Biochemical and Biophysical Research Communications **278,** 192–196 (2000)
doi:10.1006/bbrc.2000.3732, available online at http://www.idealibrary.com on IDEAL®

Isolation and Characterization of the Gene Encoding Glyceraldehyde-3-Phosphate Dehydrogenase

Mi-Jeong Jeong,[1] Soo-Chul Park, Hawk-Bin Kwon, and Myung-Ok Byun
Division of Molecular Genetics, National Institute of Agricultural Science and Technology, RDA, Suwon, 441-707, Korea

Received September 25, 2000

A 1.2-kb full-length cDNA sequence of a glyceraldehyde-3-phosphate dehydrogenase (GPD) gene was isolated from the mushroom, *Pleurotus sajor-caju*. The full-length cDNA of the *GPD* gene consists of 1248 nucleotides, predicted to encode a 36-kDa polypeptide consisting of 335 amino acid residues. Sequence analysis revealed that the *GPD* gene has more than 72–78% amino acid sequence homology with those of other Basidiomycetes. Expression of the *GPD* gene increased when *P. sajor-caju* was treated with various abiotic stresses, such as salt, cold, heat, and drought. There was an eightfold induction by drought treatment. Salt and cold stress induced four- and twofold induction of *GPD* gene expression, respectively. There was also a fivefold induction by heat stress. The *GPD* gene exhibits different expression patterns under different stress conditions. It reached its maximum expression level within two hours under cold or heat treatment. The mRNA levels of this gene increased proportionally to increasing treatment time under salt or dry conditions. Because the expression of *GPD* was significantly increased, we tested whether *GPD* could confer abiotic stress resistance when it was introduced into yeast cells. For this, a transgenic yeast harboring *P. sajor-caju GPD* was generated under the control of a constitutively expressed GAL promoter. The results from biofunctional analyses with *GPD* yeast transformants showed that *GPD* yeast transformants had significantly higher resistance to cold, salt, heat, and drought stresses.

Key Words: **osmotic stress; *GPD* gene; transgenic yeast.**

Cytosolic glyceraldehyde-3-phosphate dehydrogenase (GPD, EC 1.2.1.12) is an essential enzyme in glycolysis and gluconeogenesis. GPD functions as a tetramer of identical subunits, and converts the NAD^+-dependent oxidative phosphorylation product of glyceraldehyde-3-phosphate to 1,3-bisphosphate glyceric acid. This is one of the most important metabolic reactions in the glycolytic pathway [1–3]. The enzyme also catalyzes the reverse reaction. In *Saccharomyces cerevisiae* and *Aspergillus nidulans*, the GPD protein constitutes up to 5% of the total soluble cellular proteins [1, 3]. In maize, four *GPD* genes, *gpc1*, *gpc2*, *gpc3*, and *gpc4* have been isolated and characterized [4]. A single GPD-encoding gene has been cloned from *A. nidulans* [5] and three structural genes from yeast [6], but multiple copies of *GPD* genes have been reported in higher eukaryotes [7, 8]. The *gpd* genes from *Boletus edulis, Amanita muscaria,* and *Lactarius deterrimus* have been cloned and sequenced [9].

Living cells display rapid changes in the pattern of gene expression when they are exposed to adverse environment stresses, such as heat shock, osmotic shock, oxidative damage, and nutrient depletion. The mechanisms responsible for stress responses and adaptation to heat-shock have been intensively investigated in many organisms, including mammals. Heat shock is known to increase accumulation of the mRNAs of some glycolytic genes in yeast, *Xenopus,* and maize [10–12], and mammalian cells also show increased glycolytic gene expression under anaerobiosis [13]. In addition, it has been shown that the *Gpc 3* and *GapC* genes of maize [12, 14, 15] and the *GapC* gene of *Arabidopsis thaliana* [16] are anaerobically regulated. As in plants, activation of the *gpd* gene occurs in response to oxidative stress in the rabbit aorta [17] and to heat stress and anaerobic stress in halophyte *Atriplex mummularia* [2]. Redkar *et al.* [3] reported that, in *A. nidulans,* transcriptional levels of *GPD* expression increased in cultures that were gradually adapted to NaCl. In the facultative halophyte *Mesembryaemum crystallinum,* the *Gap1* gene is transcriptionally up-regulated during salt stress [18, 19].

