GDP-D-mannose-4,6-dehydratase	20, 21
Fuc ↓	*mur2, mur3*	none		22
Ara ↓	*mur4 – mur7*	none	UDP-D-xylose 4-epimerase (*mur4*)	22, 23
Rha ↓	*mur8*	none		22
Multiple ↓	*mur9, mur10, mur11*	reduced vigour *mur9*, *mur10*		22
Cellulose ↓	*rsw1*	short, often swollen organs	glycosyltransferase	24
Cellulose ↓	*kor-1/kor-2/rsw2*	short, often swollen organs; Incomplete walls	endo-1,4-β-glucanase	25, 26, 42
Cellulose ↓	*rsw3*	short, often swollen organs		27
Cellulose ↓	*irx1, irx3*	collapsed xylem, weak stem	glycosyltransferases	28, 29
Cellulose ↓	*irx2,*	collapsed xylem, weak stem		30
Cellulose ↓	*tbr*	altered birefringence		31
	ixr A (*ixr1*)	isoxaben resistance	glycosyltransferase	32, 33
	ixr B1 (*ixr2*)	isoxaben resistance	glycosyltransferase	33, 34
Pectin	*emb30*	pectin wrongly located	Sec7-like; ER-Golgi transport	35
	kojak	emerging root hairs burst	cellulose-synthase-like gene	126
Callose ↑	*cyt1*	incomplete cell walls		36
	knolle	incomplete cell walls	syntaxin	127, 128

dancy, mutation lethality when genes affect key processes. Redundancy has generally received more attention as a problem in *Arabidopsis* where most genes have relatives elsewhere in the genome that may serve similar functions, and where very low percentages of insertional mutants show an obvious phenotype [41]. The observation that mutations in five out of ten CesA genes have already produced major phenotypes suggests a low level of redundancy for that gene family. Defects in secondary walls will generally have less far reaching consequences under laboratory conditions, but lethality is more likely if primary wall defects severely upset growth and morphogenesis. Lethality stemming from primary wall defects can be circumvented by propagating as a heterozygote [42] or tolerated if it involves only partial loss-of-function [25] or temperature-sensitive alleles [38]. Temperature-sensitive alleles have not been widely used in higher plants but allow selection for severe phenotypes that would be lethal if constitutive. The increased difficulty of cloning them entailed by reliance on map-based methods rather than insertional mutagenesis is a diminishing problem with the availability of the genome sequence [43]. The advantages provided by temperature-sensitive alleles for phenotypic analysis will increasingly be felt when the focus moves from gene discovery to functional analysis: phenotypic severity can be graded with temperature in some cases [44]; the time course with which the phenotype develops can be studied to help disentangle primary effects [45], and the phenotype can be induced at any stage of the life cycle so that, for example, suitably timed temperature shifts can assess the impact of the mutation on floral development without confusion caused by any flow on from impaired vegetative development [44].

The abundance of candidate glycosyltransferase genes increases the attraction of mutating specific genes to determine function (reverse genetics). Available methods include finding T-DNA insert lines which amplify a PCR product using an insert-specific and a gene-specific primer [46], screening databases to identify inserts whose position in the genome has already been determined (http://arabidopsis.org.uk/blast.html), regional mutagenesis taking advantage of the propensity of transposons to reinsert close to their starting site to mutagenise a particular chromosomal region containing the target gene [47], the numerous variants of sense and antisense suppression using stable or transient expression [48, 49] and RNA/DNA heteroduplex methods for creating mutations in a specific gene [50]. The rapidly growing databases detailing changes in the expression of large numbers of genes [51, 52] will also help link cell wall genes into interacting networks, particularly when expression data become available for cell wall mutants.

Functional expression of genes in heterologous systems has proved important in characterising two plant glycosyltransferases [19, 53] and has great potential as a tool to define glycosyltransferase specificity. The activity of the encoded protein can be detected in vivo if new glycosidic linkages appear in the host, whereas in vitro activity can be assayed in material purified to varying degrees up to homogeneity. The data accumulating with purified hyaluronate synthase [54] indicate the potential of the methods.

In summary, the chemical, genetic and bioinformatic techniques are available to cross-reference genomic data with cell wall polysaccharide synthesis. The analysis of bacterial cellulose synthesis provides examples of both the power and the limitation of mutagenesis to unravel polymer synthesis and many interesting comparisons with synthesis in higher plants.

Diversity in the production of bacterial cellulose

Bacterial cellulose helps *Acetobacter xylinum* to float and *Agrobacterium tumefaciens* and *Rhizobium leguminosarum* to interact with plants. Mutants and biochemical studies that link genes to cellulose synthesis are important because the products of related genes in *A. xylinum, Escherichia coli* and *Aquifex aeolicus* probably make other polysaccharides (fig. 1). Mechanisms of synthesis may differ in different species: only genes A and B in the cellulose-producing operons of *A. xylinum*, *A. tumefaciens* and *R. leguminosarum* show common features, and studies of biochemistry and regulation reinforce the differences.

Genetic and biochemical data link cellulose production in *A. xylinum* to four polypeptides (A–D) produced from a single operon (termed *acs* or *bcs*), to the product of a fifth, upstream gene and to three operons producing enzymes that metabolise c-di-GMP. The A, B and C gene products are absolutely required for cellulose synthesis, whereas the D gene product suppresses cellulose II production [55–57]. The A gene encodes a D,D,D,QxxRW glycosyltransferase that is recovered by product entrapment and labelled by a UDP-Glc photoaffinity probe [58]; the B gene product is suggested to bind the regulator c-di-GMP (but see below); the C gene product contains one predicted trans-membrane domain, several tetratricopeptide repeats and some sequence similarities to putative pore-forming proteins such as TrbI from *E. coli* and VirB10 from *A. tumefaciens* [56]; the sequence of the D gene product provides no clues to explain how it affects the cellulose I/cellulose II balance. Mutants lacking a proline-rich protein produced by a gene 500 bp upstream of the *bcs* operon also make no cellulose [59], while a neighbouring gene encodes a secreted endo-1,4-β-glucanase (CMCax) whose presence or absence correlates with cellulose production in different strains. Mutants to test its role in synthesis have not been described.

