Available online at www.sciencedirect.com

SCIENCE DIRECT

Gene 350 (2005) 107–116

GENE
SECTION
EVOLUTIONARY GENOMICS

www.elsevier.com/locate/gene

The cellulose synthase gene *PrCESA10* is involved in cellulose biosynthesis in developing tracheids of the gymnosperm *Pinus radiata*

Erwin Krauskopf, Philip J. Harris, Jo Putterill*

School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand

Received 21 June 2004; received in revised form 2 February 2005; accepted 22 February 2005
Available online 7 April 2005
Received by W. Martin

Abstract

One full length (*PrCESA10*) and seven other cDNA clones (*PrCESA2, 3, 5–8, 11*) encoding cellulose synthases (CESAs) were isolated from the coniferous gymnosperm *Pinus radiata*. *PrCESA10* encodes a protein predicted to contain the same domains and regions as angiosperm *CESA* genes: a zinc finger domain, a hypervariable region 1 (HVR1), a plant-conserved region (CR-P), a class-specific region or hypervariable region 2 (HVR2), in addition to the four conserved domains U1–U4 that are characteristic of the family 2 processive β-glycosyltransferases. The *P. radiata* protein is also predicted to contain eight transmembrane domains. The zinc finger domain, the CR-P and the C-terminal portion of the proteins, are highly conserved between *P. radiata* and the nearest angiosperm CESA protein from *Solanum tuberosum*. Reverse transcriptase-PCR showed that all the *P. radiata* genes were expressed in all organs tested, although to different extents. In situ hybridization studies with *PrCESA10* in stems of 2- and 12-month-old seedlings showed that it was expressed in the secondary xylem in the two-to-three most recently developed tracheids, which were laying down secondary cell walls.

Keywords: Lignified secondary cell walls; Angiosperms; Phylogenetic; Evolution; *CESA* gene

1. Introduction

Cellulose, a (1→4)-β-D-glucan, is the most abundant polysaccharide in the biosphere. It is a major component of both primary and secondary cell walls of seed plants (angiosperms and gymnosperms), where it is present in a crystalline form as microfibrils (Bacic et al., 1988). All other embryophytes (land plants), many algae, some protozoa and bacteria, including *Acetobacter xylinum*, and one group of invertebrate animals, the tunicates, also produce it (Stone, 2001).

Despite the abundance of cellulose in seed plants, attempts to purify and characterize cellulose synthases from these organisms using biochemical methodology have been unsuccessful (Delmer, 1999; Doblin et al., 2002). In contrast, this approach was successfully used to isolate and characterize cellulose synthase from the bacterium *A. xylinum*. This study led to the subsequent cloning of the first cellulose synthase genes from bacteria, including the *CESA* gene that encodes the enzyme containing the catalytic site of cellulose synthase (EC 2.4.1.12) (Saxena et al., 1990). With the nucleotide sequence of this gene, Pear et al. (1996) used a bioinformatic approach to identify the first two *CESA* genes from seed plants in the angiosperm *Gossypium hirsutum*.

Definitive genetic evidence for the functionality of cellulose synthase genes in angiosperms was first demonstrated using a *CESA* mutant of *Arabidopsis thaliana*, *rsw1*,

Abbreviations: bp, base pair(s); cDNA, DNA complementary to RNA; *CESA*, cellulose synthase gene; CSR, class-specific region; CR-P, conserved region-plant; DIG, digoxigenin; EST, expressed sequence tag; HVR 1 and 2, hypervariable regions 1 and 2; *rsw*, root swelling mutant; RT-PCR, reverse transcriptase-polymerase chain reaction; UDP, uridine diphosphate; *UTR*, untranslated region(s).

* Corresponding author. Tel.: +64 9 373 7599x87233; fax: +64 9 373 7417.
E-mail address: J.Putterill@auckland.ac.nz (J. Putterill).

0378-1119/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2005.02.018

that exhibits radial swelling of its roots at restrictive temperatures due to a defect in the production of crystalline cellulose; the wild type *RSW1* (*AtCESA1*) gene restored the wild type phenotype (Arioli et al., 1998). Since then, full length *CESA* genes have been isolated from a range of other angiosperm species (Doblin et al., 2002; http://cellwall.stanford.edu), including hardwood trees such as aspen (*Populus tremuloides*) (Wang and Loopstra, 1998; Wu et al., 2000; Samuga and Joshi, 2002, 2004; Kalluri and Joshi, 2003) and monocotyledons such as *Zea mays* (Holland et al., 2000).

