BIOLOGIA PLANTARUM 51 (3): 407-413, 2007

REVIEW

Some aspects of cellulose biosynthesis

D. FINAEV

Kazan Institute of Biochemistry and Biophysics, Kazan Scientific Center, Russian Academy of Science, PO Box 30, Kazan 420111, Russia

Abstract

The paper is focused on two groups of proteins inevitably important for cellulose biosynthesis in vascular plants. These are cellulose synthases and chitinase-like proteins. Cellulose synthases have been the subject of much research, and current conceptions and recent findings are reviewed in this paper. Severe effects of mutations and expression analysis have recently shown that chitinase-like proteins are crucial components of cellulose biosynthesis. However, understanding of their precise function is missed. Further research is to be prompted by an effective idea on it. I propose that chitinase-like proteins could play a role in the assembly of nascent glucan chains into microfibrills. Therefore, cellulose synthases and chitinase-like proteins are possibly sequential elements of the cellulose biosynthesis.

Additional key words: cell wall, cellulose synthases, chitinase-like proteins, CTL.

Introduction

Cellulose is the most plentiful polymer on the Earth (Brown 2004) and vascular plants are the major producers of cellulose. The interest in cellulose comes from its importance for the economy and significance in nature. Cellulosic microfibrils serve as load-bearing elements of the cell wall (CW) and a determinant of cell growth and division. Within the near crystalline microfibrils, linear parallel β-1,4-D-glucan chains are connected via hydrogen bonding and Van der Waals forces. In vascular plants, cellulose is synthesized in the plasma membrane by hexagonal formations called rosettes. The rosette consists of six symmetrical particles with identical structure. Since each rosette produces one microfibril and each microfibril comprises 36 polysaccharide chains, it is assumed that one particle contains 6 catalytic subunits, each of which is responsible for one glucan chain. The rosette is a multisubunit complex. Aside from the subunits directly forming cellulose synthesizing complex (CSC), other agents also participate in cellulose synthesis. Substantial progress has been in unraveling function of *CesA*-genes that are thought to encode the catalytic subunits.

Cellulose synthases

Mode of action: CSC catalytic subunits (cellulose synthases; Arioli *et al.* 1998) processively join glucosyl residues (Saxena and Brown 2005) to form a β-1,4-D-glucan chain. The necessity of a primer and the possibility that sitosterol-β-glycoside is the primer have been widely debated (Peng *et al.* 2002, Schrick *et al.* 2004, Saxena and Brown 2005). After the start of synthesis some cellulase could cut off a growing glucan chain from the primer. Such a model has been suggested for cellulose synthesis in *Agrobacterium* (Matthysse *et al.* 1995) and *Acetobacter*

Received 16 June 2006, *accepted* 25 February 2007.

Abbreviations: CESA - cellulose synthase; CR-P - conserved region in plants; CSC - cellulose synthesizing complex; CSR - class-specific region; CTL - chitinase-like protein; CW - cell wall; PCW - primary cell wall; SCW - secondary cell wall; TM - transmembrane region; VR - variable region.

Acknowledgments: I wish to thank Prof. Candace Haigler (North Carolina State University, USA) for encouraging conversation on CTLs and Prof. Richard Beckett (University of KwaZulu-Natal, South Africa) for refining the style of the manuscript.

Fax: (+7) 843 2927347, e-mail: meshuga@rambler.ru

(Han and Robyt 1998). During polymerization cellulose synthases invert glucosyl units about 180 ° with respect to each neighbor allowing linear chains to be formed (Carpita and McCann 2000).

Evidence of function: Although enzymatic activity of cellulose synthases (CESAs) *in vitro* or in a heterologous system has not been shown, various data show they are indeed involved in cellulose synthesis. Firstly, these proteins share amino acid sequence similarity and general features of organization (Pear *et al.* 1996) with *Acetobacter xylinum* cellulose synthase, enzymatic activity of which has been demonstrated (Mayer *et al.* 1991). They contain the D,D,D,QxxRW-motive (Saxena *et al.* 1995), which is now believed to be completely conserved in all processive β-1,4-glycosyltransferases (Vergara and Carpita 2001). In plant cellulose synthases this motive acquires, with rare exceptions, the form of DDG,DCD,TED,QVLRW. Secondly, analysis of lines of *Arabidopsis* (Scheible and Pauly 2004, Chen *et al.* 2005) and rice (Tanaka *et al.* 2003) with mutated *CesA* genes, showed that these genes contribute in cellulose synthesis. Kimura *et al.* (1999) immunochemically showed that CESA-proteins go to make up the rosette. CESA-proteins interact directly with cellulose that has been shown in experiments with the herbicide CGA 325'625 (Peng *et al.* 2001). CGA 325'625 causes the accumulation of non-crystalline cellulose, which when incubated with cellulase, releases CESA-protein. In addition, expression profiling (Appenzeller *et al.* 2004, Burton *et al.* 2004, Hamann *et al.* 2004), its localization (Holland *et al.* 2000) and gene knockout (Burton *et al.* 2000, Burn *et al.* 2002) have been applied to investigate the roles of individual members of the *CesA*-family.