The detailed route from UDP-Glc to cellulose remains poorly documented. Additions are most likely made to

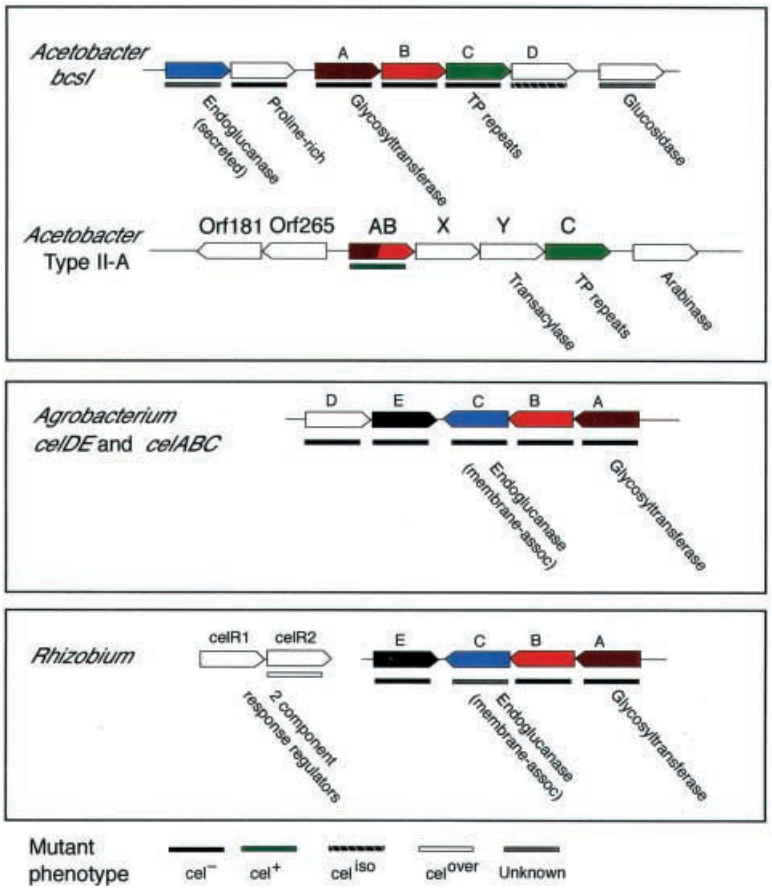

Figure 1. Bacterial operons involved in cellulose synthesis in *Acetobacter xylinum*, *Agrobacterium tumefaciens* and *Rhizobium leguminosarum* and the related type II-A genes found elsewhere in the *A. xylinum* genome which are not involved in cellulose production. The entries for each gene are from the top: gene name; boxes showing the direction of transcription and coloured to highlight similar genes in the other organisms (white indicates that no similar gene exists in the other organisms); a box, colour coded to indicate the mutant phenotype; the predicted enzyme activity or other features of the gene product apparent from the sequence (TP repeats, tetratricopeptide repeats). The abbreviations in the key to phenotypes at the bottom of the figure are: cel^-, cellulose deficient; cel^+, cellulose production unaffected; cel^{iso}, cellulose isomorph changed; cel^{over}, cellulose overproduced.

the non-reducing end of the glucan chain which faces the site of synthesis [60]. This may be general for products of D,D,D,QxxRW glycosyltransferases, since *Thallassiosira* β-chitin [61], *Pasteurella multocida* hyaluronan [62] and *Rhizobium* chito-oligosaccharides [63] show the same polarity. Proposals that polypeptide A directly transfers glucose from UDP-Glc to the growing glucan chain have not been confirmed by, for example, heterologous expression or full purification of the protein. (Kawagoe and Delmer [64] discuss the problems of excluding other proteins and lipid or other intermediates from participation). Han and Robyt [65] argued for lipid-linked intermediates following demonstrations that ^{14}C-glucose was incorporated into chloroform-methanol-soluble material and that mild acid released β-1,4-[^{14}C]-glucan from cells and membrane fractions. Their complex reaction sequence, however, supplements very limited experimental data with many comparative arguments and has 'cellulose synthase' (presumably the A gene product) transferring glucose residues between two lipid pyrophosphate glucopyranosyl units rather than utilising UDP-Glc, a property supported by photoaffinity labelling [58].

Five gene products from two adjacent operons (*celABC* and *celDE*) are required to make cellulose in *A. tumefaciens* [66] but only the glycosyltransferase celA and the celB protein of unknown function show similarities to *A. xylinum* proteins. celD and celE are suggested to catalyse early steps in the cytosol, because membrane fractions from *celD* and *celE* mutants make cellulose when mixed with soluble fractions from *celA*, *celB* or *celC* mutants but the reverse combinations do not. The early steps may build the lipo-glucose that accumulates in mutants lacking the celA glycosyltransferase. Lipo-glucose is then extended to lipo-oligosaccharides which accumulate in *celB* and *celC* mutants. Only the lipo-oligosaccharide accumulated by the *celC* mutant can be incorporated into cellulose. This suggests that the celA glycosyltransferase adds one or more additional glucose residues to gluco-lipid;

celB modifies the lipid moiety and celC (a putative endo-1,4-β-glucanase with a single predicted transmembrane domain) transfers the saccharide residue(s) to cellulose. The proposed reaction pathway is therefore very different to the direct mechanism offered for *A. xylinum*. We will return later to consider similarities between these proposals and findings with *Arabidopsis* mutants.

There is extensive evidence that c-di-GMP regulates cellulose synthesis in *A. xylinum*, conflicting findings over its role in *A. tumefaciens* [66–68] and evidence for regulation by a two-component response system in *R. leguminosarum*. c-di-GMP stimulates cellulose synthesis in *A. xylinum* extracts [69] and bcsB is suggested to bind c-di-GMP [70]. The evidence, however, is not clearcut [71], particularly given demonstrations that cellulose synthase preparations contain another c-di-GMP-binding protein [72]. Three operons encode diguanylate cyclases and phosphodiesterases to synthesise and degrade c-di-GMP [73]. They are partially redundant, but cellulose synthesis is very low when mutations inactivate two of the diguanylate cyclases. The buoyant cellulosic pellicle has been suggested to promote oxygen supply to *A. xylinum,* and sequence similarities to oxygen-sensing domains suggest that c-di-GMP regulation itself may be linked to oxygen availability. Mutants of *R. leguminosarum* point to a diversity in regulation of cellulose production with a putative two-component response system that can be mutated to overproduce cellulose [74].