Pleurotus sajor-caju is one of the most important edible mushrooms. Despite their economical importance, little study has been carried out regarding the mechanisms for mediating environmental stress responses in this fungus. In this paper, we report the

[1] To whom correspondence should be addressed. Fax: 82-331-290-0392. E-mail: mobyun@niast.go.kr.

0006-291X/00 $35.00

isolation of a *GPD* gene from *P. sajor-caju* and analyses of the gene's expressions under various environmental stress conditions, such as salt, cold, heat and drought. This study also showed that yeast cells overexpressing *P. sajor-caju GPD* gene had higher resistance to abiotic stress tolerance.

MATERIALS AND METHODS

Strain and culture conditions. *Pleurotus sajor-caju* (ASI 2070) was taken from the National Institute of Agricultural Science and Technology, Korea. It was maintained in the mushroom complete medium (MCM; 0.5 g $MgSO_4 \cdot 7H_2O$; 0.46 g KH_2PO_4; 1.0 g K_4HPO_4; 2.0 g Peptone; 2.0 g yeast extract; 20 g glucose per liter) at 28°C. *Escherichia coli* strain DH5α was used for transformation and plasmid propagation. *E. coli* were grown in Luria Bertani (LB) medium at 37°C for plasmid manipulation.

Purification of genomic DNA and RNA from P. sajor-caju. Mycelia of *P. sajor-caju* were harvested by filtration through Whatman #1 filter paper and washed with deionized water. Washed mycelia were frozen in liquid nitrogen and powdered in a mortar. Genomic DNA was isolated from the mycelia using a rapid extraction procedure described by Graham [20]. Total RNA was purified from abiotic-stressed mycelia by a LiCl precipitation protocol adapted from the procedure of Sambrook *et al.* [21].

Construction of cDNA library and phage DNA isolation. Poly(A) RNA was isolated from total RNA using the poly(A) Quik mRNA isolation kit (Stratagene, La Jolla, CA). Synthesis of cDNA was performed using the ZapII cDNA synthesis kit (Stratagene, La Jolla, CA), and a cDNA library was constructed using the ZapII cDNA GigapackII cloning kit (Stratagene, La Jolla, CA). Lambda phage DNA was isolated from the cDNA library by the method described by Sambrook *et al.* [21].

PCR isolation of GPD gene and DNA sequencing. To isolate the *GPD* gene from *P. sajor-caju*, PCR was performed using lambda DNA isolated from a *P. sajor-caju* cDNA library as a template. Sense (GPD-S1; 5′-TGTCAGACTGGTGATTGTGGTGG-3′) and antisense (GPD-AS1; 5′-CTCGATGGAGCCCTTGAAGCG-3′) primers, derived from a conserved region among the *GPD* genes of Basidiomycetes, were synthesized. Polymerase chain reaction (PCR) amplification was performed using a Peltier thermal cycler (PTC 200, MJ Research, USA) for 35 cycles. Each cycle consisted of 30 s at 94°C, 30 s at 48°C and 1 min at 72°C. The amplified fragments were subcloned into the pBluescript $KSII^+$ (pBS; Stratagene) and sequenced. To obtain full length *GPD* cDNA, end-to-end reverse transcription PCR (RT-PCR) was performed. For this RT-PCR, the sense primer (5′-TACGACTCGGTCCACGGCCG-3′) that resides on the 5′ end of the *GPD* gene and a T7 primer were used. The 1.25-kb RT-PCR product was cloned into pBS for DNA sequence determination. The DNA sequences were determined with a T7 sequencing kit (Pharmacia LKB), and analyzed with DNASIS/PROSIS software package (HITACHI), Swiss-prot and Genbank database (NIH).

Abiotic stresses. The mycelia of *P. sajor-caju* that had been grown on MCM medium for two weeks were harvested by filtration. Cold stress was applied by exposing the mycelia to a temperature of 4°C; salt stress by soaking the mycelia in 4 M NaCl solution; heat stress by exposure to a temperature of 45°C; drought by vacuum drying for 10 min followed by placing on a clean bench for 5 h. Abiotic stress condition was continued for 4 h unless indicated. Abiotic stress treated mycelia were collected and immediately frozen in liquid nitrogen for RNA isolation.