Cellulose synthase structure: Plant cellulose synthases are almost 1000 to 1100 aa in length. They have two zinc fingers (Fig. 1) at their N-end, which presumably take part in protein complex assembly *in vivo* (Kurek *et al.* 2002, Jacob-Wilk *et al.* 2006). The zinc fingers are formed of a cysteine-rich sequence commonly written as Cx(2)Cx(12)FxACx(2)Cx(2)PxCx(2)CxEx(5)Gx(3)Cx(2)C. This site is followed, after a short sequence, by a variable region (VR; Delmer 1999). Downstream of this variable region the first transmembrane domain (TM) is located. Plant cellulose synthases have 8 TMs – two at the N-end and six at the C-end – and an extensive loop turned into cytoplasmic area. The short C-tail is somehow involved in the activity of the enzyme. A mutation of the C-end impedes cellulose synthesis. There is a degree of conservation of the C-terminal sequences that spans monocotyle-donous and dicotyledonous paralogs (Wang *et al.* 2006). The cytoplasmic loop harbors the sequences directly participating in the enzyme functioning (Pear *et al.* 1996). The most prominent signature at the loop is the D,D,D,QxxRW-motive.

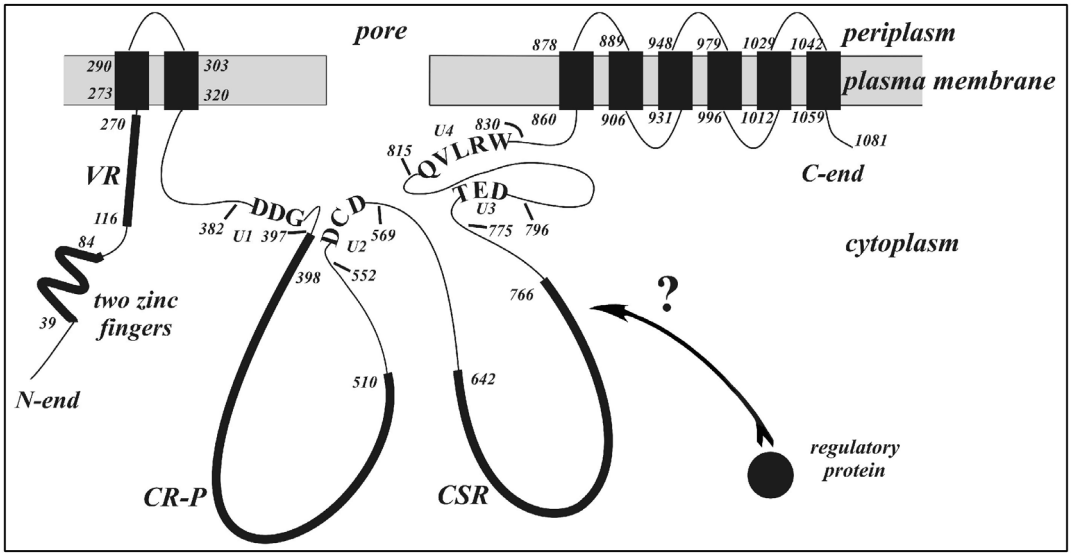

Fig. 1. Schematic representation of AtCESA1. Numbers point out the first and the last amino acids in the regions. TMs were predicted by the program PHDhtm (Rost *et al.* 2004). The boundaries of VR, CR-P and CSR were defined *via* alignment of plant and bacterial CESA-proteins. The alignment was performed using the program T-COFFEE v.4.54 by default (Notredame *et al.* 2000). The set used for the alignment: plant AmCESA1, AtCESA1, AtCESA4, AtCESA7, AtCESA10, BrCESA1, EgCESA2, EgCESA5, EgCESA6, GhCESA4, MtCESA1, MtCESA5, MtCESA10, NaCESA1, OsCESA2, OsCESA6, OsCESA8, PrCESA1, PtCESA2, PtCESA3, PtPtCESA2, PtrCESA2, StCESA3, ZmCESA7 (downloaded from the http://cellwall.stanford.edu) and bacterial Q8X5L7, P37653, P58931, Q8Z291, Q93IN2, P58932, Q7X246, Q1V8W4, Q1R570, Q5DZ42, Q6LRE9, Q2KWQ7, Q8FCH3 (downloaded from the Uniprot (http://www.ebi.uniprot.org). The U1-U4 motives are presented as described by Pear *et al.* (1996).

