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Alteration of β-Tubulin Gene Expression during Low-Temperature Exposure in Leaves of *Arabidopsis thaliana*[1]

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Responses of β-tubulin gene expression to low-temperature exposure (4°C) have been investigated in leaves of *Arabidopsis thaliana*. During low-temperature exposure, the patterns of both α- and β-tubulin isoforms are altered; the effect is smaller for the α-tubulins than for the β-tubulins, however. An examination of β-tubulin gene expression revealed that during low-temperature exposure, transcript levels of *TUB2*, *TUB3*, *TUB6*, and *TUB8* decrease, whereas those of *TUB4*, *TUB5*, and *TUB7* remain constant, and the *TUB9* transcript level increases. The changes in transcript levels of *TUB6*, *TUB8*, and *TUB9* were detectable after 6 h of low-temperature treatment. As shown by transcription-blocking experiments, the in vivo decay rates at 25°C are comparable to those at 4°C for *TUB5*, *TUB6*, and *TUB8* mRNAs, whereas *TUB9* mRNA appears to be more stable at 4°C than at 25°C. Thus, decreases in transcript levels of *TUB6* and *TUB8* in response to low temperature appear to be regulated at the transcriptional level, and the increase in *TUB9* transcript level that results from lowering the temperature from 25°C to 4°C may be due in part to its slower rate of decay at 4°C. When a chimeric gene containing 1061 base pairs of *TUB8* 5′ flanking DNA fused to the β-glucuronidase coding region was used to produce transgenic *Arabidopsis* plants, the chimeric gene expression was down-regulated in response to low temperature as assayed by histochemical localization and RNA gel blots. These results confirm that the alteration of transcript levels of *TUB8* in response to low temperature is regulated at the transcriptional level.

Microtubules are composed mainly of highly conserved α- and β-tubulin isoforms. Both α- and β-tubulins are encoded by multigene families in animals (Cleveland and Sullivan, 1985) as well as in higher plants (Silflow et al., 1987; Fosket, 1989). For example, there are at least eight α-tubulin genes in maize (Villemur et al., 1992). In *Arabidopsis thaliana*, six α- and nine β-tubulin genes have been characterized (Kopczak et al., 1992; Snustad et al., 1992). Transcripts of both α- and β-tubulin genes exhibit organ-specific and developmental stage-specific accumulation in *A. thaliana* (Ludwig et al., 1988; Carpenter et al., 1992; Snustad et al., 1992). The maize α1- and α2-tubulin genes are expressed primarily in the radicle (Montoliu et al., 1990). The β1-tubulin gene of soybean is expressed strongly only in the hypocotyl of etiolated seedlings (Han et al., 1991).

In accordance with the presence of multiple tubulin genes in higher plants, multiple tubulin isoforms have been observed in several plants, including *Phaseolus vulgaris* root tips (Hussey and Gull, 1985), carrot cell suspensions (Dawson and Lloyd, 1985; Cyr et al., 1987), carrot plant tissues (Hussey et al., 1988), various organs and tissues of maize (Joyce et al., 1992) and *A. thaliana* (Kopczak et al., 1992; Snustad et al., 1992), rye root tips (Kerr and Carter, 1990b), and spinach leaf cells (Bartolo, 1990). Certain tubulin isoforms are differentially present through various stages of development and in various tissues and organs (Hussey et al., 1988; Taylor et al., 1991; Joyce et al., 1992). Kerr and Carter (1990b) showed that both α- and β-tubulin isoforms are altered in rye root tips when seedlings are grown at low temperature for cold acclimation. Bartolo (1990) also found that β-tubulin isoforms are altered in spinach leaf cells in response to low temperature. Although posttranslational modifications such as acetylation, tyrosination, and glutamylation of α-tubulins, and phosphorylation and glutamylation of β-tubulins generate tubulin diversity in animals (reviewed by Joshi and Cleveland, 1990), none of these modifications has been confirmed in plant tubulins.