Related bacterial gene products may produce other polysaccharides

Genes related to members of the cellulose-synthesising operons occur elsewhere in the *A. xylinum* genome but do not contribute to in vivo cellulose synthesis (fig. 1) [57]. They are interspersed with *bcsX* (encoding a protein with no known homology) and *bcsY*, encoding a putative transacylase whose product has similarities to proteins that acylate sugars. This has led to suggestions that they produce acetan or some polysaccharide other than cellulose [75]. The *E. coli* and *A. aeolicus* genes are in organisms not known to produce cellulose. Looking beyond these close relatives of the cellulose synthases, more divergent D,D,D,QxxRW glycosyltransferases synthesise other bacterial polysaccharides (e. g. hyaluronic acid) and oligosaccharides (e. g. nod factors).

In summary, much has been learnt about the proteins required to make cellulose in bacteria but a great deal more remains to be understood about their functions and the reaction pathways used. *A. xylinum* and *A. tumefaciens* both use D,D,D,QxxRW glycosyltransferases and a second protein of unknown function but similar sequence. There are, however, substantial differences in the other genes involved, in the regulatory mechanisms and perhaps in the reaction pathways. Historical priority sometimes seems to confer a legitimacy on *A. xylinum* as a model organism for plant cellulose synthesis but mutational analysis of *A. thaliana* is showing interesting similarities to *A. tumefaciens*.

Mutants implicate glycosyltransferases and an endo-1,4-β-glucanase in plant cellulose synthesis

Plant cellulose synthesis has been studied by many strategies, a continuing goal being to develop a cell-free system making cellulose with high specificity and high specific activity. Further characterisation of such preparations could then identify the proteins involved and begin to resolve the reaction mechanism. However, much effort and valuable recent progress [76] has not yet identified proteins or clarified reaction mechanisms. Studies using mutants, however, have recently implicated two categories of enzymes.

Pear et al. [77] took advantage of the cotton fibre highly cellulosic wall to identify the *celA* gene product as a strong candidate glycosyltransferase for cellulose synthesis. It was a D,D,D,QxxRW glycosyltransferase, showed weak similarities to glycosyltransferases implicated in bacterial cellulose synthesis, bound UDP-Glc in a blot assay and was strongly expressed during active cellulose deposition in the fibre. *Arabidopsis* mutants now link five such genes to cellulose production. In current terminology, they belong to the ten-member CesA family of glycosyltransferases, which is one of seven families within the *Arabidopsis* D,D,D,QxxRW glycosyltransferase superfamily [15].

Mutants currently link CesA1, CesA3 and CesA6 to primary cell wall cellulose and CesA7 and CesA8 to secondary cell wall cellulose (table 2). Exclusive assignments to primary or secondary walls should be regarded as preliminary, however, until more direct methods than an obvious mutant phenotype confirm enzyme inactivity in the other type of wall. The *rsw1* mutant is cellulose deficient [24, 27] and has a one-nucleotide change that replaces Ala^{549} with Val in CesA1 [24]. The presence of Val [$(CH_3)_2CH$–] rather than the Ala (CH_3–) or Gly (–H) residues found there in the ten wild-type family members (fig. 2) causes a strongly temperature-sensitive phenotype. The extensive morphological alterations of the mutant [38, 44] suggest that CesA1 deposits cellulose in many primary cell walls, and changes in wall ultrastructure confirm that this occurs in the walls of cells in the root growth zone which swell in the mutant [45]. Antisense suppression of the widely expressed *CesA1* or *CesA3* genes reproduces many aspects of the $Rsw1^-$ phenotype such as reduced cell and organ lengths, suggesting that *CesA3* also contributes to primary cell wall cellulose in many parts of the plant [J. E. Burn, unpublished re-

Table 2. Genes encoding CesA proteins in *Arabidopsis thaliana*.

Number	Chromosome[a]	Synonym	Mutants	Gene expression
1	4 (82 cM)	*RSW1*	*rsw1* – radial swelling and reduced elongation with defective primary cell walls [24]	widely expressed mRNA and promoter GFP fusion[b]
2	4 (105 cM)	AthA		widely expressed mRNA[b]
3	5 (16 cM)	AthB	*ixr1*/*ixrA* – isoxaben resistance [32, 33]; antisense plants resemble *RSW1* antisense plants[b]	widely expressed mRNA[b]
4	5 (93 cM)			vascular-specific expression of promotor-GUS fusion in many organs [16]
5	5 (25 cM)			
6	5 (125 cM)		*ixr2* / *ixrB1*– confers isoxaben resistance [33, 34]; *prc* - radial swelling and reduced elongation [33]	*prc* mutant phenotype in roots and hypocotyl [39]
7	5 (33 cM)	IRX3	*irx3* – irregular xylem; defective in secondary cell walls [28]	mRNA in stem, not leaves [29]
8	4 (60 cM)	IRX1	*irx1* – irregular xylem [29]	mRNA in stem, not leaves [29]
9	2 (40 cM)			
10	2 (47 cM)			

[a] http://cellwall.stanford.edu/cellwall/species/index.shtml.
[b] J.E. Burn et al., unpublished results.

sults]. Moreover, *CesA3* is mutated in the *ixr1* mutant (formerly *ixrA* [32]) so that the radial swelling caused by the cellulose synthesis inhibitor isoxaben is suppressed [33]. This is again consistent with CesA3 making cellulose for primary cell walls, and suggests that CesA3 is the target for isoxaben. *CesA6* is mutated in *procuste* which has less cellulose, reduced stature and radial swelling in the root and hypocotyl [39, 78]. It is allelic to *ixr2* [33], a second locus conferring resistance to isoxaben-induced radial swelling [34] and so again is consistent with the conclusion from the *procuste* phenotype that CesA6 makes primary wall cellulose.

CesA7 and CesA8 deposit cellulose in secondary cell walls. The *irx3* and *irx1* mutants have defects in *CesA7* and *CesA8,* respectively [28, 29] and show less cellulose and collapsed xylem in the reproductive stem [30]. They lack the changes to growth and morphology of the sort seen with the mutations in *CesA1*, *CesA3* and *CesA6,* suggesting that IRX3 and IRX1 contribute little or no cellulose to primary walls. CesA4 may also contribute to secondary cell wall cellulose; its promoter region drives GUS expression in vascular tissue [16] although no evidence corroborating this expression pattern is yet available.