The residues of this motive are dispersed through the loop. Groups of conserved amino acids that surround every non-variable residue of aspartic acid and QVLRW-sequence has been named U-motives (Pear *et al.* 1996). Between the first and the second U-motives is situated the conserved region in plants (CR-P). This domain is similar in all plant cellulose synthases. A comparable extensive area between U1 and U2 is present also in the cellulose synthases of the slime mold *Dictyostelium discoideum* (Blanton *et al.* 2000) and in the cellulose synthases of some cyanobacteria (Nobles *et al.* 2001), but is absent in the cellulose synthases of bacteria (Pear *et al.* 1996) and tunicates (Nakashima *et al.* 2004). This area is similar in plant and cyanobacterial homologs; the cellulose synthases of *D. discoideum* have no significant similarity with the area of plant homologs. An extensive region between domains U2 and U3 exists exclusively in plant cellulose synthases. It is named the class-specific region (CSR) (Vergara and Carpita 2001). Based on the degree of similarity showed by this region, plant cellulose synthases can be divided into classes, and such a division is preserved at the full sequence alignment level. Also, this separation is seen when only C-ends are being aligned (Wang *et al.* 2006). Within the classes, as far as we can judge by data presently available, a single function has been kept [which can be cellulose synthesis for primary cell wall (PCW) or secondary cell wall (SCW)]. Despite its "class specificity" CSR shows significant similarity for all cellulose synthases at its distal end. This part of CSR is acidic. The proximal two thirds of CSR are more variable and enriched with basic amino acids (Vergara and Carpita 2001). CSR is a possible binding site for regulatory proteins (Pear *et al.* 1996). U1,U2 and U3,U4 form binding sites for UDP-donor and acceptor respectively (Charnock *et al.* 2001). Together, U-motives join in a structure made of two tightly coupled sheets (Coutinho *et al.* 2003). This is a GT-A fold.

Therefore, homology between plant CESA-proteins is expressed in various degrees along their sequences. There are highly homologous regions (U1-U4 motives containing areas), regions of intermediate similarity (CR-P, CSR, C-tail) and the variable region (VR).

***CesA*-genes:** *CesA*-genes vary in size from 3.5 to 5.5 kb. Genes, for which genomic sequences are available, have 8 or 9 small introns. Vascular plant cellulose synthase genes are members of a multigene family. The *CesA*-family contains 10 and 9 members in the genomes of *Arabidopsis* and rice, respectively, (http://cellwall.stanford.edu), reaching up 18 in *Populus trichocarpa* (Djerbi *et al.* 2005). Chromo-somes of maize contain *CesA*-gene clusters (Holland *et al.* 2000), while other screened genomes have no such clusters (Richmond 2000).

The rosettes are the CSC of vascular plants as well as of mosses and some algae. There exist several models of the rosette (Perrin 2001, Scheible *et al.* 2001, Robert *et al.* 2004, Saxena and Brown 2005), but they all have in common a CSC comprising different CESA-isoforms (Peng *et al.* 2002, Taylor *et al.* 2003). It has been shown for a number of plants – *Arabidopsis*, rice, barley, aspen (Burn *et al.* 2002, Tanaka *et al.* 2003, Burton *et al.* 2004, Kalluri and Joshi 2004), – that the composition of isoforms is specific for the PCW and the SCW.

The abnormalities of cellulose deposition alter mechanical features of the CW. Changes in PCW do not allow the developing cells to form their normal shapes and sizes. Severe mutants with PCW-cellulose deficiency often have radially swollen cells. On the other hand, cellulose deficiency in SCW, while generally preserving normal organ shape, causes cell surface to become roughened, as in the case of the irregular xylem vessels deformed during water transport (Taylor *et al.* 2003). The stages when changes appear, and the characteristic changes possessed by plants with cellulose synthases lesions, indicate whether the genes specific for PCW or SCW have been affected.

Based on such observations and the character of expression, a number of *CesA*-genes have been attributed to either PCW or SCW. The most complete picture has been obtained with *Arabidopsis*. Proteins AtCESA4, AtCESA7 and AtCESA8 interact non-redundantly within a single complex for cellulose synthesis for the SCW (Gardiner *et al.* 2003, Taylor *et al.* 2003). The effect of mutations and expression analyses prove that these cellulose synthases are the only SCW-related *Arabidopsis* cellulose synthases (Holland *et al.* 2000, Taylor *et al.* 2000, Ha *et al.* 2002).

A somewhat more complicated picture can be observed for the PCW. The role in cellulose synthesis for PCW has been proved for *AtCesA1*, *AtCesA3* and *AtCesA6*. *AtCesA2* works with these three genes, keeping its participation in the formation of the PCW less obvious (Persson *et al.* 2005, see also Burn *et al.* 2002, Beeckman *et al.* 2002). A changed wall ultrastructure in growing and dividing root cells of plants mutated in this gene directly proves that *AtCesA1* is responsible for producing the PCW (Sugimoto *et al.* 2001). Mutated plants have reduced cellulose content, their organs are of smaller size, and their reduced cells often protrude from the organ surface (Arioli *et al.* 1998). Mutations in *AtCesA3* (Ellis *et al.* 2002, Cano-Delgado *et al.* 2003) and *AtCesA6* (Fagard *et al.* 2000) result in similar phenotypes, also providing evidence for cellulose deposition in PCW. Antisense suppression of *AtCesA3* causes radial swelling and cell size reduction (Burn *et al.* 2002).