Changes in levels of tubulin isoforms in a plant in response to low-temperature exposure could result from altered tubulin gene transcript levels. There are several reports of low temperature-induced alterations in gene expression in plants. For example, spinach (Guy et al., 1985), winter rape (Meza-Basso et al., 1986; Johnson-Flanagan and Singh, 1987), alfalfa (Mohapatra et al., 1987, 1989), barley (Hughes and Pearce, 1988; Dunn et al., 1990), potato (Tseng and Li, 1990), and *A. thaliana* (Gilmour et al., 1988; Kurkela et al., 1988; Hajela et al., 1990; Kurkela and Franck, 1990) all show changes in gene expression in response to low-temperature exposure. In these cases, however, the identities of the gene products that change in response to low temperature are unknown. Two known genes that are preferentially expressed when plants are exposed to low temperature are an extensin gene in pea (Weiser et al., 1990) and a Suc synthase gene in wheat (Crespi et al., 1991).

In this study we used *A. thaliana* Columbia because (a)

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Abbreviation: GUS, β-glucuronidase.

Arabidopsis can be cold acclimated (Gilmour et al., 1988), (b) gene-specific hybridization probes are available for all of the α- and β-tubulin genes of this plant (Kopczak et al., 1992; Snustad et al., 1992), and (c) the small genome and transformability of *A. thaliana* should facilitate studies on the mechanism of thermoregulation of tubulin gene expression. In this paper we report the change in tubulin isoforms and β-tubulin gene transcript levels in *A. thaliana* in response to low temperature exposure and compare turnover rates for certain β-tubulin gene transcripts at moderate (25°C) and at low-temperature (4°C). Transgenic *Arabidopsis* plants containing a chimeric gene made by fusing the putative promoter of *TUB8* to the GUS coding region were examined to determine whether the putative promoter of the *TUB8* gene imparts the same temperature sensitivity to the chimeric gene as is possessed by the endogenous *TUB8* gene.

MATERIALS AND METHODS

Plant Materials

Arabidopsis thaliana Columbia plants were grown in Oasis Growing Medium (Smithers-Oasis, Kent, OH) at 25°C for 24 d under continuous illumination (50 μmol m^{-2} s^{-1}) provided by cool-white fluorescent tubes. These plants (termed NA, for nonacclimated) were then grown at 4°C under either continuous illumination or 8 h light:16 h dark for up to 14 d for cold acclimation (CA). (Both light regimes caused cold acclimation, by which is meant that the freezing tolerance of the plants increased.) Plants were watered daily and provided nutrient solution twice a week. Leaves of both NA and CA plants were used for immunofluorescence staining and for protein and RNA extractions.

Protein Extraction and Immunoblots

Proteins from NA and CA plants (grown for 24 d at 25°C under continuous light and then for 14 d at 4°C under 8 h light:16 h dark) were extracted (Guy et al., 1985), dissolved in O'Farrell (1975) lysis buffer containing 2% ampholines (0.6%, pH 3–10, and 1.4%, pH 5.0–5.5, from Bio-Rad), and stored at −80°C prior to analysis by two-dimensional gel electrophoresis. IEF gels were run at 400 V for 16 h and then at 800 V for 4 h with the acidic and basic solutions reversed. Focused IEF gels were placed in equilibration buffer briefly before carrying out gel electrophoresis in the second direction, which was done using a 4.4% stacking gel and a 10% resolving gel at constant voltage. After electrophoresis, proteins within the gel were electrophoretically transferred to nitrocellulose (Towbin et al., 1979) and incubated with a monoclonal antibody, B-5-1-2, specific for all α-tubulins (Piperno et al., 1987), and a monoclonal β-tubulin antibody (Amersham). Biotinylated second antibody was used, followed by treatment with avidin:biotinylated horseradish peroxidase (Vectastain Elite ABC, Vector Laboratories, Burlingame, CA) using 3,3′-diaminobenzidine (Sigma)/$NiCl_2$ as the substrate.

Total RNA Extraction and RNA Gel Blots

Total RNA was isolated using the phenol:chloroform method (Dean et al., 1985) with slight modifications. Purified RNA samples were dissolved in diethyl pyrocarbonate-treated water, and the concentrations of RNA were determined spectrophotometrically at 260 nm (assuming 1 A_{260} nm = 40 μg RNA/mL).

