Plant Physiology, November 1999, Vol. 121, pp. 715–721, www.plantphysiol.org © 1999 American Society of Plant Physiologists

Expression of Endoxyloglucan Transferase Genes in *acaulis* Mutants of Arabidopsis[1]

Taku Akamatsu, Yoshie Hanzawa, Yuhko Ohtake, Taku Takahashi, Kazuhiko Nishitani, and Yoshibumi Komeda*

Division of Biological Sciences, Graduate School of Science, Hokkaido University, N10, W8, Sapporo 060–0810, Japan (T.A., Y.H., Y.O., T.T., Y.K.); and Department of Biology, Graduate School of Science, Tohoku University, Aoba, Sendai 980–8578, Japan (K.N.)

A mutant of Arabidopsis with reduced internodal cell length, *acaulis5* (*acl5*), has recently been shown to have reduced transcript levels of a gene for endoxyloglucan transferase, *EXGT-A1* (Y. Hanzawa, T. Takahashi, Y. Komeda [1997] Plant J 12: 863–874). In the present study, we cloned genomic fragments of five members of the *EXGT* gene family, *EXGT-A1*, *EXGT-A3*, *EXGT-A4*, *XTR2*, and *XTR3*, and examined their expression in the wild type and in a series of *acl* mutants. In wild-type plants, the *EXGT-A3* gene showed higher expression in lower internodes (internodes between nodes bearing axillary shoots) than in upper and young internodes, in which *EXGT-A1* was highly expressed. *EXGT-A4* was preferentially expressed in roots and *XTR3* in siliques. The *XTR2* gene was constitutively expressed. In *acl1*, *acl3*, and *acl4* mutants, which have a severe defect in leaf expansion as well as in internode elongation, the *EXGT-A1* gene showed reduced levels of expression before bolting of plants. In contrast, *XTR3* was increased in these mutant seedlings. Reduction of *EXGT-A1* expression was also detected after bolting of all *acl* mutants except *acl2*, whose growth defect is restricted to lower internodes. These results suggest the involvement of each EXGT in different aspects of organ development.

The growth of plant cells depends on the balance between the turgor pressure and the extensibility of the cell wall. While the turgor pressure, which provides the driving force for cell extension, is influenced by the availability of water, the wall extensibility is to a large extent regulated by enzymes involved in the cleavage or formation of cross-links between cell wall polymers and in the turnover of certain wall components. In dicots, xyloglucan is a major structural polysaccharide of primary cell walls and is hydrogen-bonded to cellulose microfibrils to form cross-links between them (for review, see Hayashi, 1989; Carpita and Gibeaut, 1993). The cleavage and molecular grafting of xyloglucan polymers are catalyzed by endoxyloglucan transferase (EXGT) enzymes (also called xyloglucan endo-transglycosylase; XET). Therefore, EXGT has been suggested as one of the most likely agents responsible for wall loosening (for review, see Fry, 1995; Nishitani, 1995, 1997).

Cloning of *EXGT* genes from several plant species has led us to realize that plants possess a large gene family of EXGTs. They have been classified into three subfamilies based on their sequence similarities (Nishitani, 1995, 1997; Xu et al., 1996). Subfamily I includes EXGT-V1 from azuki bean epicotyls, the first enzyme proved to mediate a transglycosylation reaction between xyloglucans (Nishitani and Tominaga, 1992). Subfamily II includes Arabidopsis meristem-expressed Meri5 (Medford et al., 1991) and mechanostimulus-inducible TCH4 (Xu et al., 1995), soybean brassinosteroid-inducible BRU1 (Zurek and Clouse, 1994), maize flooding-responsive WUSL1005 (Saab and Sachs, 1996), and tomato-fruit-expressed XET-B1 (Arrowsmith and de Silva, 1995). Germinating seed-specific NXG1 of nasturtium (de Silva et al., 1993) belongs to subfamily III and was originally identified as a hydrolase (xyloglucanase) (Edwards et al., 1986). Expression patterns of these genes are in good agreement with their proposed roles in cell wall modification during cell elongation (Nishitani, 1997), fruit ripening (Redgwell and Fry, 1993), vascular differentiation (Oh et al., 1998), and adaptive growth to physical stimuli (Antosiewicz et al., 1997). Dwarf phenotypes of the Arabidopsis brassinosteroid-responsive mutants have been shown to correlate with a reduced expression of *TCH4* (Kauschmann et al., 1996). It is still not known whether each member of the *EXGT* gene family within a single plant species plays a distinct and vital role in cell morphogenesis.

We have isolated and studied Arabidopsis mutants with reduced internodal cell length, *acaulis* (*acl*), to determine the molecular basis of cell elongation in stem internodes. In rosette plants, including Arabidopsis, initiation of the internode elongation (bolting) follows flower bud formation. This process is probably mediated by phytohormones, but how their effects are exerted is not clear. Our previous study revealed that the *acl5* mutant, whose defect is sharply restricted to internodal growth, shows a reduced expression of the *EXGT-A1* gene after flowering (Hanzawa et al., 1997). We report the cloning of genomic fragments of five members of the *EXGT* gene family and their expression patterns in the wild type and in a series of *acl* mutants, to which *acl3* and *acl4* have recently been added.