Lack of available mutants for *AtCesA2*, *AtCesA5*, *AtCesA9* and *AtCesA10* implies minor contribution of these genes to the phenotype. *AtCesA2* silencing induces a weakly modified phenotype that is consistent with its role in PCW formation (Burn *et al.* 2002). *AtCesA5* is expressed throughout the plant, but at a moderate level (Hamann *et al.* 2004). Therefore, *AtCesA5* functioning

noticeably differs from the functioning of most *AtCesA*-genes, which are activated in stage-specific manner and need higher expression levels to fulfill their own roles. Genes *AtCesA9* and *AtCesA10* and their products are structural analogs of *AtCesA2* and *AtCesA1* respectively. However, the sequence similarity does not mean that these genes are redundant. This can be seen by the example of *rsw1* mutants, defective in *AtCesA*1-gene; *AtCesA10* cannot substitute its function. The situation with the couple *AtCesA2* and *AtCesA9* is less evident. Here the lack of mutants makes the situation less clear. The abnormal phenotype of *AtCesA2*-antisense lines proves that *AtCesA9* cannot substitute for *AtCesA2*, while smallness of the change rather corresponds to small impact to the phenotype than to compensation by *AtCesA9*.

The "genes of the second echelon" are likely not redundant. Members of multigene families tend to play unique roles, but not duplicate them or have been silent (Adams and Wendel 2005). Probably, latent genes are expressed at special locations or under special conditions (Carpita and Vergara 1998). The only place where the expression of *AtCesA10* has been observed was in the petioles of rosette leaves (Doblin *et al.* 2002). *AtCesA9* expression has been observed in embryonic stages of development (Beeckman *et al.* 2002) and in the region adjacent to the shoot apical meristem (Scheible and Pauly 2004). Therefore, cellulose synthesis in differentiating cells may be the responsibility of *AtCesA9*.

Cellulose synthesis *in vitro*: Lai-Kee-Him *et al.* (2002) were the first to conduct the synthesis of sizable quantities of cellulose *in vitro* using microsomal preparations of a higher plant, *Rubus fruticosus*. No addition of enzymes or activators of cellulose synthesis was required. Therefore, cellulose synthesizing system was isolated untouched. Another group succeeded in isolating intact *Gossypium hirsutum* rosettes, which after isolation retained the ability to synthesize cellulose *in vitro* (Saxena and Brown 2005). The interesting report of *in vitro* synthesis of cellulose by *Acetobacter xylinum* membrane preparations (Aloni *et al.* 1982) has greatly deepened our understanding of the mechanism of bacterial cellulose synthesis and led to the discovery of corresponding genes. For plants too, we have a right to expect a similar impact from studying *in vitro* synthesis.

Practical applications: Manipulation of the genetics of cellulose synthesis is one of the most important challenges of wood biotechnology. In an attempt to increase the efficiency of wood utilization, many researchers have directed their investigations on tension wood, because this is enriched with cellulose. This wood is laid down in many angiosperm trees under the mechanical stress such as constant winds. Tension wood is characterized by presence of an additional layer of the SCW called the gelatinous layer or G-layer consisting mainly of cellulose microfibrils oriented almost parallel to the fiber axis (Pilate *et al.* 2004). In this context, the SCW cellulose synthases, whose expressions are localized in the tension wood (Joshi 2003, Paux *et al.* 2005), are prospective candidates for genetic engineering.

Chitinase-like proteins

Apart from cellulose synthases, some other agents are important for cellulose synthesis. Current understanding of the roles of endo-β-1,4-D-glucanase KORRIGAN, proteins KOBITO and COBRA (at least two last located on plasma membrane), and also the roles of the cyto-skeleton and N-glycosylation have been discussed elsewhere (Somerville 2006). However, I would like to mention one more group of proteins that quite recently attracted attention as participants of cellulose synthesis. These are chitinase-like proteins (CTL).

Proteins of this cohort are supposed to evolve from chitinases. Having lost hydrolytic activity they have presumably preserved their chitin-binding characteristics, *i.e.*, CTLs are now thought to be chitin-binding lectins (Zhang *et al.* 2004). These are two-lobed proteins with a cleft in the middle. Proteins of similar structure such as chitinase or lysozyme often process unbranched extended molecules. In case of cellulose biosynthesis, such molecules could be β-1,4-glucan chains. Analysis *in silico* showed that CTLs are secreted through the plasma membrane (Zhong *et al.* 2002, Zhang *et al.* 2004). CTL expression is highly coordinated with cellulose synthase expression (Zhang *et al.* 2004, Aspeborg *et al.* 2005, Brown *et al.* 2005, Persson *et al.* 2005). If CTLs were regulatory molecules for cellulose synthases as proposed by Zhang *et al.* (2004) the interrelations between their expression patterns would be less apparent. There would be some lag between activation of CTL and activation of cellulose synthase. When forming PCW and SCW, different paralogs start working (Persson *et al.* 2005) that again resembles the behavior of cellulose synthases. Such parallelism suggests that an association, physical or physiological, exists between these two classes of enzymes. Mutations of these genes result in severe damage to the CW because of cellulose deficiency (Hauser *et al.* 1995, Schneider *et al.* 1997, Cano-Delgado *et al.* 2000, Mouille *et al.* 2003).