Total RNAs were denatured in 1× Mops running buffer containing 6.5% formaldehyde and 50% formamide at 60°C for 15 min. Denatured RNA samples were subjected to electrophoresis through 1.2% agarose gels in 1× Mops buffer containing 1.9% formaldehyde. Transfer of RNA from gels to nylon membranes (Nytran, Schleicher and Schuell) was carried out as described by Ausubel et al. (1987). β-Tubulin gene-specific probes were labeled by primer extension (Hu and Messing, 1982). The same membrane was used for all the gene-specific probes, being stripped after each probe was applied. An rDNA probe (from a soybean rDNA genomic clone [pKDR1], provided by R.T. Nagao and J.L. Key, University of Georgia, Athens, GA) was labeled using the random primer method (Feinberg and Vogelstein, 1983). Procedures and conditions for prehybridization and hybridization were as described by Oppenheimer et al. (1988). Quantitation of individual β-tubulin mRNA was accomplished using densitometry and a radioanalytic imaging system (Ambis, San Diego, CA).

Assessment of β-Tubulin mRNA Decay in Vivo

Plants (NA) were cut at the base and partially immersed in actinomycin D (Sigma) (100 μg/mL from a 50 mg/mL DMSO stock). Controls were immersed in 0.2% (v/v) DMSO solution for the same period of time as the actinomycin D treatment. After 2 h of incubation at 25°C under light, half of the plants were moved to 4°C while the remainder were maintained at 25°C. Leaves were harvested at various times and frozen in liquid N_2.

To test the efficiency of inhibition of transcription by actinomycin D, [^{3}H]uridine (8×10^7 cpm) was added to both the solutions (1 mL) of actinomycin D and the control solutions after the 2-h incubation at 25°C. After 5 h of [^{3}H]uridine labeling at both 25°C and 4°C, total nucleic acids were extracted and the radioactivity in the nucleic acids was determined using liquid scintillation spectrometry as described by Maniatis et al. (1982). To test whether the radioactivity measured was due to [^{3}H]uridine incorporation into RNA, the same amount of nucleic acid was treated with RNase before the scintillation counting. Inhibition of transcription was calculated as follows: $(1 - [D_1 - D_2]/[C_1 - C_2]) \times 100\%$, where D_1 is the counts for actinomycin D treatment without RNase digestion, D_2 is the counts for actinomycin D treatment with RNase digestion, C_1 is the counts for control without RNase digestion, and C_2 is the counts for control with RNase digestion.

Bacterial Strains and Media

Escherichia coli strains MV1190, used for the growth and isolation of pUC118 and pUC119 (Vieira and Messing, 1987) and their derivatives, and DH5α (Sambrook et al., 1989), used for the growth and isolation of pBI121 and its derivatives, were grown in 2 × YT medium (Sambrook et al., 1989). *Agrobacterium tumefaciens* strain AGL1 (Lazo et al., 1991),

used for all plant transformation experiments, was grown in Nutrient Broth medium supplemented with 2% Glc. Antibiotics were used at the following concentrations: for *E. coli*, ampicillin, 50 μg/mL, and kanamycin, 50 μg/mL; for *A. tumefaciens*, carbenicillin, 100 μg/mL, and kanamycin, 50 μg/mL.

Construction of pBP864I, an *Agrobacterium* Binary Plasmid Vector Harboring a *TUB8/GUS* Chimeric Gene

Plasmid pBP864I, containing a chimeric *TUB8* 5′/*GUS* coding/nopaline synthase 3′ gene (Fig. 1), was constructed by replacing the cauliflower mosaic virus 35S promoter of plasmid pBI121 with a 1061-bp *Eco*RI-*Alu*I restriction fragment from the 5′ flanking region of an *A. thaliana* Columbia *TUB8* genomic clone. Cleavage at the *Alu*I site 13 bp upstream of the *TUB8* ATG translation start triplet was verified by sequencing after insertion of the restriction fragment into *Sma*I–*Eco*RI-cut pUC118. The *Eco*RI site of the *TUB8* 5′ restriction fragment was converted to a *Hin*dIII site by producing blunt ends with Klenow enzyme and the addition of *Hin*dIII linkers. The *Hin*dIII–*Bam*HI restriction fragment excised from pUC118 was force-cloned into the *Hin*dIII and *Bam*HI acceptor sites of plasmid pBI121 after removal of the 35S promoter. The resulting plasmid, designated pBP864I, was introduced into *A. tumefaciens* strain AGLI (Lazo et al., 1991) by electroporation.