[1] This work was partially supported by a Grant-In-Aid from the Ministry of Education, Science and Culture of Japan and by a grant for the Research for the Future Program from the Japan Society for the Promotion of Science (JSPSRFTF96L00403).

* Corresponding author; e-mail ykomeda@bio.sci.hokudai.ac.jp; fax 81–11–706–2739.

MATERIALS AND METHODS

Plant Material

Arabidopsis ecotype Columbia was used in all experiments. Plants were grown on rock-wool bricks watered with Murashige and Skoog solution under continuous fluorescent light at 22°C. For RNA preparation from root tissue, seeds were surface-sterilized and sown on solidified Murashige and Skoog medium with 3% (w/v) Suc in Petri dishes. Petri dishes were kept under continuous fluorescent light at 22°C.

The *acl3-1* and *acl4-1* mutants were selected in a screen for mutants with short internodes from M2 plants derived from fast-neutron-mutagenized seeds homozygous for *gl1* (Lehle Seeds, Tucson, AZ). These were backcrossed five times into the wild-type Columbia (Col-0). Mapping was performed using molecular markers polymorphic between Columbia and Landsberg *erecta* (Konieczny and Ausubel, 1993; Bell and Ecker, 1994). Mutant alleles of *ACL1* and *ACL2* used in this study were *acl1-2* and *acl2-1*, respectively (Tsukaya et al., 1993). The *acl5-1* allele in the Landsberg *erecta* background (Hanzawa et al., 1997) was backcrossed at least five times into the Columbia background.

Isolation of Genomic Clones Encoding EXGT

Four cDNA clones with homology to *EXGT-A1* (Okazawa et al., 1993) were previously isolated from an Arabidopsis cDNA library by screening at low stringency with the *EXGT-A1* cDNA fragment, and were designated *EXGT-A2*, *EXGT-A3*, *EXGT-A4*, and *EXGT-A5* (Nishitani, 1997; S. Okamoto and K. Nishitani, unpublished data). The nucleotide sequences of *EXGT-A2* and *EXGT-A5* were found to be identical to those isolated and named *XTR2* and *XTR3*, respectively, by Xu et al. (1996). An Arabidopsis genomic library constructed in λGEM12 was generously provided by J. Mulligan and R.W. Davis (Stanford University, Stanford, CA). The library was screened by plaque hybridization using a mixture of cDNA fragments as the probes. Subclones were prepared in pBluescript SK+ (Stratagene, La Jolla, CA) and sequenced using a *Taq* dye terminator cycle sequencing kit and a DNA sequencer (model 373A, Applied Biosystems, Foster City, CA).

RNA Gel-Blot Analysis

Total RNA was isolated from different tissues as described by Takahashi et al. (1992), separated by agarose/formaldehyde gel electrophoresis, and blotted onto nylon membranes (GeneScreen, New England Nuclear, Boston). Hybridization was performed at 42°C in 50% (w/v) formamide, 10% (w/v) dextran sulfate, 1% (w/v) SDS, 1 M NaCl, 0.25 mg mL^{-1} salmon-sperm DNA, and the labeled gene-specific probe (see below). The filters were washed twice for 15 min at 65°C in 2× SSC, 1% (w/v) SDS and once at room temperature in 0.1× SSC. For all blots, equal loading was confirmed by ethidium bromide staining of ribosomal RNAs (25S, 18S).

