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Stress Responses and Metabolic Regulation of Glyceraldehyde-3-Phosphate Dehydrogenase Genes in *Arabidopsis*[1]

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We report here effects of three environmental conditions, heat shock, anaerobic treatment, and carbon source supply, on expression of nuclear genes encoding chloroplast (*GapA* and *GapB*) and cytosolic (*GapC*) glyceraldehyde-3-phosphate dehydrogenase from *Arabidopsis thaliana*. The steady-state mRNA level of the *GapC* increased when *Arabidopsis* plants were transferred from normal growth condition to heat-shock, anaerobiosis, or increased sucrose supply conditions. In contrast, the steady-state mRNA levels for *GapA* and *GapB* genes were unaffected or decreased transiently under the same treatments. To identify the *cis*-acting regulatory elements, transgenic tobacco plants containing a 820-bp *GapC* 5′-flanking DNA fragment and β-glucuronidase (*Gus*) fusion were constructed. Analyses of these transgenic plants indicate that this 820-bp DNA fragment is sufficient to confer both heat-shock and anaerobic responses. These results suggest that transcriptional level control is involved in regulation of *GapC* expression under these stress conditions. Histochemical analysis of *Gus* activity indicates that expression of the *GapC* is cell-type specific and is probably linked to the metabolic activity of the cells.

During the course of evolution, plants have developed elaborate systems to sense changing environmental conditions and to adjust their growth and development accordingly. Environmental stresses, such as heat shock and anaerobiosis, are known to alter gene expression in plants. Heat shock produces rapid changes in gene expression, reducing the RNA levels of many genes while inducing the transcription of a small set of heat-shock genes (Pelham, 1985). The pattern of induced proteins varies, but all species have HSPs with molecular masses between 65 and 75 kD, and most also have one or more HSPs of 15 to 30 kD (Pelham, 1985). The expression of HSPs is controlled primarily at the transcriptional level, and this control is achieved by the interactions between the *cis*-acting HSEs and the *trans*-acting factor HSF (Parker and Topol, 1984; Wiedderrcht et al., 1987). In addition to HSPs, heat shock is also known to increase accumulation of the mRNA for some of glycolytic genes in yeast, *Xenopus*, and maize (Lindquist and Craig, 1988; Nickells and Browder, 1988; Russell and Sachs, 1989). The physiological significance of this induction and whether transcriptional control is involved remain to be elucidated.

Similar to heat shock, anaerobiosis causes repression of preexisting protein synthesis and, in plants, induces the synthesis of about 20 proteins (ANPs) after approximately 90 min (Sachs et al., 1980). Six ANPs have been identified as alcohol dehydrogenase (*Adh1*, *Adh2*), glucose phosphate isomerase, fructose-1,6-diphosphate aldolase, pyruvate decarboxylase, and sucrose synthase (Freeling and Bennett, 1985). In addition, maize *Gpc3* and a rice *GapC* gene are also anaerobically regulated (Martinez et al., 1989; Ricard et al., 1989; Russell and Sachs, 1989). Like plants, mammalian cells also show increased glycolytic gene expression under anaerobiosis (Webster, 1987). The anaerobic control is mainly at the transcriptional level (Sachs et al., 1980; Walker et al., 1987a, 1987b). The *cis*-acting ARE has been identified in maize *Adh1* and has been shown to be responsible for anaerobic expression (Walker et al., 1987a). It has been shown that these AREs bind to *trans*-acting regulatory factors in vivo (Ferl and Nick, 1987; Ferl and Laughner, 1989).

The fact that both heat shock and anaerobiosis alter expression of glycolytic genes suggests that primary carbon metabolism is one of the pathways responding to changing environmental conditions. In addition to cytosolic glycolytic enzymes, higher plants possess a set of similar enzymes that are localized in the chloroplast and are involved in photosynthetic carbon fixation. It would be interesting to see how these two sets of genes, which are involved in opposite directions of carbon metabolism, are affected by different environmental conditions.

We report here effects of different growth conditions on expression of nuclear genes encoding chloroplast (*GapA* and *GapB*) and cytosolic (*GapC*) GAPDH from *Arabidopsis thaliana*. Our results show that, when *Arabidopsis* plants were transferred from the greenhouse-grown condition to heat shock or increased sucrose supply, the steady-state mRNA level for the *GapC* increased about 5- to 10-fold, whereas the mRNA levels for *GapA* and *GapB* genes decreased by 2- to 3-fold or remained constant. In contrast, the mRNA levels for all three *Gap* genes increased under anaerobic treatment, although the kinetics of induction were different between *GapC* and *GapA/B*. Analyses of transgenic plants containing a *GapC* promoter and *Gus* gene fusion indicate that a 820-bp 5′-flanking DNA fragment is sufficient to confer both heat-shock and anaerobic inductions. Surprisingly, histochemical analysis of the *Gus* activities in the transgenic tobacco plants

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Abbreviations: ANP, anaerobic inducible protein; ARE, anaerobic responsive element; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GUS, β-glucuronidase; HSE, heat-shock element; HSF, heat-shock factor; HSP, heat-shock protein; MS, Murashige-Skoog.

indicates that expression of the *GapC* gene is cell-type specific, although one would expect the opposite for a gene whose products are involved in glycolysis.

MATERIALS AND METHODS

Plant Materials and DNA Probes

Arabidopsis thaliana Columbia and *Nicotiana tabacum* Wisconsin 38 were used in this study. The sources of the hybridization probes were: *Arabidopsis GapA, GapB, GapC,* the cDNA inserts from pAtcGapA/10, pAtcGapB/10, and pAtcGapC/7 clones (Shih et al., 1988); *Arabidopsis Adh* gene, a 1.8-kb *Hin*dIII-*Pst*I fragment from the *ADH* genomic clone (Chang and Meyerowitz, 1986); *Xenopus* histone H4, a 1.5-kb *Pst*I fragment from pAnXH4, obtained from Dr. Dan Weeks (Department of Biochemistry, University of Iowa); rDNA, a 2.5-kb *Eco*RI insert from soybean rRNA genomic clone pKDR1, provided by Dr. J.L. Key (University of Georgia); *Gus,* a 2.0-kb *Pst*I-*Eco*RI fragment isolated from pBI101 encoding GUS (Jefferson et al., 1987).