Plant Transformation and Histochemical Assay for GUS Activity

A. tumefaciens strain AGLI containing the pBP864I construct was used to transform root explants of *A. thaliana* Columbia following the protocol of Valvekens et al. (1988) as modified by Carpenter et al. (1992). Histochemical assays for GUS activity were performed as described by Jefferson et al. (1987).

RESULTS

Tubulin Isoform Distributions at Normal and Low Temperatures

Prior to examining tubulin gene transcription in response to low temperature, tubulin isoform distribution was investigated in leaf tissues of NA (25°C, continuous light for 24 d) and CA (25°C, continuous light for 24 d followed by 4°C, 8 h light:16 h dark for 14 d) plants. Five α-tubulin isoforms were present in the immunoblots of leaf samples from both regimes (Fig. 2). There were small differences in the α-tubulin isoform patterns between leaf samples from the two regimes. For example, the small spot immediately below the most intense spot is apparently increased in amount by the CA treatment. The β-tubulin isoform distribution patterns on the immunoblots also were different for protein samples from the two regimes (Fig. 2). One large spot, which probably consists of more than one isoform, decreased in amount, while two other spots increased in amount in response to the CA treatment, although the total number of spots (five) remained the same. These changes could be due to an altered pattern of tubulin gene transcription, to posttranscriptional or posttranslational effects, or to changes in rates of protein turnover. To investigate these possibilities, we first analyzed tubulin transcript accumulation under the two regimes. We concentrated on β-tubulin transcripts because the isoform pattern of the β-tubulins was altered more than that of the α-tubulins by the change from the NA to the CA regime.

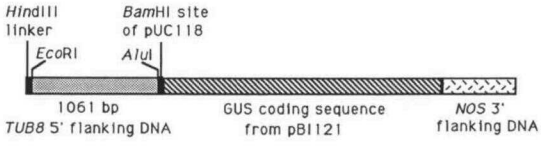

Figure 1. Structure of the chimeric reporter gene present in plasmid pBP864I. *NOS*, Nopaline synthase gene.

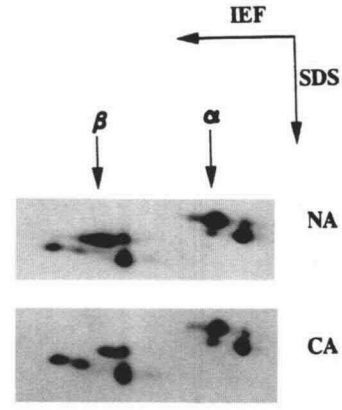

Figure 2. The overall patterns of tubulin isoforms on immunoblots. Proteins were separated by two-dimensional gel electrophoresis and then transferred to nitrocellulose papers and incubated with monoclonal anti-α- (B-5-1-2) (Piperno et al., 1987) and anti-β- (Amersham) tubulin antibodies. NA, Nonacclimated; CA, cold-acclimated (at 4°C under 8 h day:16 h night for 14 d).

Alterations in Transcript Levels of β-Tubulin Genes in Response to Temperature Decrease

Steady-state transcript levels of *TUB2*, *TUB3*, *TUB6*, and *TUB8* decreased, whereas those of *TUB4*, *TUB5*, and *TUB7* remained fairly constant during the CA treatment (Fig. 3). The amount of *TUB9* mRNA increased during the first 2 d, but by the end of 2 weeks it had declined to near the level in the 25°C-grown plants. A β-tubulin coding-sequence probe was used as an internal control (Fig. 3). The *TUB1* transcript was below the level of detection on the blot; this result was expected because *TUB1* is expressed primarily in roots (Oppenheimer et al., 1988).