Many aspects of the sequences of the CesA proteins are well conserved in *Arabidopsis* and other species [16]. The proteins appear to be integral membrane proteins with eight transmembrane helices in groups of two and six

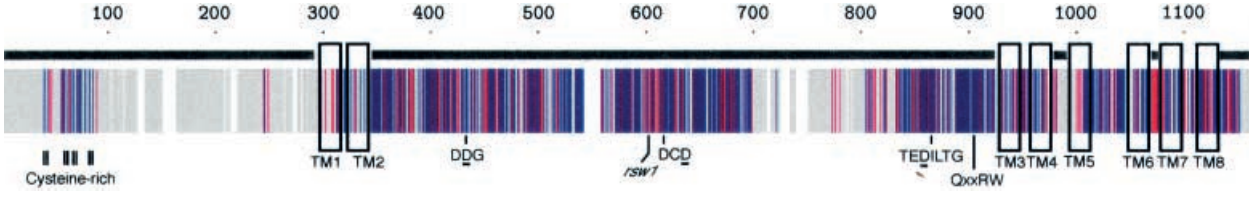

Figure 2. Features of the CesA proteins of *Arabidopsis thaliana*. A ClustalW alignment of ten *A. thaliana* CesA proteins was processed by the Fingerprint option of the Texshade package at the Biology Workbench (http://workbench.sdsc.edu). The colours are: blue, identical in all ten sequences; red, conserved in all ten; grey, not conserved in all ten. White segments represent gaps introduced into the CesA1 sequence to achieve optimal alignment. The features mapped onto the fingerprint are: the amino acid numbering of the alignment; vertical boxes crossing the fingerprint and showing the approximate sites of the eight, predicted transmembrane (TM) domains; grey horizontal bars above the fingerprint indicating parts of the sequence believed to face the cytoplasm; the cysteine-rich domain with the four pairs of vertical bars marking the four CxxC elements; short sequences containing the components of the D,D,D,QxxRW glycosyltransferase signature (the critical D residues are underlined in each short sequence); *rsw1*, the site of the Ala^{549} to Val substitution responsible for cellulose deficiency in *rsw1*. In addition to the N and C termini, variation clusters in the region between the cysteine-rich domain and the first predicted transmembrane segment (TM1) and in a region approximately between residues 700–800 which lies between the second and third D residues of the D,D,D,QxxRW glycosyltransferase signature. The gap in the region of otherwise high conservation at about residue 550 is introduced because of a sequence present in only CesA4.

bracketing a large central domain containing the D,D,D, QxxRW signature (fig. 2). Predictions [24, 77] and now experimental evidence [79] place this central region facing the cytoplasm so that only the small regions between transmembrane domains 1 and 2, 3 and 4, 5 and 6, and 7 and 8 contact the wall. A highly conserved cysteine-rich sequence precedes the first two transmembrane domains. It has similarities to zinc-binding LIM [64] and ring finger [24] domains but is not exactly duplicated in any other proteins. Its function(s) in cellulose synthesis remain unknown. In addition to the N and C termini, variations between the ten CesA proteins are concentrated in two regions: between the Cys-rich region and the first putative transmembrane domain and between the second and third Asp residues implicated in catalysis (fig. 2). A quite highly conserved region increases the spacing between the first and second Asp residues of the D,D,D,QxxRW signature in plant CesA proteins compared to the spacing in their bacterial relatives [77].

A second type of enzyme, an endo-1,4-β-glucanase has now been linked to cellulose production in *Arabidopsis*. Roots of *rsw2* make less cellulose but near normal amounts of non-cellulosic polysaccharides [27]. *RSW2* is allelic to *KOR* [26], a gene encoding a membrane-bound endo-1,4-β-glucanase [25, 42] which resembles the cel3 protein of tomato [80]. Fourier transform infra-red spectroscopy shows that the original *kor-1*mutant also has less cellulose in its hypocotyl [78]. The *Arabidopsis* genome currently contains two other putative membrane-bound endo-1,4-β-glucanases (type III in the terminology of Brummell et al. [80]) as well as numerous secreted endo-1,4-β-glucanases [81]. Membrane-bound enzymes may be involved in cellulose synthesis, whereas secreted enzymes may weaken walls during processes such as cell expansion and organ abscission.

Three other genetic loci have been linked to cellulose synthesis in *Arabidopsis* although the genes have not yet been characterised. The visual and polysaccharide phenotypes of *rsw3* resemble those of *rsw1* and *rsw2* [27, 38] suggesting a role in primary cell wall deposition; the *irx2* mutant resembles *irx1* and *irx3* in showing collapsed xylem and reduced cellulose, suggesting a role in depositing secondary cell walls [30]; *tbr* has reduced birefringent retardation in secondary walls of trichomes and reduced cellulose by chemical analyses [31].

Several other proteins are proposed to participate in cellulose synthesis [64] although the experimental evidence for their roles is not yet compelling. Sucrose synthase (EC 2.4.1.13) is perhaps the most interesting candidate. It catalyses a readily reversible reaction producing UDP-Glc, the cellulose synthase substrate; much of the enzyme is plasma membrane associated and the cotton fibre enzyme localizes in a helical pattern reminiscent of cortical microtubules or microfibrils [82]; levels of membrane-associated sucrose synthase increase when corn pulvini initiate elongation [83] and when gibberellin stimulates elongation and cellulose synthesis in pea internodes [84]; a corn mutant defective in one sucrose synthase isoform shows impaired endosperm cell wall development [85].