Taken together the findings of these investigations suggest that CTLs act until β-1,4-glucan chains are completely crystallized. Perhaps the existing cleft directs chains as they are being formed enabling them to gather neatly into supramolecular structures. In other words, CTL could be a crystallization subunit (Delmer and Amor 1995). Cellulase activity also cannot be excluded.

A protein that is evolved from a chitinase, but processes cellulose, could be a cellulase. Moreover, roles for cellulases in cellulose biosynthesis have been proposed (Delmer 1999, Peng *et al.* 2002). Possible involvements of cellulases are seen as being an "editor/chain terminator" (Delmer 1999) and/or a "primer releaser" (Peng *et al.* 2002). The first role lies in trimming off the less crystalline sites of cellulose microfibrils, while the second lies in cutting of a possible lipid primer. Earlier the membrane-bound endo-β-1,4-D-glucanase KORRIGAN was suggested for the second role (Peng *et al.* 2002), but this role of KORRIGAN is now contested (Robert *et al.* 2004, 2005). Nevertheless, another cellulase could really play this role. CTL's structural analogs hydrolyze bonds within polysaccharide chains. Therefore, it is easier to imagine that CTLs process a polysaccharide chain, rather than a lipid-oligosaccharide chain.

Mutation analysis suggests that CTLs are pivotal for cellulose biosynthesis, and expression patterns show that CTL activity is tightly coupled with cellulose synthase activity. The structure of CTLs allows them to play intriguing roles in cellulose biosynthesis. Therefore, continuing research is likely to be rewarding.

Conclusion

At present, we are unable to fully explain the mechanism of cellulose biosynthesis. Recently, significant progress has been made in the study of cellulose synthases, but even for this, the most thoroughly investigated group, we are far from fully understanding their activity. Further research will shift methodology towards understanding in more detail the unique roles of each isoforms and their spatio-temporal activity localization. Intense efforts will be applied to biochemically characterize the functions of other proteins and cellular elements involved in cellulose synthesis.

References

Adams, K.L., Wendel, J.F.: Polyploidy and genome evolution in plants. - Curr. Opinion Plant Biol. **8**: 135-41, 2005.

Aloni, Y., Delmer, D.P., Benziman, M.: Achievement of high rates of *in vitro* synthesis of 1,4-beta-D-glucan: activation by cooperative interaction of the *Acetobacter xylinum* enzyme system with GTP, polyethylene glycol, and a protein factor. - Proc. nat. Acad. Sci. USA **79**: 6448-6452, 1982.

Appenzeller, L., Doblin, M.S., Barreiro, R., Wang, H., Niu, X., Kollipara, K., Carrigan, L., Tomes, D., Chapman, M., Dhugga, K.S.: Cellulose synthesis in maize: isolation and expression analysis of the cellulose synthase (*CesA*) gene family. - Cellulose **11**: 287-299, 2004.

Arioli, T., Peng, L., Betzner, A.S., Burn, J., Wittke, W., Herth, W., Camilleri, C., Hofte, H., Plazinski, J., Birch, R., Cork, A., Glover, J., Redmond, J., Williamson, R.E.: Molecular analysis of cellulose biosynthesis in *Arabidopsis*. - Science **279**: 717-720, 1998.

Aspeborg, H., Schrader, J., Coutinho, P.M., Stam, M., Kallas, A., Djerbi, S., Nilsson, P., Denman, S., Amini, B., Sterky, F., Master, E., Sandberg, G., Mellerowicz, E., Sundberg, B., Henrissat, B., Teeri, T.T.: Carbohydrate-active enzymes involved in the secondary cell wall biogenesis in hybrid aspen. - Plant Physiol. **137**: 983-997, 2005.

Beeckman, T., Przemeck, G.K., Stamatiou, G., Lau, R., Terryn, N., De Rycke, R., Inze, D., Berleth, T.: Genetic complexity of cellulose synthase A gene function in *Arabidopsis* embryogenesis. - Plant Physiol. **130**: 1883-1893, 2002.

Blanton, R.L., Fuller, D., Iranfar, N., Grimson, M.J., Loomis, W.F.: The cellulose synthase gene of *Dictyostelium.* - Proc. nat. Acad. Sci. USA **97**: 2391-2396, 2000.

Brown, D.M., Zeef, L.A., Ellis, J., Goodacre, R., Turner, S.R.: Identification of novel genes in *Arabidopsis* involved in secondary cell wall formation using expression profiling and reverse genetics. - Plant Cell. **17**: 2281-2295, 2005.

Brown, R.M., Jr.: Cellulose structure and biosynthesis: what is in store for the 21st century? - J. Polymer Sci. Part A: Polymer Chem. **42**: 487-495, 2004.