In this experiment, plants were under an 8 h light:16 h dark photoperiod when exposed to 4°C, whereas they were under continuous light prior to the low-temperature exposure. This was done to duplicate the conditions used in preliminary cold-acclimation studies (not shown) and in the tubulin isoform experiment (Fig. 2). To determine whether the dark period is required for the changes in β-tubulin transcript levels, a second experiment was carried out in which plants at 4°C were kept under continuous illumination.

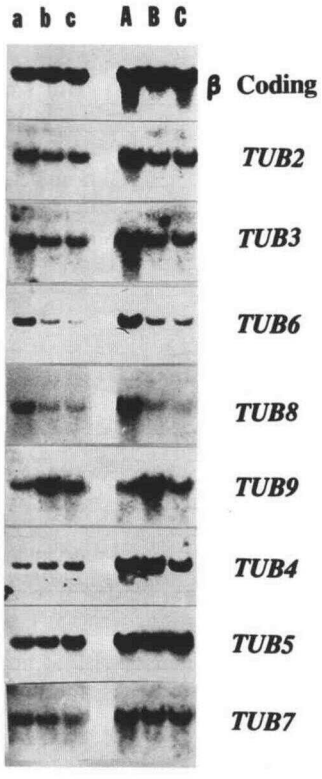

Figure 3. RNA gel-blot analysis of β-tubulin gene transcript accumulation during low-temperature exposure. Total RNAs (4 μg for lanes a, b, and c, 8 μg for lanes A, B, and C) were separated using a denaturing agarose gel (1.2%) and transferred to a nylon membrane for sequential blot hybridizations. A β-tubulin coding sequence (β-coding) probe was used to detect all β-tubulin gene transcripts as an internal control. Lanes a and A, Plants grown at 25°C under continuous illumination; lanes b and B, plants grown for 2 d at 4°C under 8 h day:16 h night; lanes c and C, plants grown for 14 d at 4°C under 8 h day:16 h night.

Changes in transcript levels of *TUB6*, *TUB8*, and *TUB9* were similar in this experiment to those observed in the previous experiment with the dark period (data not shown). Once again, the *TUB9* transcript showed the same transitory change during the 2-week period of low-temperature exposure. Therefore, low temperature alone can cause the observed changes in β-tubulin transcript levels. Moreover, the changes in these transcript levels were detectable within 6 h after the temperature was decreased from 25°C to 4°C (data not shown).

β-Tubulin mRNA Decay in Vivo

To investigate whether the changes in the levels of *TUB5*, *TUB6*, *TUB8*, and *TUB9* transcripts are regulated at the transcriptional or posttranscriptional level, rates of mRNA decay were examined in vivo at 25°C and at 4°C (continuous illumination at both temperatures) following treatment with actinomycin D to inhibit transcription. The inhibition of transcription was about 92% at 25°C and 75% at 4°C (data not shown). Overall, β-tubulin mRNAs appeared slightly more stable at 4°C than at 25°C (Fig. 4, β-coding). This could