RT-PCR and RT-PCR Southern blot analysis. Reverse transcription was carried out in 80 μl aliquots of reaction mixture containing 5× buffer (Promega, Madison, WI), 0.25 mM each of the four deoxynucleotides (dNTPs), 2 μg of synthetic poly-dT primer (5′-TTTTTTTTTTTTTTT-3′), 200 units of Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (Promega, Madison, WI) and 8 μl DNAse-treated RNA, which had been preheated at 72°C for 2 min. The reactions were performed at 37°C for 1 h. The 15 μl sample of first strand cDNA products was then employed as template DNA for the amplification of *GPD* cDNA. RT-PCR product was separated on 0.8% agarose gel and transferred onto Hybond N^+ nylon membrane (Amersham, USA) by the capillary transfer method (21). *GPD* cDNA was used as a probe. Hybridization was carried out for 16 h at 42°C. Filters were washed twice with 2× SSC buffer containing 6 M urea at 42°C, and they were washed again twice with 2× SSC buffer for 5 min at room temperature. For detection of GPD signal, the ECL detection system was used (Amersham, USA).

Construction of yeast expression vector and transformation. The yeast expression vector, pYES2, and the yeast strain INVSC2 (MATα his3$\triangle$1 leu2 trp1-289 ura3-52) were purchased from Invitrogen, Inc. (San Diego, CA). An expression vector containing *GPD* cDNA (pYES2/PsGPD) was constructed by inserting 1.2-kb full-length *GPD* cDNA into *Sac*I/*Eco*RI sites, under the control of a GAL promoter. Transformation of yeast cells with pYES2/PsGPD was performed by the lithium acetate/carrier DNA methods described by Schiestl and Gietz [22]. Briefly, the late log growth-phase yeast cells were harvested, diluted to 2×10^6 cells ml^{-1} in YPD medium, then grown to a concentration of 1×10^7 cells ml^{-1}. After centrifugation, the cells were resuspended in 0.5 ml of 10 mM Tris–HCl/1 mM EDTA/10 mM LiAC (pH 7.5). Yeast cell suspension (100 μl) was transferred to a microfuge tube followed by addition of 1 μl of pYES2/PsGPD vector DNA; 100 μl of single stranded salmon sperm carrier DNA, 600 μl of 40% PEG 4,000 solution (40% PEG 4,000 in 10 mM Tris-HCl, pH 7.5/1 mM EDTA/10 mM LiAC, pH 7.5) were then added. After incubation for 30 min at 30°C with shaking, the cells were heat shocked at 42°C for 15 min. The cells were immediately cooled on ice, and then spun down for 30 s at 12,000 rpm. The cell pellet was resuspended in 0.5 ml of TE, and spread on SC-ura plates [23].

RESULTS

Isolation of GPD Gene from P. sajor-caju

PCR using a *GPD* gene-specific antisense (GPD-AS1):T3 primer set and the GPD sense (GPD-S1):T7 primer set, using lambda DNA isolated from a *P. sajor-caju* cDNA library as template, generated 0.8-kb and 1.4-kb PCR products, respectively. These fragments, presumably 3′ and 5′ regions of *GPD* cDNA, were purified and ligated into pBS for nucleotide sequencing analysis. DNA sequences of these fragments showed high homology to other *GPD* genes of Basidiomycetous fungi. To isolate full-length *GPD* cDNA, end-to-end PCR with *GPD*-specific sense primer that resides at the 5′ end of the *GPD* cDNA and T7 primer was performed using DNA isolated from *P. sajor-caju* cDNA library as template. The sequence analysis revealed that the resulting 1.2 kb PCR product was full-length *GPD* cDNA of *P. sajor-caju*. Genomic Southern blot analysis revealed that there is a single copy of the *GPD* gene in *P. sajor-caju* (data not shown).

Sequence Analysis of P. sajor-caju GPD Gene

The *GPD* cDNA consists of a 1248-bp sequence of nucleotides, including 21-bp of the 5′ untranslated region (UTR), an ATG translation initiation codon, a

FIG. 1. Nucleotide and deduced amino acid sequences of GPD cDNA of *P. sajor-caju* (GenBank Accession No. AF087676).

TGA translation termination codon and a poly(A) tail. This suggests that the cDNA contains the full-length coding sequence (Fig. 1). The full-length cDNA of *GPD* gene contains an uninterrupted open reading frame of 1005 nucleotides, which we predicted encode a 36-kDa polypeptide consisting of 335 amino acid residues.