Stress Treatment

For anaerobic stress, *Arabidopsis* seeds were germinated for 10 d in MS liquid medium supported by membrane rafts (Sigma) with the use of a 16-h light/8-h dark cycle at 22°C. Seedlings were submerged for various times in sterile double-distilled water in the dark. Nitrogen gas was pumped in constantly to deplete oxygen. Rooted transgenic tobacco plants were grown on soil for 3 weeks in the growth chamber with a 16-h light/8-h dark cycle at 25°C before being subjected to anaerobic treatment. Anaerobic treatment was done by submerging whole plants into a Nalgene tank containing sterilized water that had been depleted of oxygen by constantly pumping nitrogen gas into it. For heat-shock treatment, *Arabidopsis* seeds were germinated on soil and grown at 22°C for 4 weeks and then transferred to a 42°C growth chamber for various lengths of time. To prevent seedling desiccation, prewarmed water was added to the trays underneath as needed. Transgenic tobacco plants were similarly treated.

Sucrose Treatment

Arabidopsis plants (3 weeks old) grown in liquid MS medium as described for anaerobic treatment were adapted to the dark for 5 d. The membrane rafts were then transferred to new GA7 vessels (Sigma) containing only MS medium or MS medium plus 2% sucrose in continuous white light or complete darkness for 24 h. Total RNA was then isolated and subjected to northern blot analysis.

RNA Analysis

RNA was isolated and analyzed by modification of procedures described by Maniatis et al. (1989). Two grams of plant tissues were ground in liquid nitrogen and homogenized in the buffer containing 4 M guanidine-HCl, 50 mM Tris, 100 mM EDTA (pH 7.6), and 2% lauryl sarcosine. The solution was extracted twice with phenol/chloroform. RNA was then precipitated by adding 0.1 volume of 3 M sodium acetate (pH 5.5) and 2 volumes of ethanol at −20°C for 2 h. After the material was centrifuged, the pellet was resuspended in buffer (10 mM Tris [pH 7.5], 1 mM EDTA) and transferred to an Eppendorf tube. RNA was precipitated by adding one-third volume of 6 M $LiCl_2$ and putting the mixture on ice for 1 h. The pellet was resuspended in 2% potassium acetate and spun to remove insoluble material. The supernatant was precipitated in ethanol at −70°C for 15 min. The pellets were rinsed, dried, and resuspended in diethyl pyrocarbonate-treated water. For northern blot analysis, 20 μg of total RNA was denatured in 1× Mops buffer (20 mM Mops, 5 mM sodium acetate, 1 mM EDTA), 6.5% formaldehyde, and 50% (v/v) formamide at 65°C for 10 min and separated on 1.2% agarose formaldehyde gels in the 1× Mops buffer, 1.5% formaldehyde. RNA was transferred in 10× SSC (1× SSC = 0.15 M NaCl, 0.015 M Na_3 citrate) and UV cross-linked (Church and Gilbert, 1984) to a Nytran membrane (Schleicher & Schuell). Prehybridization was in 50% (v/v) formamide, 4× Denhardt's solution (100× Denhardt's solution = 2% each of Ficoll 400, PVP, and BSA), 10% dextran sulfate, 5× SSC, 1% SDS, and 0.1 mg/mL of sheared salmon sperm DNA at 42°C for 2 h. Hybridization was in the same solution at 42°C overnight. Probes were labeled with [^{32}P]dATP by the random priming method (Feinberg and Vogelstein, 1983). Blots were washed twice in 2× SSC, 1% SDS for 5 min at room temperature and twice in 0.1× SSC, 1% SDS for 30 min at 42°C. For higher stringency washing, the temperature was increased to between 55 and 65°C. Blots were exposed to XAR-5 x-ray film (Kodak) overnight with an intensifying screen at −70°C. To allow reprobing, blots were boiled in 0.01× SSC and 0.5% SDS for 20 min, rinsed in 2× SSC, and stored. Quantification of the blots was done by scanning autoradiograms with a Bio-Rad model 620 densitometer. The data shown are the averages of two or three independent northern blot analyses.

Construction of *GapC* Promoter/*Gus* Fusion and Transgenic Tobacco Plants

Site-directed mutagenesis was used to create an *Nco*I restriction site in the translation initiation codons (ATG) of the *Gus* and *GapC* genes to create puc19/Gus* and pBs/GapC*, respectively. A 820-bp *Bam*HI-*Nco*I fragment from the pBs/GapC* that contains the 780-bp promoter sequence and the complete 5′-untranslated region of the *GapC* gene was ligated to the *Bam*HI-*Nco*I double-digested puc19/Gus* to create puc19/GapC-Gus*. The 3-kb *Bam*HI-*Eco*RI fragment that contains the *GapC-Gus* fusion from puc19/GapC-Gus* was then used to replace the *Bam*HI-*EcoRI Gus* fragment of pBI101 (Jefferson et al., 1987). The resulting binary vector, GapC/Gus/pBI101, was mobilized into the *Agrobacterium tumefaciens* strain LBA 4404 by triparental mating (Bevan, 1984). Sterile leaf discs of *Nicotiana tabacum* were infected by LBA 4404 harboring GapC/Gus/pBI101 vector. Transformed cells and regenerated plants were selected on solid MS medium containing 0.5 mg/L BA, 3% sucrose, 100 μg/mL of kanamycin, and 200 μg/mL of carbenicillin. Regenerated plants were transferred to soil and grown in a growth chamber using a 16-h light/8-h dark cycle at 25°C until ready for

stress treatment. Tobacco plants with leaf lengths of 5 to 10 cm were used for all experiments.