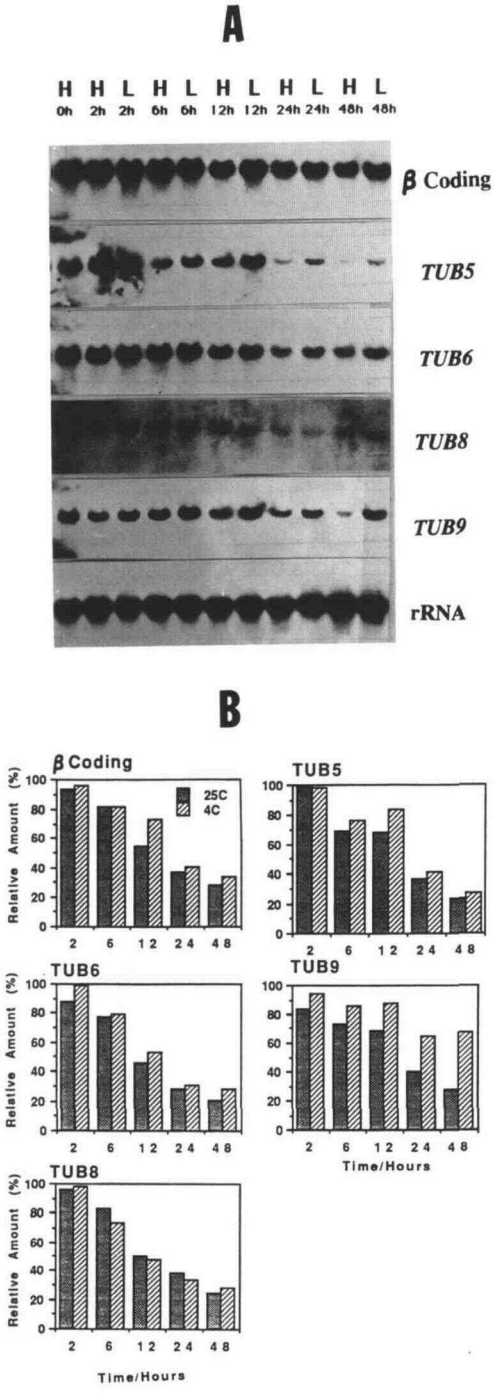

Figure 4. Comparisons of β-tubulin mRNA decay rates in vivo at two temperatures (25°C and 4°C). A, RNA gel-blot analysis of mRNA levels of total β-tubulin transcripts (β-coding), *TUB5*, *TUB6*, *TUB8*, and *TUB9* after various lengths of actinomycin D treatment at 25°C (H) and at 4°C (L). The RNA gel blot was hybridized with an rRNA probe as an internal standard to compare the amounts of total RNA in each lane. B, Quantitation of radioactivity hybridized to the individual probes by densitometric scanning of the autoradiogram. The relative amounts of total β-tubulin (β-coding), *TUB5*, *TUB6*, *TUB8*, and *TUB9* mRNA were normalized to rRNA levels for each time point. The membranes that were hybridized with the β-tubulin coding and rRNA probes were also analyzed using a radioanalytic imaging system (Ambis, San Diego, CA) that directly quantitated the hybridized radioactivity. The results obtained by the two methods were comparable.

be due in part to the lower inhibition of transcription by actinomycin D at 4°C than at 25°C. Among the individual β-tubulin mRNAs examined, the transcript decay rates of *TUB5* and *TUB6* at the two temperatures were similar to those observed for total β-coding mRNAs, and the decay rate for *TUB8* mRNA was comparable at 4°C and at 25°C. In contrast, *TUB9* mRNA appeared to be considerably more stable at 4°C than at 25°C (Fig. 4).

Expression at 25°C and at 4°C of a *TUB8* 5′ Noncoding/*GUS* Coding Chimeric Gene in Transgenic Plants

Cold treatment had a large effect on the transcript level of *TUB8*, reducing it significantly within 12 h of cooling to 4°C. Accordingly, *TUB8* was singled out for further examination to determine the mechanism by which it is regulated in response to temperature change. Changes in GUS activity in response to low temperature were examined in plants of five lines transformed with a *TUB8* 5′/*GUS* coding region fusion gene. The fusion gene contained 1061 bp of *TUB8* 5′ flanking DNA. When cooled from 25°C to 4°C, plants of all five transgenic lines showed a decrease in GUS activity, but a trace of GUS activity was still detectable in leaves after 1 week at 4°C (data not shown). Since rates of turnover of the GUS protein might be different at the two temperatures, this decrease of GUS activity upon cooling does not necessarily imply a change in transcript levels.