Lipo-glucan and models for cellulose synthesis

Cellulose synthesis mutants of *A. tumefaciens* accumulate lipo-glucose and lipo-glucans according to the genes affected. These are considered intermediates in cellulose synthesis, since membrane preparations from wild-type cells metabolise the lipo-glucan accumulated in *celC* mutants to cellulose [68]. Shoots of the cellulose-deficient mutants *rsw1*, *rsw2* and *rsw3* [27] accumulate a β-1,4-glucan that extracts with ammonium oxalate and alkali (*rsw1* [24, 86]; *rsw2* [26, 86]; *rsw3* [86]). A similar glucan is not recovered from the roots of the same mutants, or from shoots of wild type. Glucan chain lengths average about 30 residues (t-Glc:4-Glc ratio), much longer than the four or less seen in *A. tumefaciens.* Chains accumulating in *rsw1* are lipid linked [C. H. Hocart, unpublished results]. (Lipid linkage has yet to be tested for the glucans from *rsw2* and *rsw3*.) This suggests that lipo-glucan may be an intermediate in cellulose synthesis in *Arabidopsis* just as shorter lipo-glucan chains are in *A. tumefaciens,* but still leaves several possible routes for synthesis.

A scheme for cellulose synthesis should incorporate known enzymes, explain why lipo-glucan accumulates in *rsw1* and encompass the organisation of enzymes and intermediates at the plasma membrane. A basic distinction is made between direct and indirect mechanisms (fig. 3). Direct mechanisms use a processive glycosyltransferase to elongate a glucan chain to its full length (often several thousand glucose residues) whereas indirect mechanisms utilise some other steps before glucose residues are transferred to the final polymer chain. Ideas for those additional steps usually involve lipid-linked intermediates. These might be needed only at particular times such as during chain initiation (fig. 3, model B1) or they may be intrinsic to the mechanism by which all chain extensions occur even when chains are nearly full length (fig. 3, model B2). In terms of bacterial cellulose synthesis, proposals for *A. xylinum* exemplify direct mechanisms [64] whereas the proposals of Matthysse et al. [68] for *A. tumefaciens* exemplify indirect mechanisms. Detection of lipo-glucan in *rsw1* strongly increases the likelihood that lipo-glucans are synthesised as part of an indirect mechanism to make cellulose. The less likely possibility is that short glucan chains accumulate from defects in cellulose synthesis but become linked to lipid only through a process that is not part of the normal synthesis reaction.

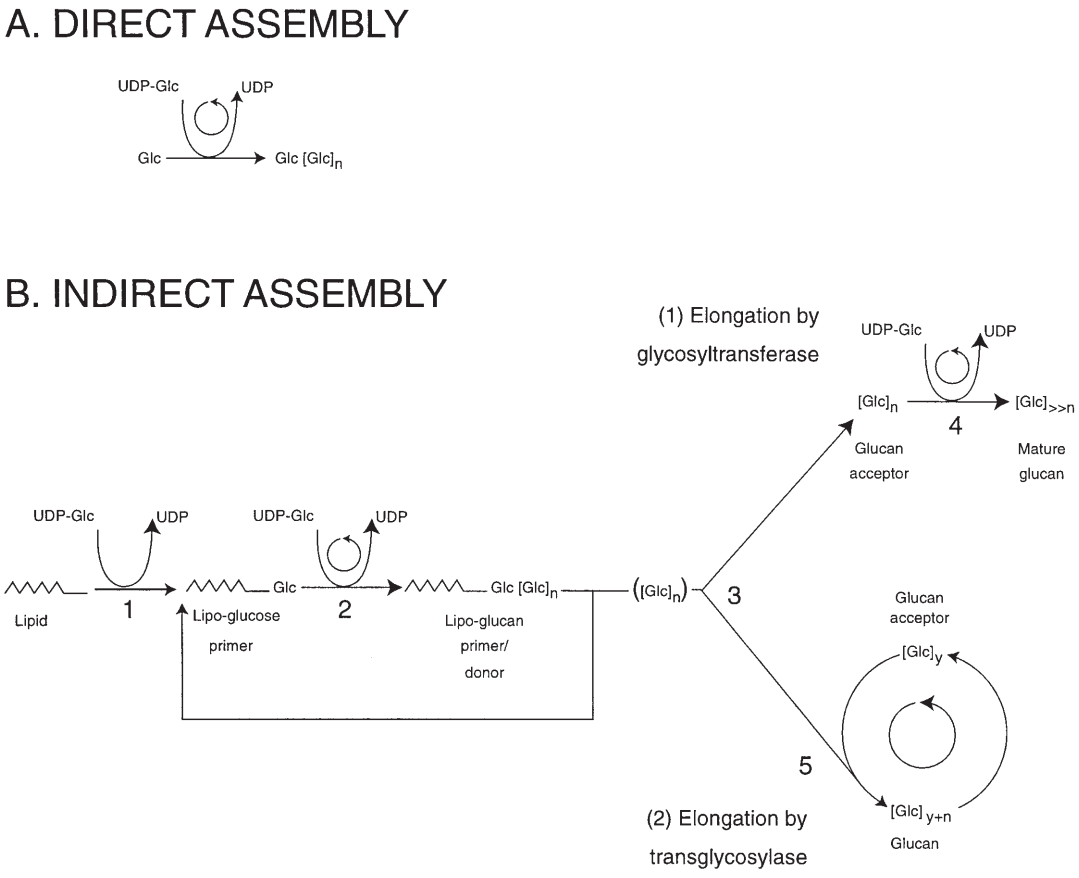

Figure 3. Diagrams summarising alternative types of pathway for cellulose synthesis. Direct mechanisms (*A*) involve only a glycosyltransferase in extending the glucan chain to full length whereas indirect mechanisms (*B*) involve the synthesis of lipo-glucan. The lipo-glucan can act either (mechanism B1) as a primer from which oligo-glucan is released by an endo-1,4-*β*-glucanase (reaction 3) for further extension by a glycosyltransferase, or (mechanism B2) as a donor from which oligo-glucan is transferred to extend the glucan chain with a block of glucose residues. This transglycosylase reaction could also be catalysed by an endo-1,4-*β*-glucanase if conditions in the synthase complex favoured the cellulose chain rather than water as the acceptor. Reactions repeated many times are indicated by a circular arrow. The number against each reaction is used for reference in the text.

The reaction catalysed by CesA

CesA protein sequences strongly suggest that they are glycosyltransferases but do not in themselves reliably define the substrate, acceptor or linkage made. Linkage and substrate are probably uncontroversial. The linkage is deduced from the reduced cellulose contents of the *rsw1*, *prc*, *irx1* and *irx3* mutants. Regarding substrate, Carpita and Delmer [87] concluded that cellulose synthesis in cotton fibres used UDP-Glc as substrate because it was the only potential substrate turned over at adequate rates and, consistent with this, proteins from fragments of the cotton *celA* gene expressed in *E. coli* bound UDP-Glc in a blot assay [77]. Heterologous expression of CesA proteins remains highly desirable to confirm the linkage made and allow quantitative studies of substrate binding, stoichiometry and specificity.