Burn, J.E., Hocart, C.H., Birch, R.J., Cork, A.C., Williamson, R.E.: Functional analysis of the cellulose synthase genes *CesA1, CesA2* and *CesA3* in *Arabidopsis*. - Plant Physiol. **129**: 797-807, 2002.

Burton, R.A., Gibeaut, D.M., Bacic, A., Findlay, K., Roberts, K., Hamilton, A., Baulcombe, D.C., Fincher, G.B.: Virus-induced silencing of a plant cellulose synthase gene. - Plant Cell **12**: 691-706, 2001.

Burton, R.A., Shirley, N.J., King, B.J., Harvey, A.J., Fincher, G.B.: The *CesA* gene family of barley. Quantitative analysis of transcripts reveals two groups of co-expressed genes. - Plant Physiol. **134**: 224-236, 2004.

Cano-Delgado, A., Penfield, S., Smith, C., Catley, M., Bevan, M.: Reduced cellulose synthesis invokes lignification and defense responses in *Arabidopsis thaliana*. - Plant J. **34**: 351-362, 2003.

Cano-Delgado, A.I., Metzlaff, K., Bevan, M.W.: The *eli1* mutation reveals a link between cell expansion and secondary cell wall formation in *Arabidopsis thaliana*. - Development **127**: 3395-3405, 2000.

Carpita, N., McCann, M.: The cell wall. - In: Buchanan, B.B., Gruissem, W., Jones, R.L. (ed.): Biochemistry and Molecular Biology of Plants. P. 58. American Society of Plant Physiologists, Rockville 2000.

Carpita, N., Vergara, C.: A recipe for cellulose. - Science **279**: 672-673, 1998.

Charnock, S.J., Henrissat, B., Davies, G.J.: Three-dimensional structures of UDP-sugar glycosyltransferases illuminate the biosynthesis of plant polysaccharides. - Plant Physiol. **125**: 527-531, 2001.

Chen, Z., Hong, X., Zhang, H., Wang, Y., Li, X., Zhu, J.K., Gong, Z.: Disruption of the cellulose synthase gene, *AtCesA8*/IRX1, enhances drought and osmotic stress tolerance in *Arabidopsis*. - Plant J. **43**: 273-283, 2005.

Coutinho, P.M., Deleury, E., Davies, G.J., Henrissat B.: An evolving hierarchical family classification for glycosyltransferases. - J. mol. Biol. **328**: 307-317, 2003.

Delmer, D.P.: Cellulose biosynthesis: exciting times for a difficult field of study. - Annu. Rev. Plant Physiol. Plant mol. Biol. **50**: 245-276, 1999.

Delmer, D.P., Amor, Y.: Cellulose biosynthesis. - Plant Cell **7**: 987-1000, 1995.

Djerbi, S., Lindskog, M., Arvestad, L., Sterky, F., Teeri, T.T.: The genome sequence of black cottonwood (*Populus trichocarpa*) reveals 18 conserved cellulose synthase (*CesA*) genes. - Planta **221**: 739-746, 2005.

Doblin, M.S., Kurek, I., Jacob-Wilk, D., Delmer, D.P.: Cellulose biosynthesis in plants: from genes to rosettes. - Plant Cell Physiol. **43**: 1407-1420, 2002.

Ellis, C., Karafyllidis, I., Wasternack, C., Turner, J.G.: The *Arabidopsis* mutant *cev1* links cell wall signaling to jasmonate and ethylene responses. - Plant Cell **14**: 1557-1566, 2002.

Fagard, M., Desnos, T., Desprez, T., Goubet, F., Refregier, G., Mouille, G., McCann, M., Rayon, C., Vernhettes, S., Hofte, H.: *PROCUSTE1* encodes a cellulose synthase required for normal cell elongation specifically in roots and dark-grown hypocotyls of *Arabidopsis*. - Plant Cell **12**: 2409-2424, 2000.

Gardiner, J.C., Taylor, N.G., Turner, S.R.: Control of cellulose synthase complex localization in developing xylem. - Plant Cell **15**: 1740–1748, 2003.

Ha, M.A., MacKinnon, I.M., Sturcova, A., Apperley, D.C., McCann, M.C., Turner, S.R., Jarvis, M.C.: Structure of cellulose-deficient secondary cell walls from the *irx3* mutant of *Arabidopsis thaliana*. - Phytochemistry **61**: 7-14, 2002.

Hamann, T., Osborne, E., Youngs, H.L., Misson, J., Nussaume, L., Somerville, C.: Global expression analysis of *CESA* and *CSL* genes in *Arabidopsis*. - Cellulose **11**: 279-286, 2004.

Han, N.S., Robyt, J.F.: The mechanism of *Acetobacter xylinum* cellulose biosynthesis: direction of chain elongation and the role of lipid pyrophosphate intermediates in the cell membrane. - Carbohydr. Res. **313**: 125-133, 1998.