To investigate whether the transcript level of the chimeric gene changed in response to the low-temperature treatment, an RNA gel-blot analysis of R_1 plants from three independently transformed lines (pBP864I-1, -14, and -18) and an RNA slot-blot analysis for R_2 plants of four other transformed lines (pBP864I-3, -7, -20, and -22) was conducted (Fig. 5, A and B). After 1 d at 4°C, the transcript level of the chimeric gene decreased as did that of *TUB8* (Fig. 5A; the GUS transcript in line pBP864I-14 is rather low). In contrast, the GUS transcript produced in transgenic plants harboring the cauliflower mosaic virus 35S promoter/*GUS* coding region fusion gene changed little, if at all, in response to the same low-temperature treatment. Thus, the cold-induced decrease in GUS transcript levels in the seven transgenic lines carrying the *TUB8* 5′ flanking DNA/*GUS* chimeric gene most likely results from a decrease in the transcription rate of the *TUB8* putative promoter rather than an increase in the rate of mRNA turnover.

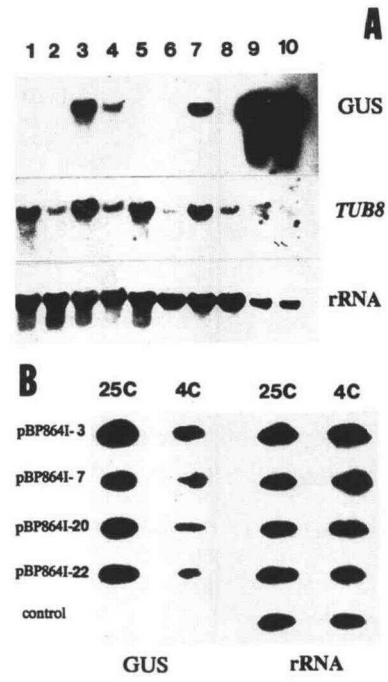

Figure 5. Changes in transcript level in response to low-temperature exposure of transgenic plants carrying a *TUB8* 5′/*GUS* chimeric gene. A, An RNA gel blot. RNAs were isolated from wild-type control plants (lanes 1 and 2), transgenic plants from three different transgenic lines (pBP864I-1, pBP864I-14, and pBP864I-18) harboring a *TUB8* 5′ region/*GUS* coding sequence fusion gene (lanes 3 and 4, 5 and 6, and 7 and 8, respectively), and transgenic plants carrying a cauliflower mosaic virus 35S promoter/*GUS* coding sequence chimeric gene (lanes 9 and 10). RNA samples analyzed in lanes 1, 3, 5, 7, and 9 were from plants grown at 25°C for 24 d, whereas the RNA samples investigated in lanes 2, 4, 6, 8, and 10 were from plants grown at 25°C for 24 d and then at 4°C for 1 d. B, An RNA slot blot shows changes in GUS transcript level in response to low-temperature exposure of four other transgenic lines harboring a *TUB8* 5′ region/*GUS* coding sequence fusion gene. Plant growth conditions were as described in A. Transgenic lines harboring a *TUB8* 5′ region/*GUS* coding chimeric gene used in A were from R_1 plants, whereas those in B were from R_2 plants.

DISCUSSION

Plant microtubules are one of several supramolecular structures that can be affected by low temperature and freezing (Bartolo and Carter, 1991, and refs. therein). Exposing plants to a given low temperature can modify the responses of microtubules to further temperature decrease (Kerr and Carter, 1990a). One possible cause of this modification is low temperature-induced changes in the synthesis of tubulin isoforms, a change that has been detected in winter rye (Kerr and Carter, 1990b), spinach (Bartolo and Carter, 1991), and winter rape (B. Chu, unpublished results). In the study reported here, the mechanism for this differential expression was investigated in *A. thaliana* after finding that levels of tubulin isoforms also change in mesophyll cells of *Arabidopsis* in response to a change from NA to CA conditions (Fig. 2).