The acceptor to which glucose residues are added is a more complex issue. Direct addition mechanisms (fig. 3A), where the acceptor is the end of a glucan chain that is assembling into a microfibril, face a perceived problem because successive glucose residues in a *β*-1,4-glucan chain are rotated through a half turn about an axis parallel to the chain's long axis. Rotation of acceptor and enzyme are probably restricted if the chain is rigidly held within a microfibril and if the enzymes are part of an oligomer (discussed below). This raises the problem as to how an enzyme with a single active site adds residues in both orientations. Delmer [71] argued that glucan chains have sufficient freedom to rotate to the correct orientation and then relax whereas Saxena et al. [13] proposed a hypothesis making rotation unnecessary. They hypothesised two substrate-binding sites whose opposite orientations prealign successive residues correctly for polymerisation and obviate the need for rotation. The two preoriented glucose residues could add sequentially or simultaneously to the end of the nascent chain or they could be linked into a dimer (cellobiose) before addition.

Two binding sites specialised to bind different sugars have now been demonstrated for the D,D,D,QxxRW hyaluronic acid synthases of *Streptococcus* spp. [88] and, consistent with direct-addition mechanisms, the purified recombinant protein makes high-molecular-weight hyaluronan in vitro apparently without primers, lipids or cofactors [54]. In contrast to the dual substrate sites of hyaluronic acid synthase, only a single site binds a nucleotide-sugar in the crystal structure of SpsA, a D,D,D, QxxRW glycosyltransferase involved in producing the *Bacillus subtilis* spore coat [89]. Hyaluronic acid synthases therefore provide precedents for two substrate-binding sites and for direct polymerisation by a D,D,D, QxxRW glycosyltransferase, but whether either or both properties can be generalised to CesA is not yet clear.

We will consider just two of many possible variants of indirect mechanisms (fig. 3B). With current knowledge, these should be considered generic models for illustrative purposes rather than specific proposals. Both use a glycosyltransferase to elongate lipo-glucose into a lipo-glucan (reaction 2) and an endo-1,4-β-glucanase to cut the glucan free of lipid (reaction 3). The lipid-free glucan can then be elongated to full length by the same or a different glycosyltransferase (reaction 4 in mechanism B1) or the oligo-glucan can be transferred as a block to elongate a glucan acceptor in a transglycosylase reaction (mechanism B2). In the first case, lipo-glucan is built only once as a primer to start each chain, whereas in the second case, the lipo-glucan is repeatedly rebuilt to serve as donor for the multiple transfers required to extend the chain to its full length. Matthysse et al. [68] proposed a block transfer mechanism (similar to B2) for *A. tumefacien*s. Their null mutant for the celA glycosyltransferase accumulates lipo-glucose and null mutants in *celB* and *celC* accumulate lipo-glucan chains that do not exceed four glucose residues in length [68]. In classical genetic reasoning, enzyme substrate and acceptor accumulate when a mutation disables the enzyme. Such reasoning and some in vitro data make the celA glycosyltransferase the enzyme that extends lipo-glucose to lipo-glucan and the celC endo-1,4-β-glucanase the enzyme that transfers oligo-glucan from lipo-glucan to build the final polymer. We cannot confidently apply such logic to the *rsw1* mutant of *Arabidopsis* since the one-amino-acid change in the CesA1 glycosyltransferase probably leaves partial function even at the restrictive temperature. Some elongation of its acceptor may therefore occur so that the lipo-glucan which accumulates may be an incompletely extended product rather than an unreacted substrate. Moreover, if there is partial redundancy, the CesA3, CesA6 and any other CesA glycosyltransferases expressed in cells containing defective RSW1 remain potentially fully functional and, perhaps, partially able to substitute. Notwithstanding such reservations, the CesA1 malfunction clearly does lead to lipo-glucan accumulation which is most simply consistent with partial blockage of reaction 2 irrespective of which mechanism (B1 or B2) operates at later stages.

Reaction catalysed by the KOR endo-1,4-β-glucanase

The endo-1,4-β-glucanase activities of KOR or the related cel3 protein in tomato [80] have not been demonstrated by direct assay, but the predicted sequence appears to be a typical endo-1,4-β-glucanase except for a trans-membrane domain near the N terminus and a proline-rich C terminus. The proteins were found in Golgi- and/or plasma membrane-enriched vesicles [25, 80]. We need to know the substrate for the endo-1,4-β-glucanase activity, whether water is the acceptor for the severed glucan chain and how the process contributes to cellulose synthesis.

Direct polymerisation (fig. 3A) leaves room for an endo-1,4-β-glucanase in roles such as cutting chains in disordered areas of microfibrils with low crystallinity to relieve strains on the synthase complex [71] or 'proof reading' to remove mistaken linkages [90] (although what seems to be an endo-1,4-β-glucanase does not seem well suited to a 'proof-reading' role). A central role in synthesis is more easily conceived at reaction 3 in indirect-assembly models (fig. 3B). Here, the lipo-glucan is cleaved and either elongated to full length by a glycosyltransferase (mechanism B1) or transferred to the final chain by the block transfer pathway (mechanism B2).

The block transfer function envisaged for the celC endo-1,4-β-glucanase of *A. tumefaciens* transferred cello-oligosaccharides from a short lipid-linked intermediate to the end of a glucan chain in the cellulose polymer. Xyloglucan endotransglycosylase performs a comparable transfer reaction in higher plants, although in this case, the glucan is decorated with xylose and other substitutions and the substrate is not lipid linked. Mechanistically, both the endotransglycosylase and the endo-1,4-β-glucanase cut glucan chains and they share motifs [91] but the reactions differ in whether the favoured acceptor for the chain is water (endo-1,4-β-glucanase) or another glucan chain (transglycosylase). The difference, however, is not absolute. Mass action manipulations (high percentages of non-aqueous solvent and high concentrations of an alternative acceptor) make endoglucanases (and other glycosidases) efficient vehicles for in vitro synthesis [92, 93]. If conditions at the cellulose synthase complex favour a glucan chain over water as the acceptor, an endoglucanase (plant or bacterial) could well act as a transglycosylase in the way Matthysse et al. [68] proposed.