GUS Assay

GUS enzymic activity assay and histochemical staining were performed according to the procedures described by Jefferson et al. (1987). GUS activities were measured by enzymic conversion of 4-methylumbelliferyl glucuronide to 4-methylumbelliferone, which was quantified with a Hoefer TK100 spectrofluorimeter.

RESULTS

Heat Shock Affects Expression of the *GapC* Gene

To investigate whether heat-shock treatment affects expression of *Gap* genes, mRNA levels in *Arabidopsis* plants grown under greenhouse conditions or with different durations of heat-shock (42°C) treatment were compared by northern blot analysis (Fig. 1a) and quantitated by scanning the autoradiograms with a densitometer (Fig. 1b). As shown in Figure 1, although *GapC* expressed (panel GapC, lane 1) under normal greenhouse conditions, its expression can be further induced to higher levels by heat-shock treatment. The mRNA level for *GapC* increased about 5-fold after 1 h of heat shock (lane 2) and then decreased slightly within 2 to 4 h of heat shock (lanes 3 and 4). When plants were shifted to the normal growth temperature after 2 h of heat shock, the mRNA level for *GapC* was rapidly reduced to approaching the basal level, as in the control plants (lanes 5 and 6). In contrast, the steady-state mRNA levels for *GapA* and *GapB* decreased slightly under heat-shock conditions (panels GapA and GapB, and Fig. 1b).

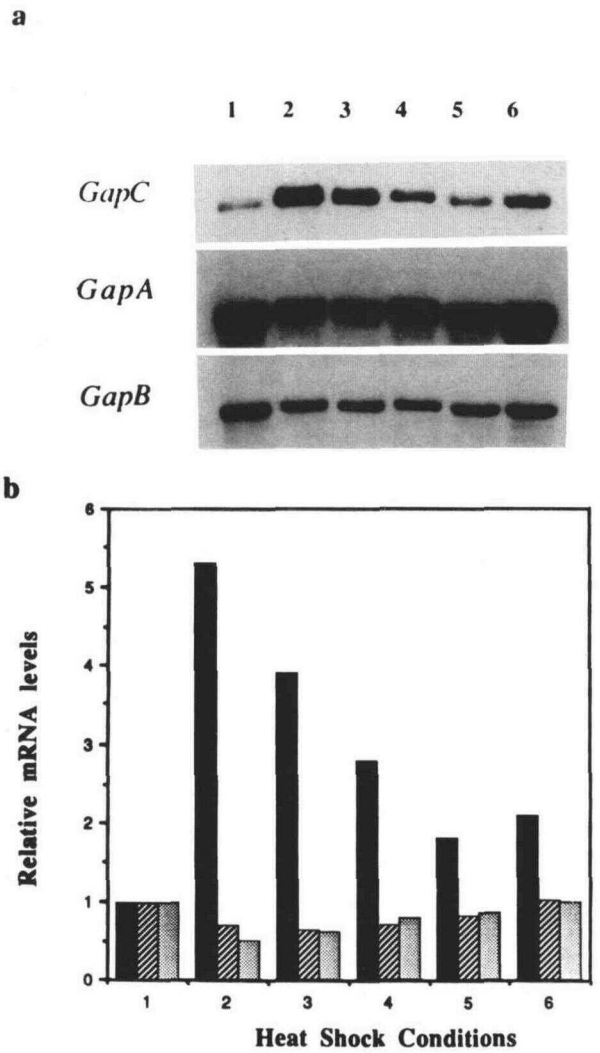

Figure 1. Heat-shock induction of GapC gene. a, Total RNA samples (10 μg) isolated from *Arabidopsis* plants exposed to different temperatures were separated by formaldehyde agarose gel electrophoresis. RNAs were transferred and UV cross-linked to a Nytran membrane according to the method of Church and Gilbert (1984). The membrane was then hybridized to ^{32}P-labeled cDNA clones of *GapA*, *GapB*, and *GapC* from *Arabidopsis*. The treatments are as follows: lane 1, 22°C; lane 2, 1 h at 42°C; lane 3, 2 h at 42°C; lane 4, 4 h at 42°C; lane 5, 2 h at 42°C and then 1 h at 22°C; lane 6, 2 h at 42°C and then 2 h at 22°C. b, Quantitation of the northern blot analysis was done by scanning autoradiograms with a Bio-Rad model 620 densitometer. The mRNA levels for each of the Gap genes from greenhouse-grown plants (lane 1) were designated as 100%. The data shown are the averages of three independent northern blot analyses.

Differential Effects of Anaerobiosis on Expression of *Gap* Genes

To determine whether anaerobic treatment affects expression of *Gap* genes, 3-week-old *Arabidopsis* plants grown on the membrane rafts in GA7 vessels (Sigma) were completely submerged in oxygen-depleted water (see "Materials and Methods" for details) for 2 or 4 h. The steady-state mRNA levels for the three *Gap* genes from these anaerobically treated plants were compared by northern blot analysis. As illustrated in Figure 2, the *GapC* gene responded quickly to anaerobic treatment. The mRNA for *GapC* started to increase after 2 h of anaerobic treatment and increased 6-fold above the basal level by 4 h. The mRNA level for the *Adh* gene, which is known to be induced by anaerobiosis (Chang and Meyerowitz, 1986), began to accumulate only after 4 h of anaerobiosis (panel ADH, Fig. 2). It is surprising that, after an initial decrease, mRNA levels for *GapA* and *GapB* genes also increased 3- and 5-fold above the basal level, respectively, after 4 h of anaerobic treatment (panels GapA and GapB). In contrast, mRNA levels for the histone 4 gene (panel H4) and rRNA gene (panel rDNA) either decreased continuously (H4) or remained unchanged (rDNA) during anaerobic treatment.