Hauser, M.-T., Morikami, A., Benfey, P.N.: Conditional root expansion mutants of *Arabidopsis*. - Development **121**: 1237-1252, 1995.

Holland, N., Holland, D., Helentjaris, T., Dhugga, K.S., Xoconostle-Cazares, B., Delmer, D.P.: A comparative analysis of the plant cellulose synthase (*CesA*) gene family. - Plant Physiol. **123**: 1313-1324, 2000.

Jacob-Wilk, D., Kurek, I., Hogan, P., Delmer, D.P.: The cotton fiber zinc-binding domain of cellulose synthase A1 from *Gossypium hirsutum* displays rapid turnover *in vitro* and *in vivo*. - Proc. nat. Acad. Sci. USA **103**: 12191-12196, 2006.

Joshi, C.P.: Xylem-specific and tension stress-responsive expression of cellulose synthase genes from aspen trees. - Appl. Biochem. Biotechnol. **105**: 17-26, 2003.

Kalluri, U.C., Joshi, C.P.: Differential expression patterns of two cellulose synthase genes are associated with primary and secondary cell wall development in aspen trees. - Planta **220**: 47-55, 2004.

Kimura, S., Laosinchai, W., Itoh, T., Cui, X., Linder, C.R., Brown, R.M., Jr.: Immunogold labeling of rosette terminal cellulose-synthesizing complexes in the vascular plant *Vigna angularis*. - Plant Cell **11**: 2075-2086, 1999.

Kurek, I., Kawagoe, Y., Jacob-Wilk, D., Doblin, M., Delmer, D.: Dimerization of cotton fiber cellulose synthase catalytic subunits occurs via oxidation of the zinc-binding domains. - Proc. nat. Acad. Sci. USA **99**: 11109-11114, 2002.

Lai-Kee-Him, J., Chanzy, H., Muller, M., Puteaux, J.-L., Imai, T., Bulone, V.: *In vitro* versus *in vivo* cellulose microfibrils from plant primary wall synthases: structural differences. - J. biol. Chem. **277**: 36931-36939, 2002.

Matthysse, A.G., Thomas, D.L., White, A.R.: Mechanism of cellulose synthesis in *Agrobacterium tumefaciens*. - J. Bacteriol. **177**: 1076-1081, 1995.

Mayer, R., Ross, P., Weinhouse, H., Amikam, D., Volman, G., Ohana, P., Calhoon, R.D., Wong, H.C., Emerick, A.W., Benziman, M.: Polypeptide composition of bacterial cyclic diguanylic acid-dependent cellulose synthase and the occurrence of immunologically crossreacting proteins in higher plants. - Proc. nat. Acad. Sci. USA **88**: 5472-5476, 1991.

Mouille, G., Robin, S., Lecomte, M., Pagant, S., Hofte, H.: Classification and identification of *Arabidopsis* cell wall mutants using Fourier-transform infra-red (FT-IR) microspectroscopy. - Plant J. **35**: 393-404, 2003.

Nakashima, K., Yamada, L., Satou, Y., Azuma, J., Satoh, N.: The evolutionary origin of animal cellulose synthase. - Dev. Genes Evol. **214**: 81-88, 2004.

Nobles, D.R., Romanovicz, D.K., Brown, R.M., Jr.: Cellulose in cyanobacteria. Origin of vascular plant cellulose synthase? - Plant Physiol. **127**: 529-542, 2001.

Notredame, C., Higgins, D.G., Heringa, J.: T-coffee: a novel method for fast and accurate multiple sequence alignment. - J. mol. Biol. **302**: 205-217, 2000.

Paux, E., Carocha, V., Marques, C., Mendes de Sousa, A., Borralho, N., Sivadon, P., Grima-Pettenati, J.: Transcript profiling of *Eucalyptus* xylem genes during tension wood formation. - New Phytol. **167**: 89-100, 2005.

Pear, J.R., Kawagoe, Y., Schreckengost, W.E., Delmer, D.P., Stalker, D.M.: Higher plants contain homologs of the bacterial *celA* genes encoding the catalytic subunit of cellulose synthase. - Proc. nat. Acad. Sci. USA **93**: 12637-12642, 1996.

Peng, L., Kawagoe, Y., Hogan, P., Delmer, D.: Sitosterol-beta-glucoside as primer for cellulose synthesis in plants. - Science **295**: 147-150, 2002.

Peng, L., Xiang, F., Roberts, E., Kawagoe, Y., Greve, L.C., Kreuz, K., Delmer, D.P.: The experimental herbicide CGA 325'615 inhibits synthesis of crystalline cellulose and causes accumulation of non-crystalline beta-1,4-glucan associated with CesA protein. - Plant Physiol. **126**: 981-992, 2001.

Perrin, R.M.: Cellulose: how many cellulose synthases to make a plant? - Curr. Biol. **11**: 213-216, 2001.

Persson, S., Wei, H., Milne, J., Page, G.P., Somerville, C.R.: Identification of genes required for cellulose synthesis by regression analysis of public microarray data sets. - Proc. nat. Acad. Sci. USA **102**: 8633-8638, 2005.

