

Preliminary

## Cloning of Cotton Homologs of *bcsA* Gene Encoding Cellulose $\beta$ -1, 4-Glucosyltransferase\*<sup>1</sup>

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Cellulose is the most abundant biopolymer on the earth and synthesized in plants and some algae, fungi and bacteria. Even though so many efforts have been done, the mechanism of cellulose biosynthesis in higher plants remains obscure. Bacterial cellulose synthase operon, already isolated from *Acetobacter xylinum*<sup>1)</sup> and *Agrobacterium tumefaciens*<sup>2)</sup>, forms a complex from four subunits. One of the subunits, *bcsA* gene product, is believed to be a  $\beta$ -1, 4-glucosyltransferase which has a binding site for UDP-glucose, precursor of cellulose biosynthesis<sup>3)</sup>.

An cDNA library was constructed in the plasmid vector pBluescript with mRNA obtained from cotton (*Gossypium hirsutum*) fiber cells harvested at 18 to 21 days post-anthesis<sup>4,5)</sup>. In cotton fiber cells, a high rate of cellulose synthesis starts at the onset of secondary wall synthesis<sup>6)</sup>. Random sequencing of 1,000 clones from the cDNA library identified fifteen clones that show sequence similarity with *bcsA*. These clones are classified into three types, *pcsA2*, *pcsA3* and *pcsA4*, respectively and are partially homologous each other (60 to 70%) at nucleotide level. This corresponds to the previous report, in which Southern blot analysis of cotton genomic DNA shows that the cotton *pcsA* genes constitute a small gene family, consisting of 3 to 4 genes<sup>7)</sup>. Since cotton fiber cells are tetraploid, it is possible to consider that these clones are due to allele.

The full length cDNA of *pcsA2* has been obtained by the 5' RACE method<sup>8)</sup> and the PCR technique. The cotton *pcsA2* which appears to be a full length clone of 3,311 bp contains an open reading frame of 3,120 bp that encodes a polypeptide of 1,039 amino acids with calculated molecular mass of about 125 kDa.

The cellulose  $\beta$ -1, 4-glucosyltransferase of *Acetobacter xylinum* shows 42.8% identity at the nucleotide level and 26.6% identity at the whole deduced amino acid level to *pcsA2* gene product. The cotton *celA1* gene product shows 53.9% identity at the nucleotide level and

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68.7% identity at the whole deduced amino acid level to the *pcsA2* gene product.

The hydrophathy profiles suggest at least two transmembrane helices, one is located in the N-terminal region and the other is in the C-terminal region. The hydrophilic regions have the conserved UDP-glucose binding motif which is required for the binding of UDP-glucose to catalyze the transfer of glucose into  $\beta$ -1, 4-glucan. Three conserved Asp residues and a conserved sequence motif (QXXRW), where X represents any amino acid, were identified from the comparison of a number of amino acid sequences of  $\beta$ -glycosyltransferases including cellulose synthase<sup>9</sup>. These conserved residues were suggested as essential for enzyme activity from the results of site-directed mutagenesis<sup>10,11</sup>. The cotton *pcsA2* gene product contains all these residues at the four regions, suggesting that *pcsA2* is a  $\beta$ -1, 4-glucosyltransferase.

The *pcsA2* gene product also contains Cys-rich sequences in the N-terminal region as well as *celA1* gene product<sup>12</sup>. These sequences are similar to the N-terminal LIM-like domain of soybean transcription factors (STF)<sup>13</sup>. Many kinds of proteins containing a LIM domain have been found in a wide range of species, among which the sequences of LIM domains are a little differed<sup>14,15</sup>. The LIM-like domains found in *pcsA2*, *pcsA1* and STF are characteristic in Cys residues for all putative metal-binding residues and in a space between two zinc fingers. Since the LIM domain may be involved in protein-protein interactions<sup>16,17</sup>, *pcsA2* gene product is potentially associated with some proteins such as the other components of cellulose synthase complex or microtubule-associated proteins (MAPs). In fact, the cellulose microfibrils in the walls are arranged along the microtubules. It should be noted that the length between the LIM-like domain and transmembrane site was calculated as 10 nm. Therefore, cellulose synthase *pcsA2* may reach the cortical microtubules because most cortical microtubules exist about 10 nm away from the inner membrane surface.

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