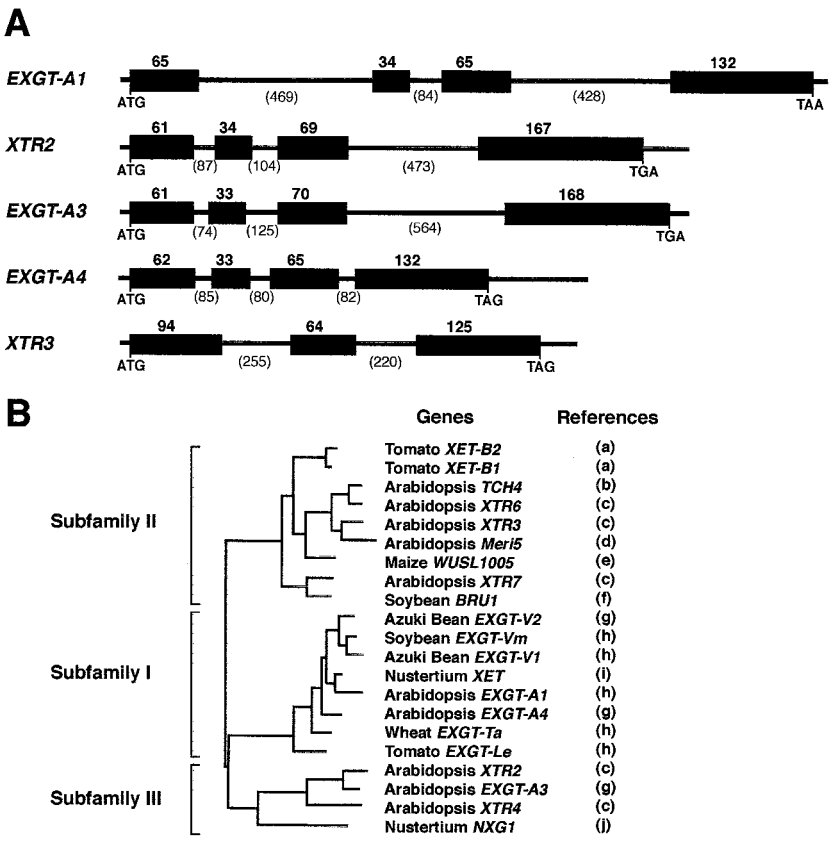

Figure 1. Comparison of *EXGT* genes. A, Genomic structure of the *EXGT* genes cloned in this study. Protein coding regions are shown by black boxes with the number of amino acid residues encoded by each exon. Numbers in parentheses indicate the number of nucleotides for intron. Intron splice sites in genomic sequences were deduced by comparison with their corresponding cDNA sequences, *EXGT-A1* (Okazawa et al., 1993; accession no. D16454), *XTR2* (Xu et al., 1996; accession no. U43487), *EXGT-A3* (Nishitani, 1997; accession no. D63509), *EXGT-A4* (Nishitani, 1997; accession no. AB026486), and *XTR3* (Xu et al., 1996; accession no. U43485). The accession numbers for genomic sequences determined in this study are AF163819 (*EXGT-A1*), AF163820 (*XTR2*), AF163821 (*EXGT-A3*), AF163822 (*EXGT-A4*), and AF163823 (*XTR3*), respectively. B, Phylogenetic relationship between the Arabidopsis and other EXGT-related protein sequences. The entire deduced amino acid sequences were compared using the malign program of DNA Data Bank of Japan (Nishitani, 1997). References: a, Arrowsmith and de Silva (1995); b, Xu et al. (1995); c, Xu et al. (1996); d, Medford et al. (1991); e, Saab and Sachs (1995); f, Zurek and Clouse (1994); g, Nishitani (1997); h, Okazawa et al. (1993); i, Rose et al. (1996); and j, de Silva et al. (1993).

Probe Preparation

To specifically detect each of the *EXGT* transcripts in the RNA gel-blot hybridization, 3′-end-specific probes were synthesized by PCR using cDNA clones as templates. The PCR primers were A1F (5′-GGCGGTTTAGAGAAGAC CAA-3′), A1R (5′-GTAACTTATGCGTCTCTGTC-3′), A2F (5′-AAGCGTCTCAGGGTCTATGA-3′), A2R (5′-GTTCAT-AAAATGGAGGAAATC-3′), A3F (5′-CAGTTTCCGAGGT-GCG ATGA-3′), A3R (5′-GGCCAAATCTCACCCATACT 3′), A4F (5′-TTGCACTGA CCGCGTCCG-3′), A4R (5′-CCA AACTTTTCTAGATTAAATTG-3′), A5F (5′-TAGCTAC-GA GAATTAATGTG-3′), and A5R (5′-AACCAACATAA-CT-CACGCCC-3′). The specificity of each probe was confirmed by DNA gel-blot analysis. No cross-hybridization was observed (data not shown). The PCR products were agarose gel purified and labeled by the random-primer protocol (BcaBest Labeling Kit, Takara, Kyoto).

RESULTS

Genomic Structure of *EXGT* Genes

Five *EXGT* cDNA clones (Okazawa et al., 1993; Nishitani, 1997; S. Okamoto and K. Nishitani, unpublished data) were used as probes to screen an Arabidopsis genomic library in λGEM12. Sequence analysis of subcloned genomic DNA fragments revealed the presence of two or three introns whose placement within each of the *EXGT* coding regions is conserved (Fig. 1A). The phylogenetic tree for these genes and those identified from other plant species is shown in Figure 1B. Genomic DNA-blot analysis indicated that 3′-end-specific probes prepared from these *EXGT* genes (see "Materials and Methods") hybridized to a single-copy gene at high-stringency conditions (data not shown).

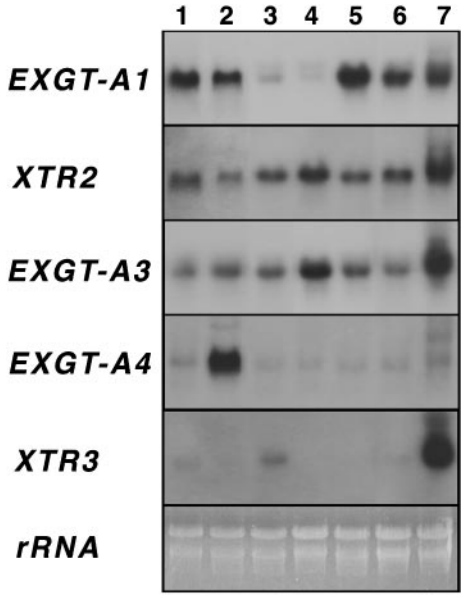

Figure 2. Analysis of the expression of *EXGT* genes in different organs. Total RNA (10 μg per lane) was prepared from 7-d-old seedlings (lane 1), roots (lane 2), rosette leaves (lane 3), internodes between nodes bearing axillary shoots (lane 4), internodes between nodes bearing flowers (lane 5), flower buds (lane 6), and siliques (lane 7).