Pilate, G., Dejardin, A., Laurans, F., Leple, J.C.: Tension wood as a model for functional genomics of wood formation. - New Phytol. **164**: 63-72, 2004.

Richmond, T.: Higher plant cellulose synthases. - Genome Biol. **1**: 3001-3005, 2000.

Robert, S., Bichet, A., Grandjean, O., Kierzkowski, D., Satiat-

Jeunemaıtre, B., Pelletier, S., Hauser, M.-T., Hofte, H., Vernhettesa, S.: An *Arabidopsis* endo-1,4-β-D-glucanase involved in cellulose synthesis undergoes regulated intracellular cycling. - Plant Cell **17**: 3378-3389, 2005.

Robert, S., Mouille, G., Hofte, H.: The mechanism and regulation of cellulose synthesis in primary walls: lessons from cellulose-deficient *Arabidopsis* mutants. - Cellulose **11**: 351-364, 2004.

Rost, B., Yachdav, G., Liu, J.: The predict protein server. - Nucl. Acids Res. **32**(Web Server issue): W321-W326, 2004.

Saxena, I.M., Brown, R.M., Jr.: Cellulose biosynthesis: current views and evolving concepts. - Ann. Bot. **96**: 9-21, 2005.

Saxena, I.M., Brown, R.M., Jr., Fevre, M., Geremia, R.A., Henrissat, B.: Multidomain architecture of beta-glycosyl transferases: implications for mechanism of action. - J. Bacteriol. **177**: 1419-1424, 1995.

Scheible, W.R., Eshed, R., Richmond, T., Delmer, D., Somerville, C.: Modifications of cellulose synthase confer resistance to isoxaben and thiazolidinone herbicides in *Arabidopsis Ixr1* mutants. - Proc. nat. Acad. Sci. USA **98**: 10079-10084, 2001.

Scheible, W.R., Pauly, M.: Glycosyltransferases and cell wall biosynthesis: novel players and insights. - Curr. Opinion Plant Biol. **7**: 285-295, 2004.

Schneider, K., Wells, B., Dolan, L., Roberts, K.: Structural and genetic analysis of epidermal cell differentiation in *Arabidopsis* primary roots. - Development **124**: 1789-98, 1997.

Schrick, K., Fujioka, S., Takatsuto, S., Stierhof, Y.-D., Stransky, H., Yoshida, S., Jurgens, G.: A link between sterol biosynthesis, the cell wall and cellulose in *Arabidopsis*. - Plant J. **38**: 227-243, 2004.

Somerville, C.: Cellulose synthesis in higher plants. - Annu. Rev. cell dev. Biol. Plant **22**: 53-78, 2006.

Sugimoto, K., Williamson, R.E., Wasteneys, G.O.: Wall architecture in the cellulose deficient *rsw1* mutant of *Arabidopsis*: microfibrils but not microtubules lose their transverse alignment before microfibrils become unrecognizable in the mitotic and elongation zones of roots. - Protoplasma **215**: 172-183, 2001.

Tanaka, K., Murata, K., Yamazaki, M., Onosato, K., Miyao, A., Hirochika, H.: Three distinct rice cellulose synthase catalytic subunit genes required for cellulose synthesis in the secondary wall. - Plant Physiol. **133**: 73-83, 2003.

Taylor, N.G., Howells, R.M., Huttly, A.K., Vickers, K., Turner, S.R.: Interactions among three distinct CesA proteins essential for cellulose synthesis. - Proc. nat. Acad. Sci. USA **100**: 1450-1455, 2003.

Taylor, N.G., Laurie, S., Turner, S.R.: Multiple cellulose synthase catalytic subunits are required for cellulose synthesis in *Arabidopsis*. - Plant Cell **12**: 2529-2539, 2000.

Vergara, C.E., Carpita, N.C.: Beta-D-glycan synthases and the *CesA* gene family: lessons to be learned from the mixed-linkage (1→3),(1→4)beta-D-glucan synthase. - Plant mol. Biol. **47**: 145-160, 2001.

Wang, J., Howles, P.A., Cork, A.H., Birch, R.J., Williamson, R.E.: Chimeric proteins suggest that the catalytic and/or C-terminal domains give CesA1 and CesA3 access to their specific sites in the cellulose synthase of primary walls. - Plant Physiol. **142**: 685-695, 2006.

Zhang, D., Hrmova, M., Wan, C.H., Wu, C., Balzen, J., Cai, W., Wang, J., Densmore, L.D., Fincher, G.B., Zhang, H., Haigler, C.H.: Members of a new group of chitinase-like genes are expressed preferentially in cotton cells with secondary walls. - Plant mol. Biol. **54**: 353-372, 2004.

Zhong, R., Kays, S.J., Schroeder, B.P., Ye, Z.H.: Mutation of a chitinase-like gene causes ectopic deposition of lignin, aberrant cell shapes, and overproduction of ethylene. - Plant Cell **14**: 165-79, 2002.