Effects of Sucrose on Expression of GAPDH Genes

We have shown previously that expression of the *GapC* gene in tobacco calli can be enhanced by growing them in a medium containing a high sucrose concentration, regardless of light conditions (Shih and Goodman, 1988). To investigate whether sucrose affects expression of GAPDH genes in mature plants, 3-week-old *Arabidopsis* plants grown in MS me-

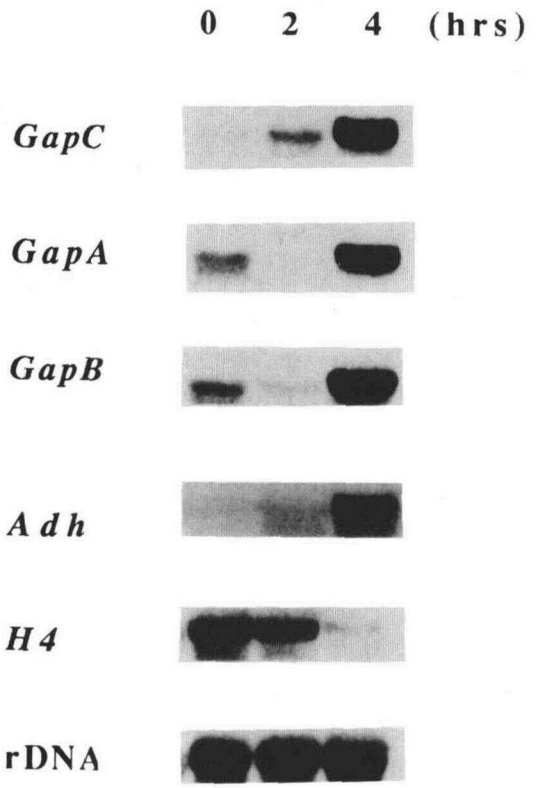

Figure 2. Anaerobic induction of GAPDH genes. Total RNA (10 μg) from *Arabidopsis* plants that received anaerobic treatment for different lengths of time (numbers represent hours after plants were transferred to anaerobic condition) were subjected to northern blot analysis. The hybridization probes were ^{32}P-labeled cDNA clones of *Arabidopsis GapC*, *GapA*, and *GapB* and genomic clones of *Arabidopsis Adh*, *Xenopus* histone 4 (H4), and soybean 28S rDNA.

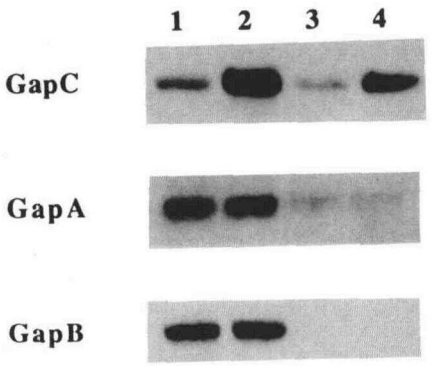

Figure 3. Sucrose effects on expression of GAPDH genes from *Arabidopsis*. Total RNA (10 μg) purified from dark-adapted *Arabidopsis* plants grown under different light and sucrose conditions (for details see "Materials and Methods") were subjected to northern blot analysis and hybridized to ^{32}P-labeled *GapC*, *GapA*, and *GapB* cDNA clones. The growth conditions were: Lane 1, MS medium, light; lane 2, MS medium plus 2% sucrose, light; lane 3, MS medium, dark; lane 4, MS medium plus 2% sucrose, dark.

dium were dark adapted for 5 d and then transferred to a medium containing MS salts plus 2% sucrose, either in continuous white light or in the dark for 24 h. The mRNA levels of the three *Gap* genes from these plants were compared by northern blot analysis. Figure 3 shows that mRNA levels for the *GapC* from plants grown in the presence of 2% sucrose, either in light (lane 2) or dark (lane 4), were at least 10-fold higher than those from plants grown without sucrose (lanes 1 and 3). In contrast, the mRNA levels for the *GapA* and *GapB* genes were not affected by the increased sucrose supply, either in the light (lanes 1 and 2, panels GapA and GapB) or in the dark (lanes 3 and 4). The data also show that light was absolutely required for expression of *GapA* and *GapB* (compare lanes 1 and 2 with lanes 3 and 4), whereas sucrose can mimic light in allowing efficient expression of the *GapC* gene.

Construction of Transgenic Tobacco Containing the *GapC* Promoter/*Gus* Fusion

To identify *cis*-acting regulatory elements of the *GapC* gene, *Agrobacterium* Ti plasmid-mediated transformation was used to construct transgenic tobacco plants containing the chimeric gene that links the 5′-flanking sequences of the *GapC* gene to the *Gus*-coding region (for details, see "Materials and Methods"). To eliminate potential effects of variable polylinker sequences on the translational efficiency of *Gus* messages in different constructs (Taylor et al., 1987), site-directed mutagenesis was used to create an *Nco*I restriction site in the translation initiation codons (ATG) of the *Gus* and *GapC* genes, respectively. The resulting binary vector, therefore, links the DNA fragment containing the 780-bp promoter sequences and the complete 5′-untranslated region of the *GapC* gene to the *Gus* coding sequences. The leaf disc transformation was then used to obtain transgenic tobacco plants. For subsequent experiments, transgenic tobacco plants with leaf lengths of 5 to 10 cm were used.

We randomly chose 10 independent transgenic plants to assay for expression of the *Gus* gene. The GUS activities of these transformants are variable and are not correlated with the copy numbers of the chimeric gene that was inserted into tobacco genomes (data not shown). A high expression line, *GapC*/44, and a medium expression line, *GapC*/14, were propagated vegetatively to produce sufficient isogenic plants for further analysis. The results of heat-shock and anaerobic inductions of the two lines are qualitatively similar. Only the results from *GapC*/44 line are presented below.