Organisation of enzymes and products at the plasma membrane

Proteins immunologically related to the central catalytic domain of a cotton CesA occur in characteristic clusters of six particles on the cytoplasmic face of the *Vigna angularis* plasma membrane [79] where they could use cytoplasmic UDP-Glc. Whether any other proteins occur in the same structures remains to be determined. Mueller et al. [94, 95] first described these clusters in 1976 and they are referred to as terminal complex rosettes or, simply, rosettes. Several lines of evidence linked rosettes to cellulose production even before the immunolabelling for CesA. They are, for example, abundant when cellulose production is high [96], concentrated in areas of plasma membrane overlying wall regions undergoing secondary thickening [97], present in protonemata of *Funaria hygrometrica* during growth and cellulose synthesis but absent when neither is occurring [98, 99] and rare in cellulose-deficient mutants [24, 100]. Many algae have larger, linear arrays of particles [101, 102] that apparently correlate with the larger, more highly crystalline microfibrils seen in those algae and that often clearly lie at the ends of microfibrils.

The catalytic domains of the CesA proteins and of the type III endo-1,4-β-glucanase are exposed at the inner [79] and outer faces of the plasma membrane [80], respectively. If synthesis proceeds from cytoplasmic substrate (UDP-Glc) to external product (cellulose), the endo-1,4-β-glucanase-dependent step will therefore follow the glycosyltransferase-dependent step. This order is consistent with roles proposed for the endo-1,4-β-glucanase in both direct and indirect schemes for cellulose synthesis (fig. 3). It leaves open the form in which glucose residues transit the plasma membrane. The direct hypothesis for cellulose synthesis sees β-1,4-glucan chains emerge either on the cytoplasmic face to transit the membrane through a pore in the centre of the six-particle rosette, or emerge from the outer face of the individual CesA proteins after crossing the bilayer through a pore formed from the transmembrane domains in CesA [71]. Brown and Saxena [103] canvassed several options for membrane transfer as part of indirect mechanisms of cellulose synthesis. These include schemes inspired by bacteria making polysaccharides or liposaccharides in which lipid-linked glucose or oligoglucans cross the membrane assisted by mechanisms that can include flippases [104] and ABC transporters [105].

The present reaction schemes remain speculative but are now based on known gene products and a known lipoglucan. They should lead to genuine advances if further mutants identify additional enzymes and/or intermediates and if genetic interpretations are tested and refined by other methods such as in vitro metabolic studies. Maximum information will be obtained from mutational analysis if metabolic defects remain specific to the mutated enzyme alone. If enzymes of different types are organised into rosettes or in the membrane around rosettes, however, the loss of rosette structure seen with some mutations [24] may flow on to impair other enzyme activities. This would make the metabolic phenotype no longer wholly specific for the primary enzyme defect and would complicate interpretation of the metabolic phenotypes of single and double mutants.

An interesting aspect of indirect mechanisms is that even simple schemes (such as B1) have three glycosyltransferase reactions (reactions 1, 2 and 4 in fig. 3) which raises the question as to which step(s) CesA proteins catalyse and, indeed, whether all CesA proteins catalyse the same reaction. The dramatic phenotypes already recognised for five of the ten *CesA* genes suggest that they show little redundancy. Each CesA (or perhaps subclasses of the whole CesA family) may, therefore, have unique attributes which prevent simple substitution of one gene for another. Substrate preference could be one such attribute. Several other pieces of evidence point in the same direction. Turner and coworkers found that the *irx1* and *irx3* mutants (affecting CesA7 and CesA8, respectively) are both required for cellulose production in secondary cell walls of xylem elements [29]. They also found that CesA7 and CesA8 copurified from detergent extracts, suggesting that the two may associate in vivo in single rosettes.

Cellulose synthesis in plant development

Although the processes that make cellulose have high intrinsic interest we should also be mindful of the role that cellulose synthesis plays in plant function. Deposition of cellulose in secondary cell walls motivates most industry interest but deposition in primary cell walls has broad interests to developmental biologists interested in its relationship to growth and morphogenesis. Growth is normally the product of cell division (which requires assembly of a new wall between the daughter nuclei) and cell expansion (which requires polysaccharide deposition to maintain the integrity of the original wall as it yields to turgor-generated forces). Analyses with mutants are beginning to expose linkages between cellulose synthesis, cell division, growth and its degree of anisotropy.

Cellulose synthesis and cytokinesis

Cellulose is deposited in cell plates at quite a late stage after callose and matrix polysaccharide deposition [106]. Plants treated with the cellulose synthesis inhibitor dichlorobenzonitrile show incomplete and wavy cell plates. These can appear as ingrowths from parent walls

[107–111] contrasting with the normal outward progression of the developing cell plate. Faults in the KOR endo-1,4-β-glucanase cause similarly irregular cell plates and increase the number of cells seen in root transverse sections [26, 42]. Additional cell divisions are not seen with dichlorobenzonitrile. Extra rounds of division perhaps ensue if faulty divisions do not properly isolate daughter cells and would be more easily picked up with the longer term studies possible with mutants. There are as yet no reports of cell division abnormalities in mutants with defective CesA glycosyltransferases.

Although KOR may be targeted to cell plates [42], several arguments support the view that completion of cell plates is unlikely to be the sole function of KOR: untargeted KOR corrects the height defects but not the cytokinesis defects in *kor*-2 [42]; post-germination expansion of cotyledons and hypocotyls is reduced when seedlings homozygous for temperature-sensitive alleles are grown at the restrictive temperature [26] even though these organs completed cell division before germination [112–114] and so at the permissive temperature; birefringence is reduced in radial longitudinal walls well back into the expansion-only zone of roots [26]; total root cellulose is reduced by 56% [27], which seems too large to reflect defects in cell plates only.