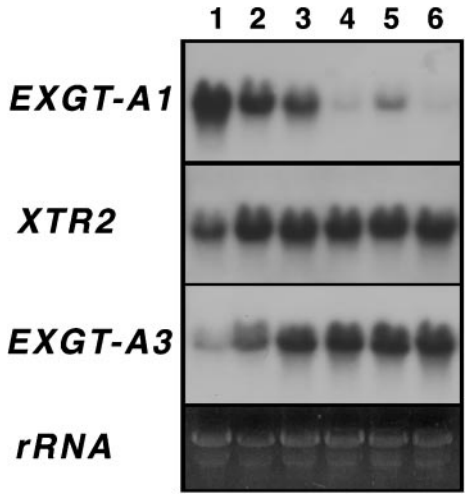

Figure 3. Analysis of the expression of *EXGT* genes during internode elongation. Total RNA (10 μg per lane) was prepared from internodes between nodes bearing flowers (lanes 1, 3, and 5) and internodes between nodes bearing axillary shoots (lanes 2, 4, and 6). Tissues were harvested at 5 d (lanes 1 and 2), 10 d (lanes 3 and 4), and 15 d (lanes 5 and 6) after bolting.

Developmental Regulation of *EXGT* Gene Expression

Steady-state levels of *EXGT* transcripts were measured in different organs of adult flowering plants and in young seedlings before bolting. The results of RNA-blot hybridization using 3′-end-specific probes are shown in Figure 2. The *EXGT-A1* gene was highly expressed in 7-d-old seedlings and in the roots, upper internodes (internodes between nodes bearing flowers), flower buds, and green siliques of 30-d-old flowering plants. Transcript levels in fully expanded leaves and lower internodes (internodes between nodes bearing axillary shoots) were reduced, indicating the preferential expression of the *EXGT-A1* gene in young, developing tissues. On the other hand, *XTR2* showed a constitutive expression. *EXGT-A3* showed a pattern similar to that of *XTR2*, but was higher in lower internodes. The *EXGT-A4* gene was mainly expressed in roots. *XTR3* was restricted to siliques and only weakly expressed in mature leaves. We further examined the expression of *EXGT-A1* and *EXGT-A3* genes during the internode elongation. RNA samples were prepared from upper and lower internodes at 5, 10, and 15 d after bolting, respectively. Our results revealed that, while the *EXGT-A3* expression was increased as the day proceeded, the *EXGT-A1* expression, especially in lower internodes, was drastically decreased (Fig. 3).

Identification of New *acl* Loci

The *acl* mutants have been characterized by a defect in elongation growth of stem internodes after flowering, from which the name "acaulis" originates. In addition to the

previously described mutants *acl1*, *acl2*, and *acl5*, two mutants derived from fast-neutron-mutagenized plants were found to represent new recessive loci by complementation tests and defined as *acl3* and *acl4*, respectively (Fig. 4). Mapping experiments revealed that *acl3* is tightly linked to the marker GL1 (Konieczny and Ausubel, 1993) on chromosome III and that *acl4* is tightly linked to the marker SC5 on the lower arm of chromosome IV (data not shown). These two mutants have a severe defect in rosette leaf expansion before flowering and are phenotypically indistinguishable from the allele of *acl1-2* (Fig. 5A).

We found that, like the phenotype of *acl1* (Tsukaya et al., 1993), the phenotype of *acl3* and *acl4* could not be rescued by the exogenous addition of phytohormones, but was drastically supressed by elevated growth temperature (28°C; Fig. 5B). On the other hand, *acl2* and *acl5* mutants were nearly wild-type in appearance before bolting and their defect was only detected in the growth of stem internodes (Fig. 4). In contrast to *acl1*, *acl3*, and *acl4* mutants, whose internodal growth was markedly restored at 28°C, *acl2* and *acl5* mutants showed no restoration of the internodal growth at 28°C. The reduction in leaf expansion and/or stem elongation in all of these *acl* mutants is primarily due to the reduction in cell size (Tsukaya et al., 1993; Hanzawa et al., 1997; data not shown).

EXGT Gene Expression in *acl* Mutants

The effect of *acl* mutations on the expression of *EXGT* genes was examined by RNA blots. Figure 6A shows that the *EXGT-A1* expression was reduced in aerial portions of 7-d-old seedlings of *acl1*, *acl3*, and *acl4* mutants with the leaf phenotype. Interestingly, these three mutant seedlings exhibited elevated levels of the *XTR3* transcript. Reduced expression of *EXGT-A1* was also observed in *acl5* mutants after flowering, as well as in *acl1*, *acl3*, and *acl4* flowering plants (Fig. 6B). The transcript levels of *XTR3* in 30-d-old flowering plants, which seems mainly attributable to the