Figure 4. Histochemical localization of GUS activity in transgenic tobacco plants containing the *GapC*/*Gus* fusion. A, Cross-section of leaf midrib region (×60). B, Cross-section of leaf petiole (×13). C, Cross-section of stem showing the leaf gap region (×18). D, Cross-section of stem (×31). E, Cross-section of root tip (×67). F, Cross-section of upper root region (×52). G, Longitudinal section of root tip (×22). H, Longitudinal section of upper root region (×22). bl, Leaf blade base; c, cortex; e, epidermis; ep, external phloem; ip, internal phloem; lg, leaf gap; lt, leaf trace; p, pith (for D) or phloem (for F); pp, palisade parenchyma; rc, root cap; ram, root apical meristem; sp, sponge parenchyma; t, trichome; vb, vascular bundle; vc, vascular cylinder; x, xylem.

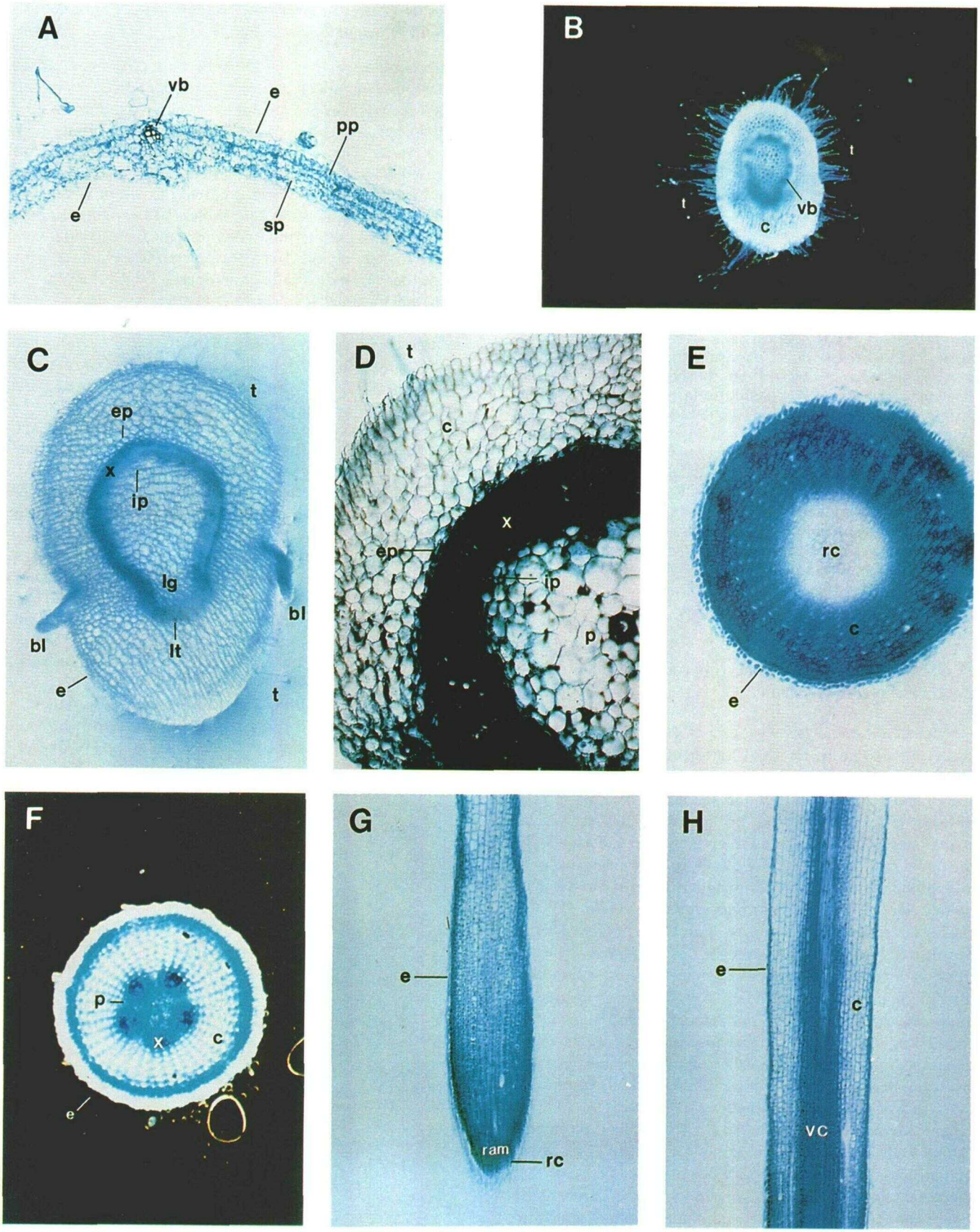

Figure 4. (Legend on facing page.)

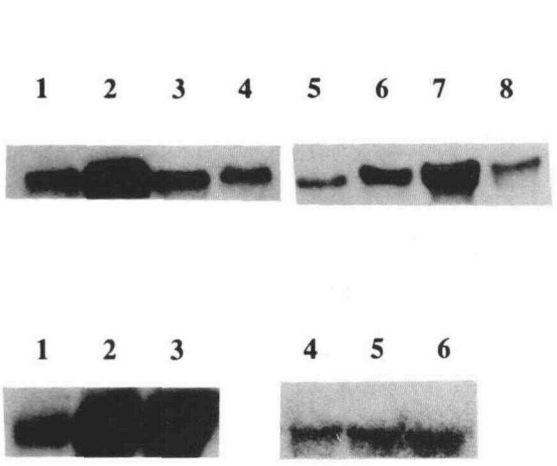

Figure 5. Stress responses of transgenic tobacco plants. a, Total RNA samples (20 μg) isolated from tobacco plants after various durations of different temperature treatments were separated by agarose gel electrophoresis and hybridized with ^{32}P-labeled *Gus* (lanes 1–4) or *GapC* cDNA clones from tobacco (lanes 5–8) as probes. The heat-shock conditions were as follows: Lanes 1 and 5, 22°C; lanes 2 and 6, 42°C, 1 h; lanes 3 and 7, 42°C, 2 h; lanes 4 and 8, 42°C, 4 h. b, Total RNA samples (20 μg) isolated from roots (lanes 1–3) or leaves (lanes 4–6) of anaerobically treated transgenic tobacco plants were subjected to northern blot analysis and hybridized to ^{32}P-labeled *Gus*. The sources of RNA were as follows: Lanes 1 and 4, control plants; lanes 2 and 5, 2 h of anaerobic treatment; lanes 3 and 6, 4 h of anaerobic treatment.