Homology analysis showed that this cDNA has high sequence homology (72–78%) with known *GPD* genes from Basidiomycetes at amino acid levels (Table 1). It shows about 70% sequence homology with those of Ascomycetes. *GPD*s of *S. cerevisiae* and *Schizosaccharomyces pombe* share 62.7 and 70.9% homology with *P. sajor-caju GPD* cDNA at the amino acid level (Table 1).

Expression Pattern of the GPD Gene under Various Abiotic Stress Conditions

To investigate whether or not the *GPD* gene becomes activated by environmental stresses, such as salt, cold, heat shock and drought, *P. sajor-caju* mycelia that had been cultured in MCM broth for three days at 28°C were transferred to NaCl solution (2 M), low (4°C) and high temperature (45°C) and an evaporator (Bioneer, Chungwon, Korea) for 10 min to dry. The change of expression of *GPD* gene was detected by RT-PCR Southern blot analysis, using RNA isolated from various stress-treated *P. sajor-caju* mycelia. All four stress treatments increased the expression of *GPD* (Fig. 2). The drought treatment (10 min vacuum drying) increased induction of *GPD* gene expression eight-fold. Salt (4 M NaCl for 4 h) and cold (4°C for 4 h) stress induced four- and twofold induction, respectively (Fig. 2B). There was also a fivefold induction by heat stress (45°C for 4 h) (Fig. 2A).

TABLE 1

Analysis of Sequence Homology of the *P. sajor-caju GPD* Gene with Those of Other Microorganisms

Organisms	Identity (%)	
	Nucleotide	Amino acid
Basidiomycetes		
Schizophyllum commune	75.4	78.4
Agaricus bisporus	74.0	75.1
Lentinus edodes	70.0	78.1
Xanthophyllomyces dendrorhous	55.6	73.7
Lactarius deterrimus	53.6	78.2
Boletus edulis	53.4	76.3
Amanita phalloides	53.1	72.5
Ustilago maydis	67.8	74.6
Ascomycetes		
Aspergillus nidulans	59.8	69.8
Aspergillus niger	61.1	68.7
Yeasts		
Saccharomyces cerevisiae	59.3	62.7
Schizosaccharomyces pombe	66.0	70.9

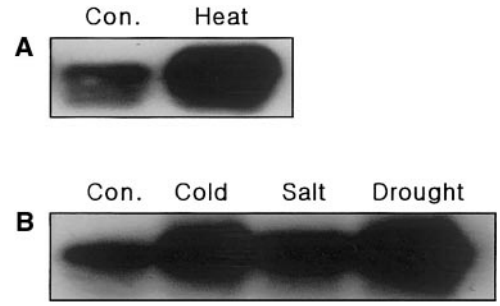

FIG. 2. RT-PCR Southern analysis for the expression pattern of *GPD* gene in the mycelia of *P. sajor-caju* upon various abiotic stresses. (A) Heat shock induced expression of *GPD* gene. The mycelia of *P. sajor-caju* were exposed at 45°C for 4 h. (B) *GPD* gene expression pattern upon salt, cold and drought stresses. For salt and cold stresses the cells were incubated in 4 M NaCl and at 4°C for 4 h, respectively. For drought stress, the cells were vacuum dried for 10 min.

Time-Course of Gene Expression

Since each of the various abiotic stresses significantly increased expression of *GPD* gene, the time-course pattern of gene expression was then tested (Fig. 3). At different time points, mycelia were collected and frozen in liquid nitrogen. RT-PCR Southern blot analysis showed that *GPD* gene expression significantly increased due to environmental stresses. The gene had different expression patterns under different stress

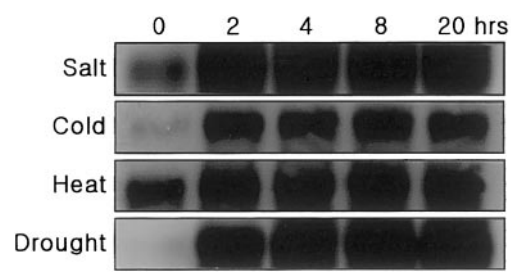

FIG. 3. Time course expression pattern of *GPD* gene in mycelia of *P. sajor-caju* upon various abiotic stresses.