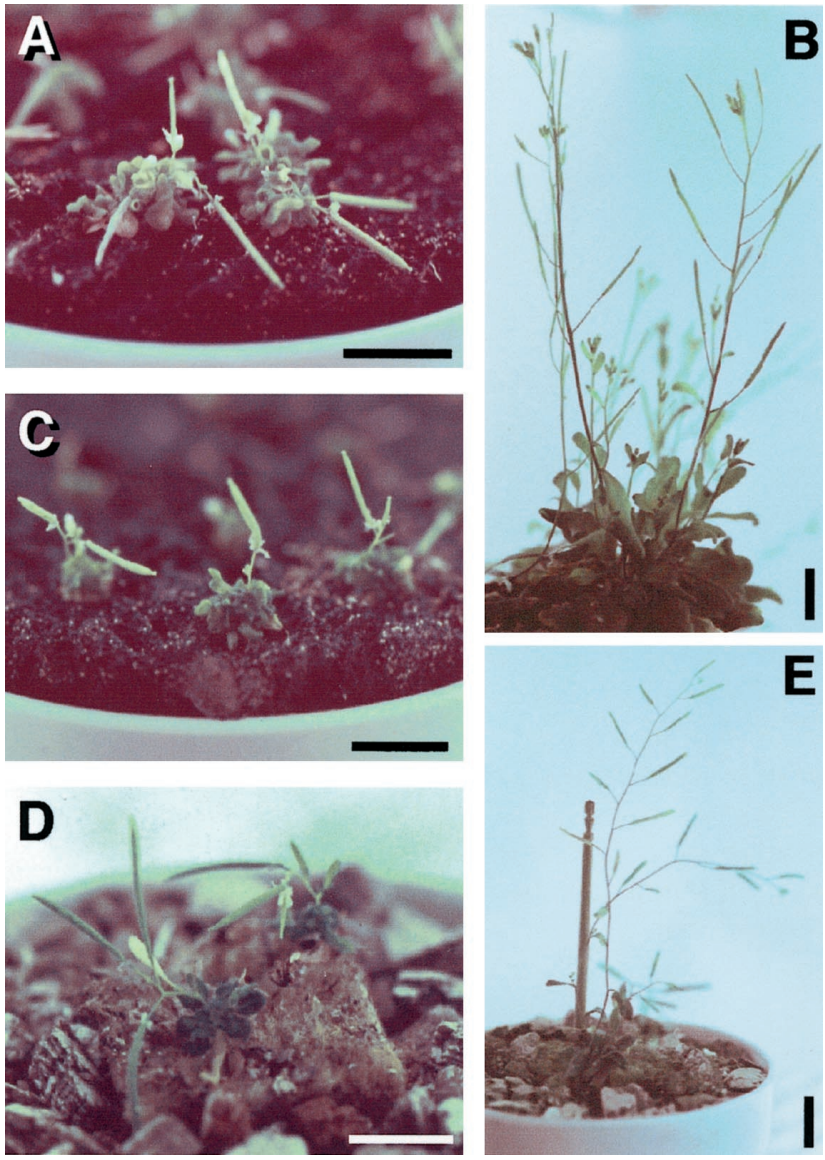

Figure 4. Morphology of adult flowering plants with *acl* mutations. Plants were grown at 22°C under continuous light for 40 d. A, *acl1-2*; B, *acl2-1*; C, *acl3-1*; D, *acl4-1*; E, *acl5-1*. Scale bars = 1 cm.