Characterization of Transgenic Tobacco Plants with the *GapC* Promoter/*Gus* Fusion

Histochemical analysis was used to determine patterns of *Gus* expression in the *GapC*/44 transgenic plants. The results show that, although GUS activity can be detected in leaves, stems, and roots, its distribution within each organ is very different (Fig. 4). In the root tip, high GUS activity can be stained uniformly throughout the region (Fig. 4, E and G). In the upper root region, high GUS activity can be detected only in epidermal and xylem cells, whereas there is very low or no detectable GUS activity in the cortex region (Fig. 4, F and H). In stems, petioles, and leaves, high levels of GUS activity can be detected only in xylem cells and trichomes, whereas there is low or undetectable GUS activity in epidermal, mesophyll, and phloem cells (Fig. 4, A–D). These results are quite unexpected, because one would expect constitutive expression of the *GapC* gene, whose products are so-called housekeeping enzymes, in all the cell types. However, there is a consistent pattern in which cells with high respiration rates and/or low photosynthetic activity, such as root tip cells, epidermal cells in roots, and xylem cells of all organs, exhibit high level expression of the *GapC* gene. This distribution pattern is consistent with our previous proposal that the metabolic state of the cell is one of the factors that regulate expression of the *GapC* gene in higher plants (Shih and Goodman, 1988).

Stress Responses of the *GapC* Promoter/*Gus* Chimeric Gene in Transgenic Tobacco

To examine whether the chimeric *GapC*/*Gus* gene can be induced by heat-shock treatment, steady-state levels of *Gus* mRNA from leaves of greenhouse-grown control and stress-treated *GapC*/44 tobacco plants were compared by northern blot analysis. Figure 5a shows that the *Gus* mRNA level increased about 10-fold after 1 h of heat-shock treatment (lane 2) and gradually decreased after 2 to 4 h of heat shock (lanes 3 and 4). The same data show that heat-shock induction of the endogenous tobacco *GapC* gene (lanes 5–8) is very similar to that of the *Arabidopsis GapC*/*Gus* chimeric gene except that the induction of the tobacco *GapC* gene did not reach the maximal level until 2 h after the onset of heat treatment. However, it has to be pointed out that there are multiple copies of *GapC* genes in tobacco (our unpublished results), and it is not known whether all the *GapC* genes respond similarly to environmental stresses. The kinetics and the extent of the heat-shock induction of the *Gus* in the transgenic tobacco are similar to those observed for the *GapC* gene of *Arabidopsis*, suggesting that similar regulatory elements are involved in heat-shock induction in both plants.

To investigate effects of anaerobic induction, *GapC*/44 tobacco plants were transferred from greenhouse to anaerobic conditions for 2 or 4 h. Total RNA isolated from roots and leaves of these plants were used in the northern blot analyses. Previous reports have shown that anaerobic induction of gene expression may show organ specificity, depending on plant species (Russell and Sachs, 1989; Xie and Wu, 1989). Our data show that anaerobic induction of *GapC*/*Gus* in transgenic tobacco occurs primarily in the roots. As illustrated in Figure 5b, the *Gus* mRNA level increased about 10-fold above the basal level after 2 or 4 h of anaerobiosis (lanes 1–3). In contrast, the *Gus* mRNA level remained unchanged in leaves after 2 or 4 h of anaerobic treatment (Fig. 5b, lanes 4–6). These results indicate that patterns of heat-shock and anaerobic induction of *GapC*/*Gus* fusion in the transgenic tobacco plants are similar to those of the *GapC* gene in *Arabidopsis*, and the 820-bp 5′-upstream region of *GapC* is sufficient to confer both heat-shock and anaerobic responses. Therefore, we conclude that transcriptional control is in-

Table I. *GUS activity of GapC/44 transgenic tobacco plants under stress conditions*

The unit for GUS activity is nmol 4-methyl umbelliferone min^{-1} μg^{-1} of protein. The data shown are the average of three measurements.

Treatment Time	Heat-Shock Leaf	Anaerobiosis	
		Root	Leaf
h			
0	0.21 ± 0.04	0.31 ± 0.05	0.21 ± 0.04
1	0.26 ± 0.06	N.D.[a]	N.D.
2	0.40 ± 0.07	0.40 ± 0.07	0.24 ± 0.04
4	0.44 ± 0.07	0.60 ± 0.09	0.18 ± 0.04

[a] N.D., Not determined.

volved in heat-shock and anaerobic induction of *GapC* expression in *Arabidopsis*.

In addition to mRNA levels, heat-shock and anaerobic treatments are known to affect translatability of mRNAs (Sachs et al., 1980; Walker et al., 1987b). To investigate whether these two stress conditions affect translation of the *Gus* mRNAs, GUS enzyme activities from different stress-treated *GapC/44* plants were determined by spectrofluorimetric assays (Jefferson et al., 1987). Table I shows that the GUS activity increased about 2-fold in leaves after 4 h of heat-shock treatment. In contrast, after 4 h of anaerobic condition, the GUS activity increased about 2-fold in roots but remained constant in leaves throughout the experiments. These results are consistent with the northern blot analysis that anaerobic induction of the *GapC* occurs primarily in roots. The timing and extent of the increases in GUS enzyme activity under stress conditions are slower and lower than those of mRNA levels. This is probably due to the high stability of the GUS protein in plants. The fact that the GUS enzyme activity shows significant increase suggests that the *Gus* mRNAs can be translated efficiently under these two stress conditions. However, whether this is due to the intrinsic property of the *Gus* mRNA or the inclusion of the 5′-untranslated region of the *GapC* gene to the *Gus* mRNA in the transgenic plants remains to be elucidated.

