Preliminary

Changes in Levels of mRNAs for Cell Wall-related Enzymes in Growing Cotton Cells

Yoshinori SHIMIZU*[1], Yuri IHARA*[1], Rumi TOMINAGA*[1], Fukumi SAKAI*[1] and Takahisa HAYASHI*[1]

(Received May 31, 1998)

Keywords: cotton fiber, *pcsA2*, cellulase, expansin, endoxyloglucan transferase, sucrose synthase

cDNAs for cell wall-related enzymes, endo-1, 4-β-glucanase, expansin, endoxyloglucan transferase, endo-1, 3-β-glucanase, sucrose synthase and cellulose synthase (CelA1 and PcsA2) have been isolated from the cDNA library of cotton fiber cells[1]. These enzymes may have a function in cell wall loosening during cell elongation and as well as the massive deposition of cellulose at the secondary wall synthesis. The endo-1, 4-β-glucanase activity hydrolyzes polysaccharides possessing a 1, 4-β-glucan backbone and may be responsible for solubilization of xyloglucan in the plant cell wall[2]. Expansins have been identified as wall-loosening proteins that can promote long-term extension of cell walls[3]. The endoxyloglucan transferase activity may modify xyloglucan cross-linkings between cellulose microfibrils by internal cleavage and linkage of the newly generated ends to other xyloglucan polymer ends[4]. A sucrose synthase may exist in a complex with cellulose synthases and serve for channel carbon from sucrose via UDP-glucose to cellulose and/or callose[5]. Cellulose synthase catalyzes the polymerization of glucose residues to form cellulose microfibrils.

The levels of mRNAs for cell wall-relating enzymes (endo-1, 4-β-glucanase, endo-1, 3-β-glucanase, endoxyloglucan transferase, sucrose synthase, expansin and cellulose synthase *pcsA1*[6] and *pcsA2*[7]) were determined in both fibers and seedlings of cotton by reverse transcription-PCR analysis. The amounts of first strand cDNA in the PCR reaction mixture were corrected by the levels of amplified DNA for actin because the levels of actin mRNA are constant during cell growth. The levels were estimated from the linear portion of the amplifying curve. Each PCR reaction contained a certain amounts of first strand cDNA equivalent to 16 to 75 ng of total RNA, derived from 2 to 75 fiber cells. This suggests that the reverse transcription-PCR analysis of mRNA is a highly sensitive assay for small amounts of differentiated cells (tissues) as well as low copy number RNA species[8].

*[1] Laboratory of Gene Expression.

Signals for endo-1, 4-β-glucanase clearly appeared at 27 cycles of DNA amplification at the stage of cell elongation (9 to 15 days post-anthesis) in fibers and at 32 cycles of DNA amplification at the elongating regions in seedlings. Signals for expansin were strong even at 30 cycles during the primary wall synthesis (9 to 15 days post-anthesis) in fibers and appeared at 32 cycles of DNA amplification at the elongated regions in seedlings. Signals for endoxyloglucan transferase appeared to be just visualized at 15 cycles and showed constant intensities during both the primary and secondary wall syntheses in fibers. However, those were high at the elongating regions and decreased in the elongated regions in seedlings. These findings suggest that the levels of mRNAs for endo-1, 4-β-glucanase were always high during cell elongation in both fibers and seedlings and decreased gradually when cell elongation ceased. The level of endoxyloglucan transferase mRNA was decreased during seedling elongation. Expression pattern for expansin showed an opposite tendency between fibers and seedlings. The phenomenon is correlated with earlier observation[9] that the level of rice-*EXP* 2 mRNA is much lower in the elongating region of internode than that in the elongated region. The data suggest that the cotton expansin plays different roles between fibers and seedling. Signals for endo-1, 3-β-glucanase appeared strong at 15 cycles at the secondary wall synthesis in fibers and also in the elongated regions in seedlings, accompanying the massive deposition of cellulose. Signals for sucrose synthase appeared at 30 cycles at all stages of cell growth in fibers. Since the level was not increased during the secondary wall synthesis, the sucrose synthase may not be a membrane-bound form which may be associated with cellulose synthase. Signals for sucrose synthase also appeared at 40 cycles at the portion of the elongated regions in seedlings. The generation of such an assimilate concentration gradient is probably controlled by the rate of sucrose utilization in sink tissues. Signals for *pcsA1* and *pcsA2* appeared at 20 and 22 cycles in fibers during the secondary wall synthesis and at 25 and 22 cycles at the portion of the elongated regions in seedlings. This suggests that cellulose synthase genes are highly expressed not only in developing fibers, but also in elongated seedlings. There are slightly different levels of these mRNA in fibers and seedlings, i.e., the level for *pcsA1* is higher than that for *pcsA2* in fibers, whereas the level for *pcsA2* is higher than that for *pcsA1* in seedlings.

In conclusion, Endo-1, 4-β-glucanase mRNA accumulated specifically in the elongating cells, and much lower abundance in the elongated cells and changed at relatively low copy numbers during cell growth. It is uncertain whether the level for endo-1, 4-β-glucanase is relatively low or its mRNA is unstable in fibers and seedlings during growth. Levels of expansin mRNA showed opposite tendency between fibers and seedlings. Expansin may have developmental functions in addition to promoting cell elongation. Endoxyloglucan transferase mRNA level was constant at all stages of growth in both fibers and seedlings. This suggests that the gene for endoxyloglucan transferase is always expressed to maintain

the cell wall reconstruction, which may be essential process in the cell growth and development. An increase in the amount of cellulose occurred synchronously with the expression for *pcsA1* and *pcsA2* encoding cellulose synthase gene in both fibers and seedling. Gene expression of *pcsA1* and *pcsA2* may be controlled directly by the timing of cellulose synthesis.

References

1) O. HASEGAWA, S. AOTSUKA, F. TADOKORO, S. TAKENISHI and H. UCHIMIYA: *In* kyoto Conference on Cellulose. Edited by Shiraishi, N. pp. 87. The Cellulose Society of Japan Kyoto (1994).
2) T. HAYASHI: *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **40**, 139–168 (1989).
3) S.J. MCQUEEN-MASON and D.J. COSGROVE: *Plant Physiol.*, **107**, 87–100 (1995).
4) K. NISHITANI and R. TOMINAGA: *J. Biol. Chem.*, **267**, 2158–2164 (1992).
5) Y. AMOR, C.H. HAIGLER, S. JOHNSON, M. WAINSCOTT and D.P. DELMER: *Proc. Natl. Sci. USA*, **92**, 9353–9357 (1995).
6) J.R. PEAR, Y. KAWAGOE, W.E. SCHRECKENGOST, D.P. DELMER and D.M. STAKER: *Proc. Natl. Sci. USA*, **93**, 12637–12642 (1996).
7) Y. IHARA, F. SAKAI and T. HAYASHI: *Wood Research*, **84**, 1–6 (1996).
8) J. CHELLY, J.C. KAPLAN, P. MAIRE, S. GAUTRON and A. KAHN: *Nature*, **333**, 858–860 (1998).
9) C. HYUNG-TAEG and K. HANS: *Plant Cell*, **9**, 1661–1671 (1997).