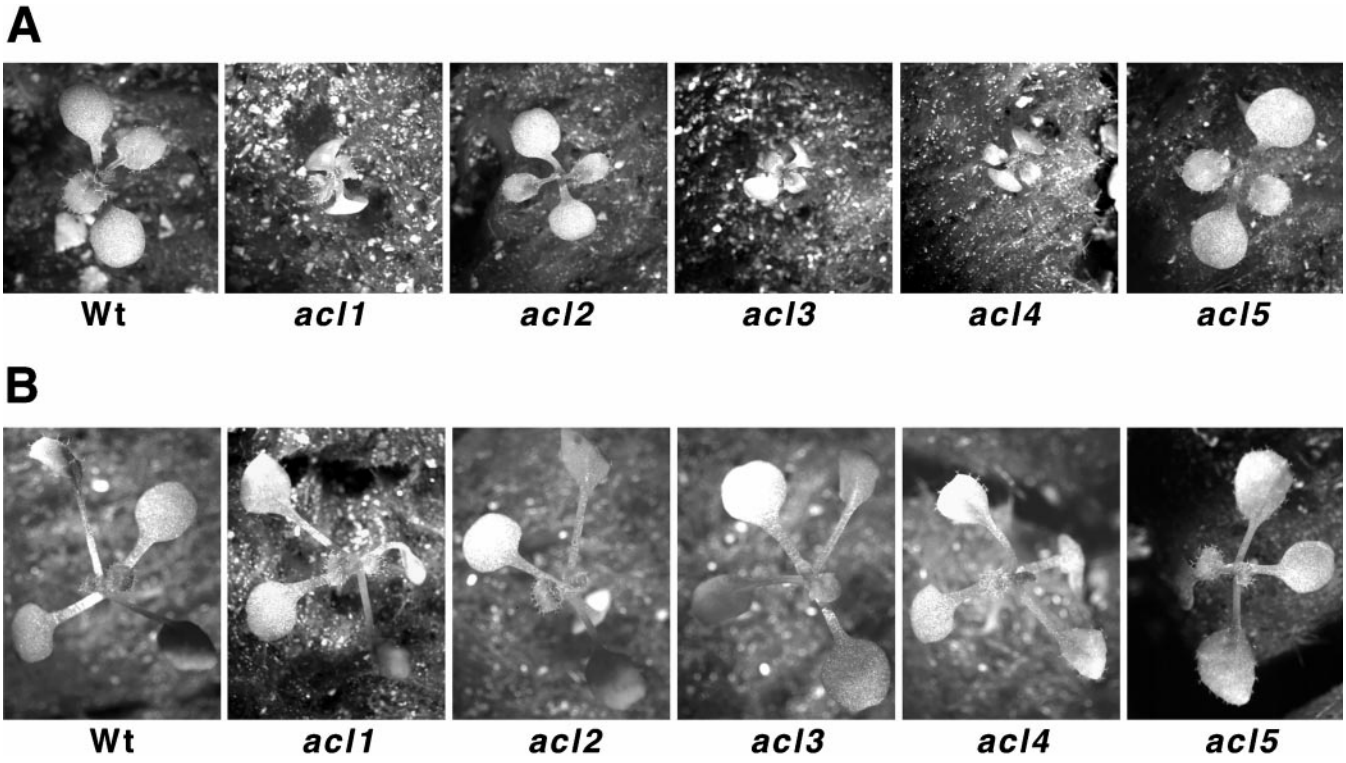

Figure 5. Morphology of 10-d-old wild-type and *acl* seedlings. Plants were grown under continuous light at 22°C (A) or 28°C (B).

expression in siliques (Fig. 2), and those in rosette leaves of flowering plants were unaffected by these *acl* mutations (Fig. 6B; data not shown). There were no obvious influences of *acl* mutations on the transcript levels of *XTR2* and *EXGT-A3* in aerial tissues (Fig. 6, A and B) or those of *EXGT-A1* and *EXGT-A4* in roots (Fig. 6C).

We further found that the transcript levels of *EXGT-A1* in *acl1*, *acl3*, and *acl4* seedlings grown at 22°C were restored by the growth at 28°C, in parallel with their morphological phenotypes (Fig. 6D). An elevated level of *EXGT-A1* expression was also seen in wild-type seedlings grown at 28°C, in which leaf expansion and petiole elongation were also enhanced (Fig. 5).

DISCUSSION

One of our major interests was to identify actual molecules involved in the rapid cell growth of stem internodes in Arabidopsis. Previously, we observed that the *acaulis5* (*acl5*) mutant showed a marked reduction of the *EXGT-A1* gene expression after flowering, as well as a severely reduced length of stem internodes (Hanzawa et al., 1997). To evaluate the relationship between the expression of *EXGT* genes and plant cell growth, we extended our analysis to the expression of other members of the *EXGT* gene family in the wild type and in a series of *acl* mutants.

This study revealed that the members of the *EXGT* gene family are under the differential control of expression during development of wild-type plants. Expression of *EXGT-A3* appeared to be high in lower (old) internodes, in contrast to that of *EXGT-A1* in upper (young) internodes. According to the phylogenic tree established from related protein sequences (Fig. 1B; Nishitani, 1997), EXGT-A1 and root-expressed EXGT-A4 belong to subfamily I, while XTR2 and EXGT-A3 belong to subfamily III. In nasturtium, NXG1 (subfamily III) and XET1 (subfamily I) exhibit mutually exclusive patterns of gene expression and possess different substrate specificities (Rose et al., 1996). NXG1 has been suggested to act predominantly as a hydrolytic enzyme in the mobilization of xyloglucan seed storage reserves in germinating seed cotyledons (Edwards et al., 1986). If hydrolytic action toward xyloglucans is a major role of members of subfamily III, then EXGT-A3, together with XTR2, could be required for the regulated degradation of xyloglucan networks for the maturation and/or maintenance of the fine structure of cell walls, which follows the elongation growth. It will be necessary to determine whether these EXGTs possess different enzyme activities against different xyloglucan substrates and whether they exhibit cell-type-specific patterns of expression.

The significance of EXGT-A1 in cell elongation was strengthened by our analysis of the expression in *acl* mutants. Two loci, *acl3* and *acl4*, were newly identified in this study. The phenotypes of these two mutants could not be restored by exogenously applied phytohormones (data not shown), suggesting that neither of these mutations represent genes involved in hormone biosynthesis. Based on their phenotypes, which are almost identical to the *acl1* phenotype, we suggest that these three gene products act in a common regulatory pathway of cell elongation.

Our results showed that the defects of *acl1*, *acl3*, and *acl4* in leaf expansion and in stem elongation are accompanied