DISCUSSION

We have shown that different environmental conditions affect expression of the three GAPDH genes in *Arabidopsis* differently. The steady-state mRNA level of the *GapC* increased about 5- to 10-fold after *Arabidopsis* plants were transferred from normal growth conditions to heat-shock, anaerobiosis, or increased sucrose supply conditions for 2 to 4 h. In contrast, the steady-state mRNA levels for *GapA* and *GapB* genes were unaffected or decreased slightly under heat-shock or increased sucrose conditions. It is surprising that, after an initial decrease, accumulation of *GapA* and *GapB* mRNA can also be induced to 5-fold above their basal levels by anaerobiosis. To the best of our knowledge, they are the first nonglycolytic genes shown to be induced by anaerobic treatment. It has been postulated that under anaerobic stress plants depend solely on the glycolysis for ATP production, whereas the oxidative phosphorylation in mitochondria is inhibited (Roberts et al., 1984; Walker et al., 1987a). The regeneration of NAD^+ or $NADP^+$, which is catalyzed by GAPDH and is required for continued glycolysis and ATP production, becomes the essential step in the anaerobic response. If this were true, it is reasonable to expect that *GapA* and *GapB* would also be induced by anaerobiosis, because their gene products are involved in chloroplast $NADP^+$ regeneration.

Although expression of both *GapA/B* and *GapC* genes can be stimulated by light (Fig. 3), the following observations suggest that different mechanisms may be involved. First, light is absolutely required for expression of *GapA* and *GapB* genes, whereas it is not absolutely required for expression of the *GapC* gene. In the presence of high sucrose in the growth medium, steady-state mRNA levels for the *GapC* gene are relatively high regardless of light conditions. Second, expression of *GapA* and *GapB* genes is organ specific, i.e. the mRNA levels for *GapA* and *GapB* are highest in leaves, lower in stems, and undetectable in roots (H.-B. Kwon and M.-C. Shih, unpublished data). In contrast, *GapC* mRNA can be detected in all three organs, with highest expression level in roots. The mRNA distribution patterns for *GapA* and *GapB* are hallmarks for genes that are regulated by light through receptor-mediated pathways. We have shown that light-regulated expression of *GapA* and *GapB* genes is mediated by both phytochrome and blue light receptors, with blue light the more efficient activator (M.-C. Shih et al., manuscript in preparation).

Histochemical analyses of transgenic tobacco plants containing the *GapC/Gus* chimeric gene yield unexpected results. In the transgenic plants, although GUS activity can be detected in roots, stems, and leaves, its distribution within each organ is cell-type specific. The distribution patterns indicate that nonphotosynthetic cells with high metabolic rates, such as root tip cells, trichomes, and xylem, have the highest levels of GUS activity. These results, in conjunction with effects of sucrose on *GapC* mRNA level, give further support to the idea that *GapC* expression is linked to the metabolic state of plants and light-stimulated *GapC* expression is due to the enhanced metabolic state of the light-grown plants. Whether these effects are regulated at the transcriptional level remains to be determined.

Analysis of the transgenic plants also showed that the 820-bp *GapC* 5′-end flanking region contains sequence information necessary for heat-shock and anaerobic induction. In *Drosophila*, a consensus 5′-CTnGAAnnTTCnAG-3′ sequence (HSE) in the 5′-upstream region of HSP genes binds tightly to an HSF that results in heat-inducible transcription of these genes (Wu et al., 1987). Recently, it was shown that the core sequence of 5′-nGAAnnTTCn-3′ is sufficient to confer heat-shock induction (Wu et al., 1987; Perisic et al., 1989). In plants, similar HSE sequences were found in the promoter regions of the HSP genes from soybean, maize, *Petunia*, *Arabidopsis*, and tomato (Rochester et al., 1986; Wu et al. 1988; Czarnecka et al., 1989; Scharf et al., 1990). A search of the *Arabidopsis GapC* promoter sequences revealed that two inverted 5′-nGAAn-3′ repeats are located 80 bp upstream of the putative TATA box (Y. Yang and M.-C. Shih, unpublished). Deletional and mutational analyses are currently underway to determine the functions of these repeats. However, these results suggest that the *trans*-acting regulatory factors involved in heat-shock induction are highly conserved among eukaryotic organisms.

Although both heat shock and anaerobiosis induce a set of proteins, most of the ANPs are not related to the HSPs (Sachs et al., 1980). Our results indicate that *GapC* can be induced by both heat shock and anaerobiosis. Similar results were obtained in maize in which one of the three cytosolic GAPDH genes, *Gpc3*, can be induced by both heat-shock and anaerobic treatments (Russell and Sachs, 1989). The facts that the *GapC* promoter sequences contain one copy of the consensus ARE (Y. Yang and M.-C. Shih, unpublished) and the *GapC/Gus* chimeric gene can be induced by anaerobiosis in transgenic tobacco plants suggest that the *cis*- and *trans*-acting

regulatory elements are conserved among different plant species. However, anaerobic induction of the *GapC/Gus* chimeric gene can be observed only in roots, and not in leaves, of transgenic tobacco plants. In contrast, the *Adh* gene from rice and the *Gpc3* gene from maize can be induced in both roots and leaves by anaerobic treatment (Russell and Sachs, 1989; Xie and Wu, 1989). This organ-specific induction in the transgenic tobacco plants could be due to either the intrinsic specificity of tobacco or the sequence information contained in the promoter region of the *Arabidopsis GapC* gene. We are currently investigating the anaerobic induction profiles of the tobacco *GapC* genes and transgenic *Arabidopsis* plants containing the same *GapC/Gus* gene fusion to distinguish between these possibilities.