Cellulose synthesis and organ elongation

Mutants deficient in cellulose because of defects in a CesA glycosyltransferase (*rsw1, prc*) or in KOR endo-1,4-β-glucanase (*kor-1*, *rsw2*) activity show radial swelling in seedling roots and hypocotyls. Radial swelling, however, is pronounced only in the root and the dark-grown hypocotyl, so that the much reduced length of almost all vegetative and reproductive organs is the dominant visual phenotype over the full life cycle [25, 44]. There is probably a quite direct effect of reduced cellulose synthesis on cell expansion: relative elemental growth rates measured at positions along the *rsw1* root confirm that transfer to the restrictive temperature rapidly reduces the maximal rate which is normally seen at about 700 μm behind the root tip and shortens the length of the elongation zone until elongation is almost undetectable after 24 h [45].

The results are less consistent where chemical inhibitors of cellulose synthesis have been applied to various whole plants and excised segments: elongation can be reduced [115, 116], promoted [117] or unaffected [118, 119]. Current views of the mechanism of cell expansion [120] emphasise changes to non-cellulosic carbohydrates as rate controlling steps but recognise that local rates of cell expansion usually correlate strongly with local rates of cell wall synthesis (of which cellulose production will be a major component). Several excised tissues incorporate precursors into cellulose and other carbohydrates at increased rates when elongation is experimentally stimulated [118, 121, 122] but reducing cellulose synthesis with dichlorobenzonitrile does not always inhibit growth even in these tissues [118, 122]. However, when we consider the alignment of cellulose, we will see that these changes cannot be considered only in terms of reduced rates of synthesis and that altered orientation of the products of synthesis must be considered.

CesA and cellulose alignment

Any idea that the *rsw1* mutant could be used to analyse how the rate of cellulose synthesis impacts on growth has to be abandoned with the finding that a prominent feature

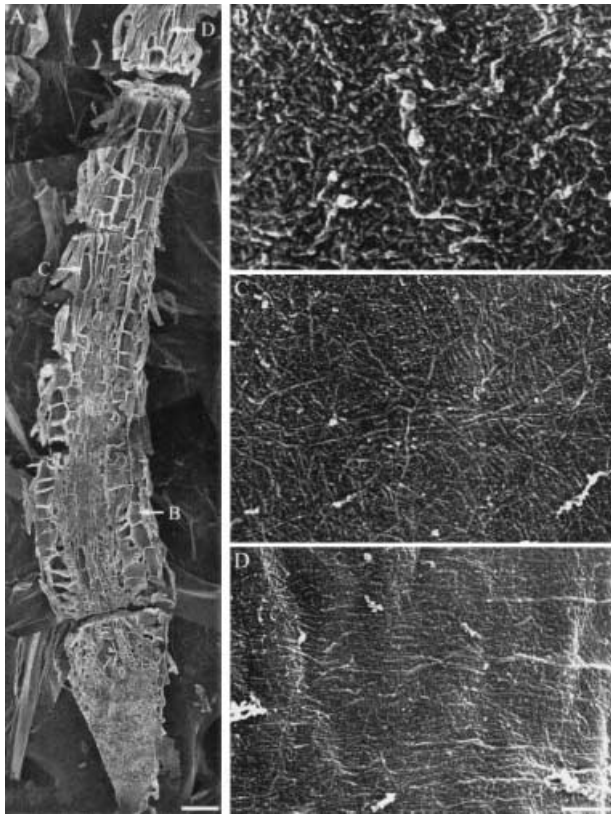

Figure 4. Field emission scanning electron micrographs showing the inner faces of the radial walls of epidermal cells in the cellulose-deficient mutant *rsw1*. The plants have been grown for 24 h at the restrictive temperature (29 °C). A low-power view of a whole root from which slices had been removed by cryomicrotomy to expose internal cells (*A*). At its widest point, the root has swollen to about double its original diameter. The inner faces of walls of the epidermal cells labelled B–D are examined at higher magnification in the following images. Walls in cells from the early elongation zone (*B*) show no cellulose microfibrils of typical appearance. Walls in cells from the late elongation zone (*C*) retain clear microfibrils but they are aligned randomly. Walls in cells from behind the elongation zone (*D*) show clear microfibrils which retain transverse orientation. Such transverse orientation would be seen in wild type cells at all developmental stages. Bar represents 60 μm in (*A*) and 300 nm in (*B–D*). [Reproduced with permission from ref. 45.]

of the phenotype of *rsw1* and of roots treated with dichlorobenzonitrile is the loss of the strongly transverse alignment of microfibrils [45] (fig. 4). This occurs at a relatively early stage after a temperature shift when many clear microfibrils are still visible in the field emission scanning electron microscope and certainly early enough to contribute to the reduction in elongation and the promotion of radial expansion. We are investigating whether this linkage between synthesis and alignment holds for *rsw2* and *rsw3* but it is seen in roots treated with the cellulose synthesis inhibitor dichlorobenzonitrile [45]. Two proposed microfibril alignment mechanisms link alignment to the rate of cellulose synthesis *per se*. Satiat-Jeunemaitre [115] proposed that the ratio of cellulose to hemicellulose had to be within certain limits to align cellulose microfibrils into helicoidal arrays via self-assembly. *rsw1* roots do show large changes in the cellulose:-hemicellulose ratio [27] but newly deposited microfibrils are always transverse in the wild type rather than forming helicoids [123]. The winding mechanism for cellulose alignment [124, 125] envisages that concurrent synthesis of multiple cellulose microfibrils constrains them into parallel arrays and that the orientation of the parallel array is set by the number of microfibrils and the size which each microfibril has when invested in its covering of matrix polysaccharides. Both of these parameters may change in *rsw1* or after dichlorobenzonitrile treatment. There is no explicit prediction of how random microfibrils would arise and predicting the consequences for microfibril alignment is not easy when, as in the first hours of growth at the restrictive temperature or the application of dichlorobenzonitrile, the number of microfibrils and the rate of cell elongation are falling but the rate of radial expansion is rising.

Conclusions

Mutants now link cellulose production to proteins with two putative enzyme activities and show that lipo-glucan accumulates when cellulose synthesis is impaired by a glycosyltransferase defect. There is much scope for further mutants to identify other proteins required for synthesis and for analysis of the biochemical phenotypes of mutants and double mutants to shed further light on the reaction pathways. In addition, mutants allow the role of cellulose synthesis in plant development to be explored with greater precision than was possible with chemical inhibitors. The time seems opportune for synergism to emerge between genetic, biochemical and structural approaches to cellulose synthesis that will further increase the rate of progress in this area.

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