



Cell wall biosynthesis in barley suspension cultures and coleoptiles

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Abstract

The plant cell wall is a complex, diverse and dynamic entity, that changes throughout the processes of cell division, growth and differentiation. While cell walls are of central importance in plant growth and development, they also represent the single major determinant of plant structure, resistance to pathogens, quality of plant-based foods and the properties of fibres and fuels.

We are using barley suspension culture cells to study cell wall synthesis during wall regeneration by protoplasts and elongating coleoptiles to study wall expansion. We have focussed our efforts on the *CesA/Csl* superfamily as likely candidate genes for the major cell wall polysaccharide synthases.

To date we have identified *CesAs* and *Csls* belonging to the C and D family and will describe their expression patterns in barley tissues.

Introduction

There are two types of cell walls found in higher plants. Type I cell walls are found in dicots, gymnosperms and most monocots, and type II walls are found in grasses and their close relatives (Bacic *et al.*, 1988; Carpita and Gibeaut, 1993; Carpita, 1996).

Cellulose, a linear (1,4)- β -D-glucan,

is the main load-bearing polysaccharide in both types of walls. In the type II wall, noncellulosic-polysaccharides such as glucuronoarabinoxylans and 'mixed-linkage' (1,3;1,4)- β -D-glucans form a matrix that holds the cellulose microfibrils together. These polysaccharides are only found in type II walls, and even though they make up

a relatively minor part of the total weight of cereal grains, they have a surprisingly large influence on the quality of grain.

The structural features of the polysaccharides in type II walls have been well characterised; however little is known about their synthesis and deposition. This is primarily because to date no cell wall polysaccharide synthase has been isolated. In plants, the first step in identifying the catalytic sub unit of cellulose synthase was the cloning of candidate cDNAs from a cotton fibre cDNA library (*GhCesA1* and *GhCesA2*). The encoded protein sequences contained three domains with similarity to bacterial cellulose synthase genes (Pear *et al.*, 1996). Cutler and Somerville (1997) and Saxena and Brown (1997) described a family of related higher plant genes that showed similarity to the *CesA* sequences. These genes, now known as the *Csl* (cellulose synthase-like) genes, have been classified into 6 subfamilies, named *CsIA*, *CsIB*, *CsIC*, *CsID*, *CsIE* and *CsIG*. They speculated that the six classes of *Csl* genes may either contribute to the synthesis of non-cellulosic polysaccharides, or they may also have a role in the synthesis of cellulose (Richmond and Somerville, 2000).

The aim of this study is to clone *CesA* and *Csl* genes from the C and D families in barley. Representatives from these families will be analysed to determine expression and possible function.

Materials and Methods

Initially, gene isolation was achieved by PCR on barley cDNA preparations and screening of a barley tissue culture cDNA library. Primers were designed to conserved regions of alignments of *CesA* and *Csl* sequences such as the D,D,D, QxxRW motifs from other plant species, or to particular barley ESTs. Information on the *CesA*, *CsIC* and *CsID* gene sequences was obtained from the website maintained by Dr. Todd Richmond (<http://cellwall.stanford.edu>). Isolated fragments were cloned and sequenced, and confirmed as *CesA*, *CsIC* or *CsID* by BLAST searches. Genes are being isolated by screening a barley BAC library and sequencing the genomic clone. RFLP analysis of barley mapping populations from the breeding program at the University of Adelaide will be used to identify the location of these genes in the barley genome.

Northern blotting and PCR have been used to identify tissues expressing isolated genes. Recombinant peptides made from fragments of the *CsIC* and *CsID* sequences have been expressed in *Escherichia coli*, and were used to generate polyclonal antibodies in rabbits. These antibodies will enable immunolocalisation of *Csl* proteins *in vivo*.

Functional analysis will involve a range of systems. These will include gain-of-function by transformation of *CesA* and *Csl* genes into different plant species or yeast cells. Loss-of-function in barley will also be achieved by gene silencing techniques such as dsRNAi.

Results and Discussion

We have isolated more than 7 *CesA* genes, 3 *CslC* genes and 2 *CslD* genes from barley. Some of these genes are full length, and efforts to complete the missing sequences from other genes are continuing.

Northern analysis has been unsuccessful, but RT-PCR has revealed expression sites of the genes in a variety of barley tissues, including stem, root, spike and suspension culture cells.

Rabbit antibodies have been generated to *CslC* and *CslD* recombinant *E. coli* proteins. Immunomicroscopy will be used to determine the subcellular localisation of proteins in barley protoplasts and elongating coleoptiles.

Conclusion

The work presented here is at an early stage, but the potential is exciting. The isolation of *CesA* and related genes from barley will allow comparison with the corresponding gene families in other plant species. The comparison with dicots species such as *Arabidopsis* will be especially interesting, because important differences between the enzymes involved in the synthesis of type I and type II cell walls may become apparent.

Acknowledgments

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References

1. Bacic A, Harris PJ, Stone BA (1988) Structure and function of plant cell walls. In: J Priess (ed) *The Biochemistry of Plants*. Academic Press, p. 297-371.
2. Carpita NC (1996) Structure and biogenesis of the cell wall of grasses. *Annual Review of Plant Physiology and Plant Molecular Biology*, 47:445-476.
3. Carpita NC, and Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal*, 3:1-30.
4. Cutler S, and Somerville, CR (1997) Cloning in silico. *Current Biology*, 7(2):R108-111.
5. Pear JR, Kawagoe Y, Schreckengost WE, Delmer DP, and Stalker DM (1996) Higher plants contain homologs of the bacterial *celA* genes encoding the catalytic subunit of cellulose synthase. *PNAS USA*, 93(22):12637-12642.

5. Richmond TA, and Somerville CR (2000) The Cellulose Synthase Superfamily. *Plant Physiology*, 124(2): 495-498.
 6. Saxena IM and Brown, RM (1997) Identification of cellulose synthase(s) in higher plants: sequence analysis of processive beta-glycosyltransferases with the common motif 'D,D,D35Q(R,Q)XRW'. *Cellulose*, 4:33-49.
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