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LITERATURE CITED

Bevan MW (1984) Binary *Agrobacterium* vectors for plant transformation. Nucleic Acids Res **12:** 8711–8721

Chang C, Meyerowitz EM (1986) Molecular cloning and DNA sequence of the *Arabidopsis thaliana* alcohol dehydrogenase gene. Proc Natl Acad Sci USA **83:** 1408–1412

Church G, Gilbert W (1984) Genomic sequencing. Proc Natl Acad Sci USA **81:** 1991–1995

Czarnecka E, Key JL, Gurley WB (1989) Regulatory domains of the Gmhsp 17.5-E heat shock promoter of soybean. Mol Cell Biol **9:** 3457–3463

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem **132:** 6–13

Ferl RJ, Laughner BH (1989) *In vivo* detection of regulatory factor binding sites of *Arabidopsis thaliana Adh*. Plant Mol Biol **12:** 357–366

Ferl RJ, Nick HS (1987) *In vivo* detection of regulatory factor binding sites in the 5′ flanking region of maize Adh1. J Biol Chem **262:** 7947–7950

Freeling M, Bennett DC (1985) Maize Adh1. Annu Rev Genet **19:** 297–323

Jefferson RA, Kavanagh TA, Bevan MW (1987) *GUS* fusion: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J **6:** 3901–3907

Lindquist S, Craig EA (1988) The heat-shock proteins. Annu Rev Genet **22:** 631–677

Maniatis T, Fritsch EF, Sambrook J (1989) Molecular Cloning. A Laboratory Manual, Ed 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Martinez P, Martin W, Cerff R (1989) Structure, evolution, and anaerobic expression of the nuclear gene for cytosolic glyceraldehyde-3-phosphate dehydrogenase (GapC) from maize. J Mol Biol **208:** 551–565

Nickells RW, Browder LW (1988) A role for glyceraldehyde-3-phosphate dehydrogenase in the development of thermotolerance in *Xenopus laevis* embryos. J Cell Biol **107:** 1901–1909

Parker CS, Topol J (1984) A *Drosophila* RNA polymerase II transcription factor specific for the heat-shock gene binds to the regulatory site of an hsp 70 gene. Cell **37:** 273–283

Pelham HR (1985) Activation of heat-shock genes in eukaryotes. Trends Gen Sci **1:** 31–35

Perisic O, Xiao H, Lis JT (1989) Stable binding of *Drosophila* heat shock factor to head-to-head and tail-to-tail repeats of a conserved 5 bp recognition unit. Cell **59:** 797–806

Ricard B, Rival J, Pradet A (1989) Rice cytosolic glyceraldehyde-3-phosphate dehydrogenase contains two subunits differentially regulated by anaerobiosis. Plant Mol Biol **12:** 131–139

Roberts JK, Callis J, Wemmer D, Walbot V, Jardetsky O (1984) Mechanisms of cytoplasmic pH regulation in hypoxic maize root tips and its role in survival under hypoxia. Proc Natl Acad Sci USA **81:** 6029–6033

Rochester DE, Winter JA, Shah DM (1986) The structure and expression of maize genes encoding the major heat shock protein, hsp 70. EMBO J **5:** 451–458

Russell DA, Sachs MM (1989) Differential expression and sequence analysis of the maize glyceraldehyde-3-phosphate dehydrogenase gene family. Plant Cell **1:** 793–803

Sachs MM, Freeling M, Okimoto R (1980) The anaerobic proteins of maize. Cell **20:** 761–767

Scharf KD, Rose S, Zott W, Schoff F, Nover L (1990) Three tomato genes code for heat stress transcription factors with a region of remarkable homology to the DNA-binding domain of the yeast HSF. EMBO J **9:** 4495–4501

Shih M-C, Goodman HM (1988) Differential light regulated expression of nuclear genes encoding chloroplast and cytosolic glyceraldehyde-3-phosphate dehydrogenase in *Nicotiana tabacum*. EMBO J **7:** 893–898

Shih M-C, Heinrich P, Goodman HM (1988) Introns existence predated the divergence of eukaryotes and prokaryotes. Science **242:** 1164–1166

Taylor JL, Jones JD, Sandler S, Muller GM, Bedbrook J, Dunsmuir P (1987) Opitimizing the expression of chimeric gene in plant cells. Mol Gen Genet **210:** 572–577

Walker JC, Howard EA, Dennis ES, Peacock WJ (1987a) DNA sequences required for anaerobic expression of the maize alcohol dehydrogenase-1 gene. Proc Natl Acad Sci USA **84:** 6624–6628

Walker JC, Llewellyn DJ, Mitchell E, Dennis ES (1987b) Anaerobically regulated gene expression: molecular adaptations of plants for survival under flooded conditions. Oxford Surv Plant Mol Cell Biol **4:** 71–93

Webster KA (1987) Regulation of glycolytic enzyme RNA transcriptional rates by oxygen availability in skeletal muscle cells. Mol Cell Biochem **77:** 19–28

Wiederrecht G, Shuey DJ, Kibbe WA, Parker CS (1987) The *Saccharomyces* and *Drosophila* heat-shock transcription factors are identical in size and DNA binding properties. Cell **48:** 507–515

Winter J, Wright R, Duck N, Gasser C, Fraley R, Shah D (1988) The inhibition of Petunia hsp 70 mRNA processing during CdCl stress. Mol Gen Genet **211:** 315–319

Wu C, Wilson S, Walker B, Dawid I, Paisley T, Zimarino V, Ueda H (1987) Purification and properties of Drosophila heat shock activator protein. Science **238:** 1247–1253

Wu CH, Caspar T, Browse J, Lindquist S, Somerville C (1988) Characterization of an Hsp 70 cognate gene family in *Arabidopsis*. Plant Physiol **88:** 731–740

Xiao H, Lis JT (1988) Germline transformation used to define key features of the heat shock response element. Science **239:** 1139–1142

Xie Y, Wu R (1989) Rice alcohol dehydrogenase genes: anaerobic induction, organ specific expression and characterization of cDNA clones. Plant Mol Biol **13:** 53–68