CESA proteins from bacteria and angiosperms are characterized by having four conserved domains U1–U4: U1 to U3 each contain a conserved aspartic acid residue (D) and U4 contains a conserved QXXRW motif. The three D residues and QXXRW motifs are characteristic of family 2 processive β-glycosyltransferases (Saxena et al., 1995). In cellulose synthases they bind the substrate, UDP-glucose and the cofactor Mn^{2+}, and catalyse the formation of cellulose (Karnezis et al., 2000; Doblin et al., 2002). They form part of the cytoplasmic domain of the protein that is between the two groups of predicted transmembrane domains, two near the N-terminus and six near the C-terminus (Delmer, 1999). Compared with the bacterial CESA proteins, angiosperm CESA proteins have an extended N-terminal region that includes a zinc finger domain and an adjacent hypervariable region (HVR1), a plant-conserved region (CR-P) between domains U1 and U2, and a class-specific region (CSR) between U2 and U3. The CSR is also known as the hypervariable region 2 (HVR2) and has a limited homology among different angiosperm CESA proteins (Vergara and Carpita, 2001). Recent evidence indicates that the zinc finger domains are involved in the interactions between individual CESA proteins (Kurek et al., 2002). In angiosperms, the CESA proteins are located in rosette structures in the plasma membrane (Kimura et al., 1999). Similar rosettes occur in all embryophytes and charophycean algae, but not in bacteria (Roberts et al., 2002).

Mutant, antisense and expression analyses of the 10 *A. thaliana CESA* genes suggest that at least three of them (*AtCESA1*, *3* and *6*) encode proteins that function to synthesize cellulose in cell types with only primary cell walls, whereas at least three genes (*AtCESA4*, *7* and *8*) encode proteins that synthesize cellulose in cell types with secondary walls such as xylem tracheary elements (Doblin et al., 2002; Williamson et al., 2002; Gardiner et al., 2003; Taylor et al., 2003). Moreover, pull-down assays indicate that the proteins encoded by *AtCESA4*, *7* and *8* all interact, suggesting that they may be in the same rosette (Taylor et al., 2003). Expression analyses of *CESA* genes in other angiosperm species also associate specific genes with the synthesis of cellulose in cells that are either depositing a primary or a secondary cell wall (Doblin et al., 2002). For example, in situ hybridization analysis showed that *PtrCESA6* and *PtrCESA7* were expressed in all expanding cells depositing primary cell walls (Samuga and Joshi, 2004), whereas *PtrCESA1* was expressed in xylem tracheary elements during secondary cell wall deposition in *P. tremuloides* (Wu et al., 2000). Mutant and expression analyses have also implicated three rice (*Oryza sativa*) *CESA* genes, *OsCESA4*, *7* and *9*, in the synthesis of cellulose in cells with lignified secondary walls (Tanaka et al., 2003).

Despite the importance of coniferous gymnosperms as sources of timber and pulp, as far as we are aware, no expression studies of their *CESA* genes have been reported. In the present study, we report the isolation, characterization and expression of eight *CESA* genes from the coniferous gymnosperm *Pinus radiata*. Our in situ hybridisations show that *PrCESA10*, encoded by a full length cDNA clone, is expressed in recently developed tracheids, which are laying down secondary cell walls.

2. Materials and methods

2.1. Plant material

Buds and developing xylem from *P. radiata* D. Don were collected in August 2000 from 4-year-old trees grown in Woodhill Forest, South Kaipara Peninsula, New Zealand, and immediately frozen in liquid nitrogen and stored at − 80 °C. The developing xylem was collected by peeling off the bark from the trunk and gently scraping off the xylem with a knife. Seedlings and young trees were also grown in a glasshouse under ambient conditions and in a growth cabinet maintained at 22 °C with 16 h light and 8 h dark periods.

2.2. Construction and screening of the P. radiata cDNA library

Total RNA was extracted from buds of 4-year-old trees of *P. radiata* (Chang et al., 1993). Poly (A+) RNA was isolated from total RNA (2.5 mg) using an mRNA purification kit (Amersham Pharmacia Biotech, Amersham, UK) following the manufacturer's instructions. From this poly (A+) RNA (5 μg), a cDNA library was constructed using the ZAP-cDNA Gigapack III Gold Cloning Kit (Stratagene, La Jolla, CA, USA). The cDNA library, which consisted of approximately 10^6 independent clones, was amplified once to obtain a high-titer stock.

The cDNA library was screened twice to isolate full-length genes. The first screening was performed using a probe (196 bp) amplified by PCR from total RNA of developing xylem from *P. radiata*. The primers used for PCR were designed to amplify the highly conserved region from U3 to U4 (Fig. 1). A multiple alignment was made using the three available *Pinus taeda* ESTs (GenBank accession nos. AA556522, AA556640, AA556746) downloaded from the University of Minnesota website (http://