

Identification and Expression Analysis of a Cellulose Synthase gene in *Physcomitrella patens*

Hua Zhang and R. Malcolm Brown, Jr.

The University of Texas at Austin, Molecular Genetics and Microbiology,

1 University Station A5000, Austin, TX 78712

e-mail: zhanghua72@mail.utexas.edu phone: +1 512 471-3364 fax: +1 512 471 3573

Cellulose is found in plants, algae, slime molds, bacteria and animals. Cellulose synthase (CesA) genes have been identified in more than 250 species. CesA proteins are integral membrane proteins containing D, D, D, and QXXRW motifs and highly conserved domains. Cellulose synthase-like proteins show similarity to cellulose synthase and a few CSL proteins are shown to be involved in the synthesis of non-cellulosic cell wall polysaccharides. Together they belong to a superfamily. In *Arabidopsis*, there are 10 CesA genes and 6 families of CSL genes. Although many cellulose synthase mutants have been identified in vascular plants, many facts remain unknown about cellulose biosynthesis.

The moss *Physcomitrella patens* has a relatively short life cycle dominated by the haploid gametophyte and it has a high rate of homologous recombination. These attributes make *Physcomitrella patens* an ideal system for genetics analysis. There are a number of fundamental questions about cellulose biosynthesis that possibly can be answered using *Physcomitrella* as an experimental system. For example, what are the differences, if any, between the cellulose produced in the various life cycle stages of this organism? What genes function in cellulose biosynthesis among the various life cycle stages?

A full-length cDNA of CesA gene was identified. It was named CesA6 and was deposited in the GenBank (Accession number: DQ160224). CesA6 is 4055 bp long with a coding region of 3288 bp. The 5'-UTR contains 160 bp and the 3'-UTR contains 607 bp. Its predicted amino acid sequence shares a maximum identity of 73% with vascular plant CesAs (with the gymnosperm *Pinus radiata*) and 68% with *Arabidopsis*. The genomic DNA of CesA6 has 13 exons and 12 introns. Its first intron is more than 1kb long. All other introns are small.

A C-terminal GFP fusion construct of CesA6 was made. Our primary data showed that GFP fluorescence was detected in two locations in the gametophore. One location was the mucilage hair cells. The other location was probably the rhizoids initial cells. We need further histological studies to verify these locations.

There are over 40 members in the *Arabidopsis* CesA superfamily and at least 15 matches in *Physcomitrella*. AtCSLD3 (cellulose synthase-like gene subfamily D) is important for root hair growth. Molecular genetic studies in *Arabidopsis* have shown that many genes regulate the patterning of both trichomes and root hair cells. Our results show that CesA6 is expressed both in hair cells and rhizoid cells and that CesA6 expression is tissue-specific.

Currently, we are carrying out a gene knockout study of CesA6 to determine the role of CesA6 in cell wall formation.