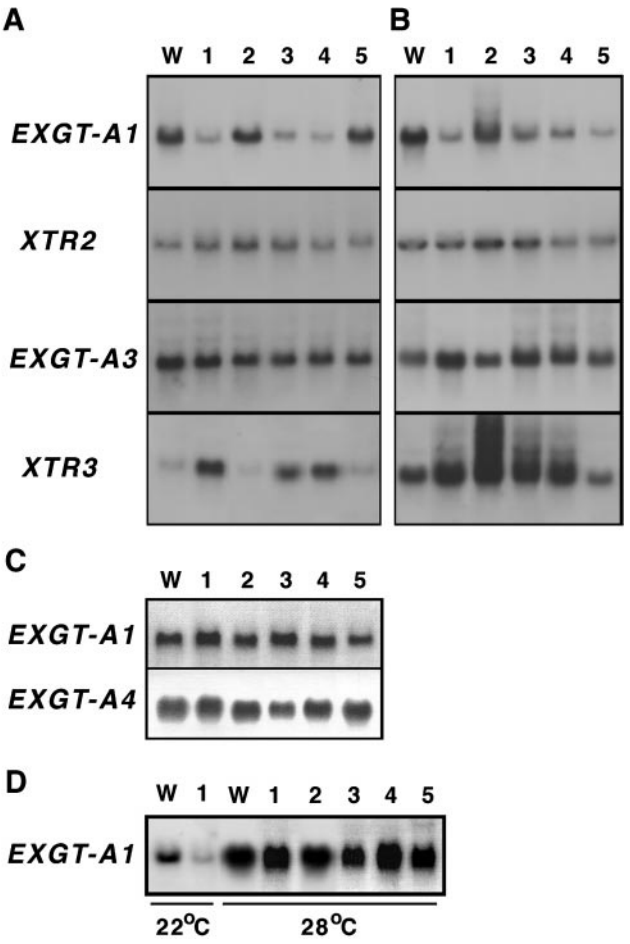

Figure 6. Analysis of the expression of *EXGT* genes in *acl* mutants. Total RNA (10 μg per lane) was prepared from aerial tissues of 7-d-old seedlings (A and D) and 30-d-old flowering plants (B) and from root tissues of 7-d-old seedlings (C). Plants were grown at 22°C (A–C) or at the indicated temperature (D). Lanes W, Wild type; lanes 1, *acl1-2*; lanes 2, *acl2-1*; lanes 3, *acl3-1*; lanes 4, *acl4-1*; lanes 5, *acl5-1*.

by the reduced expression of the *EXGT-A1* gene. When grown at 28°C, these mutants restore both the phenotype and the transcript level of *EXGT-A1*. The high temperature also enhances both petiole elongation and *EXGT-A1* expression in wild-type seedlings. Xu et al. (1996) have reported that the *EXGT-A1* gene (referred to as *EXT*) is up-regulated in response to touch, auxin, and darkness, all of which can facilitate elongation growth. It is possible that EXGT-A1 functions in the process of cell elongation in young leaves and stem internodes. Further genetic approaches, including the isolation of knockout mutants of this gene and the creation of transgenic plants with altered levels of expression are required to define the exact role. Moreover, it remains to be clarified whether the reduction in cell length, which can be caused by mutations in a vast variety of genes, is generally associated with reduced expression of *EXGT-A1*. The possibility cannot be ruled out that *EXGT-A1* expression is changed as a consequence of altered cell morphology.

There were no detectable alterations in *EXGT-A1* expression in semidominant *acl2* mutants. This can be explained by the limited defect of *acl2* within the internode elongation between nodes bearing axillary shoots (Tsukaya et al., 1995), which might be accompanied by a temporal and slight reduction in *EXGT-A1* expression. However, it is also likely that the *acl2* mutation has a negative effect on other molecules involved in cell elongation, while having no influence on EXGT-A1.

Preferential expression of the *XTR3* gene in wild-type siliques is consistent with the fact that the corresponding cDNAs have been identified as expressed-sequence-tag clones derived from dry seeds by Xu et al. (1996). XTR3, as well as stress-responsive Meri5 and TCH4 (Xu et al., 1996), belongs to subfamily II. We found that, in contrast to *EXGT-A1*, the *XTR3* transcript levels were elevated in *acl1*, *acl3*, and *acl4* seedlings. Such opposite effects on *EXGT* genes may reflect the complexity of environmental and hormonal regulation of the *EXGT* gene expression (Xu et al., 1996). Cloning of the *ACL* genes is currently in progress and will help to answer the question of how *acl* mutations affect regulatory pathways of *EXGT* gene expression.

In summary, our data on the expression of *EXGT* genes (especially on their responsiveness to environmental stimuli), which are supported by data reported by others, support the possibility that many kinds of mutations can affect the regulatory pathways of *EXGT* gene expression, resulting in altered cell morphology. The molecular processes underlying the cell wall architecture consist of various biochemical steps, indicating the involvement of many enzymes other than EXGT. It should be noted that there is increasing evidence suggesting the importance of expansins (Cosgrove, 1998) and endo-1,4-β-glucanases (Shani et al., 1997; Nicol et al., 1998) in plant cell growth. Expansins have been identified as a catalyst for acid growth and have been shown to induce the extension of isolated cell walls (McQueen-Mason and Cosgrove, 1995).

ACKNOWLEDGMENTS

We are grateful to Drs. John Mulligan and Ronald W. Davis (Stanford University, Stanford, CA) for the gift of the Arabidopsis genomic library. We also thank Dr. Shigehisa Okamoto (Kagoshima University, Kagoshima, Japan) for help with the cloning of *EXGT* genes.

Received April 21, 1999; accepted July 8, 1999.