Alteration of β-Tubulin Gene Transcription Rate in Response to Low-Temperature Exposure

The temperature effect on β-tubulin gene transcript levels was examined using specific probes for *Arabidopsis* β-tubulin genes for RNA gel-blot analysis (Fig. 3). Changes in transcript levels of *TUB6* and *TUB8* following the temperature change are most likely controlled at the transcriptional level because the decay rates of the two transcripts are very similar at 4°C and 25°C. The higher level of *TUB9* mRNA at 4°C than at 25°C during actinomycin D incubation could be due to a higher level of synthesis at 4°C than at 25°C, because the inhibition of transcription by actinomycin D was less effective at 4°C. The likelihood that this explanation is correct is diminished by the fact that the level of *TUB5* mRNA re-

mained relatively constant during the CA regime (Fig. 3; compare β-coding with *TUB5*), whereas *TUB5* mRNA decay rates at 25°C and 4°C were comparable (Fig. 4B, *TUB5*; note the relative amounts of transcript after 24 and 48 h of actinomycin D treatment). The *TUB5* mRNA results suggest that the higher level of *TUB9* mRNA at 4°C compared with 25°C is not due merely to a lower inhibition of transcription by actinomycin D at 4°C than at 25°C, but to slower turnover of *TUB9* transcript at 4°C.

Because incubation with actinomycin D at 4°C inhibited less than 50% of transcription (data not shown), plants were incubated with actinomycin D at 25°C for 2 h before exposing them to 4°C in this study. If some factors that affect β-tubulin mRNA stability at 4°C were produced only at 4°C, then this experiment would probably have failed to reveal the differences in β-tubulin mRNA stability between the two temperatures, because actinomycin D would have inhibited the production of the factors. It is unknown whether such factors exist in *A. thaliana*. In *Xenopus* liver cells, estrogen preferentially stabilizes vitellogenin mRNA against cytoplasmic degradation (Brock and Shapiro, 1983).

In spite of the limitations of the data comparing β-tubulin mRNA decay rates at 25°C and 4°C, the results from studies of the β-tubulin mRNA decay rates at 25°C provide information on β-tubulin mRNA half-life in plants. The estimated half-life of total β-tubulin mRNAs, which is about 12 h (Fig. 4B, β-coding), is slightly longer than those of chloroplast mRNAs in spinach (Klaff and Gruissem, 1991). It is comparable to those of total mRNA in *Xenopus* liver cells (Brock and Shapiro, 1983). The actual half-lives of β-tubulin mRNAs in the present study is probably slightly less than the estimate of 12 h, because actinomycin D did not completely inhibit transcription (92% rather than 100%). Moreover, rRNA was chosen as an internal standard, assuming that rRNA level remains roughly constant during the 2 d of actinomycin D incubation. A slight decay of rRNA during the actinomycin D incubation would also result in an overestimation of the β-tubulin mRNA half-lives. Fortunately, rRNAs generally are very stable. The 16S chloroplast rRNA was also chosen as an internal standard in Klaff and Gruissem's (1991) study of chloroplast mRNA decay.

Expression of the *TUB8* 5′/*GUS* Chimeric Gene Is Decreased by Cooling Plants from 25°C to 4°C

The initial RNA gel-blot survey revealed several changes in transcript levels of the β-tubulin genes in response to cooling. *TUB8*, for example, produced significantly less transcript at 4°C than at 25°C. Recognizing that a complete understanding of how plants respond to cold means learning how genes are down-regulated as well as how they are up-regulated, in response to temperature decrease, we studied the mechanism of the down-regulation of *TUB8* in greater detail.

To test the hypothesis that the change in *TUB8* transcript levels observed after cooling plants from 25°C to 4°C is regulated at the transcriptional level, we have investigated the response of the putative promoter of *TUB8* to low temperature by constructing a chimeric gene containing the 5′ flanking region of *TUB8* fused to the *GUS* coding region and analyzing the expression of this chimeric gene in transgenic plants. This fusion gene exhibited the same pattern of down-regulation of transcript levels in response to low-temperature exposure as the endogenous *TUB8* gene, even though the fusion gene transcript contained primarily *GUS* coding sequence and no down-regulation of *GUS* transcript level was observed when transcription was controlled by the 35S promoter of cauliflower mosaic virus. The down-regulation of the expression of *TUB8* 5′/*GUS* chimeric gene on cooling indicates that the 1061 bp of 5′ flanking DNA of *TUB8* contains the sequence(s) responsible for down-regulation of *TUB8* gene expression in response to the temperature decrease. Efforts are now underway to locate and characterize any *cis*-acting element(s) in this 1061-bp DNA segment that are involved in this thermal regulation.

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