LITERATURE CITED

Antosiewicz DM, Purugganan MM, Polisensky DH, Braam J (1997) Cellular localization of Arabidopsis xyloglucan endotransglycosylase-related proteins during development and after wind stimulation. Plant Physiol **115:** 1319–1328

Arrowsmith DA, de Silva J (1995) Characterisation of two tomato fruit-expressed cDNAs encoding xyloglucan endo-transglycosylase. Plant Mol Biol **28:** 391–403

Bell CJ, Ecker JR (1994) Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. Genomics **19:** 137–144

Carpita NC, 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 J **3:** 1–30

Cosgrove DJ (1998) Cell wall loosening by expansins. Plant Physiol **118:** 333–339

de Silva J, Jarman CD, Arrowsmith DA, Stronach MS, Chengappa S, Sidebottom C, Reid JSG (1993) Molecular characterization of a xyloglucan-specific endo-(1→4)-β-D-glucanase (xyloglucan endo-transglycosylase) from nasturtium seeds. Plant J **3:** 701–711

Edwards M, Dea ICM, Bulpin PV, Reid JSG (1986) Purification and properties of a novel, xyloglucan-specific endo-(1→4)-β-D-glucanase from germinated nasturtium seeds (*Tropaeolum majus* L.). J Biol Chem **261:** 9489–9494

Fry SC (1995) Polysaccharide-modifying enzymes in the plant cell wall. Annu Rev Plant Physiol Plant Mol Biol **46:** 497–520

Hanzawa Y, Takahashi T, Komeda Y (1997) *ACL5*: an *Arabidopsis* gene required for internodal elongation after flowering. Plant J **12:** 863–874

Hayashi T (1989) Xyloglucans in the primary cell wall. Annu Rev Plant Physiol Plant Mol Biol **40:** 139–168

Kauschmann A, Jessop A, Koncz C, Szekeres M, Willmitzer L, Altmann T (1996) Genetic evidence for an essential role of brassinosteroids in plant development. Plant J **9:** 701–713

Konieczny A, Ausubel FM (1993) A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J **4:** 403–410

McQueen-Mason S, Cosgrove DJ (1995) Expansin mode of action on cell walls: analysis of wall hydrolysis, stress relaxation, and binding. Plant Physiol **107:** 87–100

Medford JI, Elmer JS, Klee HJ (1991) Molecular cloning and characterization of genes expressed in shoot apical meristems. Plant Cell **3:** 359–370

Nicol F, His I, Jauneau A, Vernhettes S, Canut H, Höfte H (1998) A plasma membrane-bound putative endo-1,4-β-D-glucanase is required for normal wall assembly and cell elongation in *Arabidopsis*. EMBO J **17:** 5563–5576

Nishitani K (1995) Endo-xyloglucan transferase, a new class of transferase involved in cell wall construction. J Plant Res **108:** 137–148

Nishitani K (1997) The role of endoxyloglucan transferase in the organization of plant cell walls. Int Rev Cytol **173:** 157–206

Nishitani K, Tominaga R (1992) Endoxyloglucan transferase, a novel class of glycosyltransferase that catalyzes transfer of a segment of xyloglucan molecule to another xyloglucan molecule. J Biol Chem **267:** 21058–21064

Oh M-H, Romanow WG, Smith RC, Zamski E, Sasse J, Clouse SD (1998) Soybean *BRU1* encodes a functional xyloglucan endotransglycosylase that is highly expressed in inner epicotyl tissues during brassinosteroid-promoted elongation. Plant Cell Physiol **39:** 124–130

Okazawa K, Sato Y, Nakagawa T, Asada K, Kato I, Tomita E, Nishitani K (1993) Molecular cloning and cDNA sequencing of endoxyloglucan transferase, a novel class of glycosyltransferase that mediates molecular grafting between matrix polysaccharides in plant cell walls. J Biol Chem **268:** 25364–25368

Redgwell RJ, Fry SC (1993) Xyloglucan endotransglycosylase activity increases during kiwifruit (*Actinidia deliciosa*) ripening. Plant Physiol **103:** 1399–1406

Rose JKC, Brummell DA, Bennett AB (1996) Two divergent xyloglucan endotransglycosylases exhibit mutually exclusive patterns of expression in nasturtium. Plant Physiol **110:** 493–499

Saab IN, Sachs MM (1996) A flooding-induced xyloglucan endotransglycosylase homolog in maize is responsive to ethylene and associated with aerenchyma. Plant Physiol **112:** 385–391

Shani Z, Dekel M, Tsabary G, Shoseyov O (1997) Cloning and characterization of elongation specific endo-1,4-β-glucanase (*cel*1) from *Arabidopsis thaliana*. Plant Mol Biol **34:** 837–842

Takahashi T, Naito S, Komeda Y (1992) Isolation and analysis of the expression of two genes for the 81-kilodalton heat shock proteins from Arabidopsis. Plant Physiol **99:** 383–390

Tsukaya H, Inaba-Higano K, Komeda Y (1995) Phenotypic characterization and molecular mapping of an *acaulis2* mutant of *Arabidopsis thaliana* with flower stalks of much reduced length. Plant Cell Physiol **36:** 239–246

Tsukaya H, Naito S, Rédei GP, Komeda Y (1993) A new class of mutations in *Arabidopsis thaliana, acaulis1,* affecting the development of both inflorescences and leaves. Development **118:** 751–764

Xu W, Campbell P, Vargheese AK, Braam J (1996) The *Arabidopsis XET*-related gene family: environmental and hormonal regulation of expression. Plant J **9:** 879–889

Xu W, Purugganan MM, Polisensky DH, Antosiewicz DM, Fry SC, Braam J (1995) *Arabidopsis TCH4,* regulated by hormones and the environment, encodes a xyloglucan endotransglycosylase. Plant Cell **7:** 1555–1567

Zurek DM, Clouse SD (1994) Molecular cloning and characterization of a brassinosteroid-regulated gene from elongationg soybean epicotyls. Plant Physiol **104:** 161–170