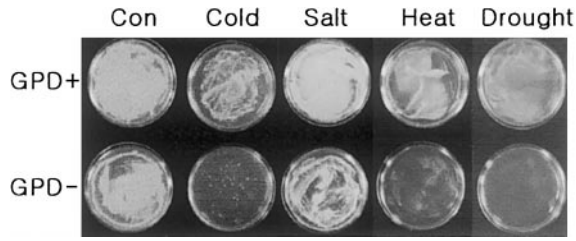

FIG. 4. Biofunctional analysis of yeast cell overexpressing *P. sajor-caju GPD* gene upon various abiotic stresses. Salt, 4 M NaCl treated for 4 h. Cold, incubated at 4°C for 4 h. Heat, treated at 45°C for 4 h. Drought, cells were vacuum dried for 10 min. GPD+ is the transgenic cell transformed with *P. sajor-caju* GPD gene. GPD− is the transgenic cell transformed with pYES2 yeast expression vector only.

conditions. It reached its maximum expression level within 2 h under cold or heat. The mRNA level of this gene increased proportionally with increasing treatment times under either salt or dry conditions.

Biofunctional Assay of GPD Gene in Transgenic Yeast Cell Overexpressing P. sajor-caju GPD

To test whether the *GPD* gene confers various stress resistance to the transgenic yeast cells, GPD transformant yeast cells were cultured in SC-ura medium [23] containing 4% raffinose at 30°C. The cells were harvested by centrifugation for 5 min at 12,000 rpm, and then resuspended in SC-ura medium containing 2% galactose. They were then grown for an additional 16 h for overexpression of *GPD* gene. Cells were collected by centrifugation and washed with 10 mM $MgSO_4$ solution. The resulting cells were used in biofunctional assay. For this experiment, transgenic yeast cells were exposed to cold, salt, heat, and drought stresses. Cold stress was conducted by exposing the cells to 4°C for 5 h. High-salt stress was carried out by resuspending the cells in 4 M NaCl solution and incubated for 5 h at 30°C. For heat stress, cells were incubated in a 55°C water bath for 5 h. Drought stress by vacuum drying the transgenic cells for 10 min. After samples were taken, they were diluted appropriately and then plated on the YPD medium. Colonies were examined after two days of incubation (Fig. 4).

DISCUSSION

Most organisms are inevitably confronted during their life cycle with numerous environmental stresses that can affect their survival. Many genes that mediate response to drought, high salt levels, and high or low temperature have been cloned [24–26]. The proteins encoded by these genes are thought to function in protecting cells from these stresses [27]. Heat-shock and anaerobic stress are known to alter expression of glycolytic genes, suggesting that primary carbon metabolism is one of the pathways that respond to changing environmental condition [2, 16, 28]. Among them is the glyceraldehyde-3-phosphate dehydrogenase (*GPD*) gene, coding for an enzyme in the glycolytic pathway [1–3, 16, 28]. The *GPD* gene from all kinds of organisms, including plants, microbes, and animal is known to be responsive to heat and anaerobic stress [2, 4, 16, 17]. The activation of the *GPD* gene under heat and anaerobic stress condition maybe an adaptive response to heat-stress-related mitochondrial damage, or anaerobic stress-induced inhibition of oxidative phosphorylation. *A. nidulans* accumulates glycerol as a compatible osmolyte and exhibits induced GPD expression in salt-adapted culture [3]. Analysis of the maize *gpc* gene family (*gpc1, gpc2, and gpc3*) for the anoxic response, showed that *gpc1* and *gpc2* transcripts either remained at the aerobic level or decreased with prolonged anoxia. However, the transcript levels of *gpc3* increased and reached 15-fold above the aerobic levels after six hours of anoxia [12]. Transcript levels for Arabidopsis *gpc* not only increased under anaerobiosis, but also during heat shock or increased sucrose supply conditions [16]. This is in addition to an increased transcript accumulation in response to light [29].

In this study, GPD cDNA clone was isolated from *P. sajor-caju* and the expression pattern under various environmental conditions such as cold, salt, heat, and dehydration stresses was analyzed (Fig. 2). The results of the *P. sajor-caju GPD* mRNA expression analysis are interesting, because the expression of this gene is highly induced not only by heat, salt, and dehydration stresses but also by cold stress. The mRNA induction level was higher in salt- or dry-treated mycelia than that in heat- or cold-treated ones. It has also been observed that potato *GapC* mRNA level is regulated by biological stress conditions and elicitor treatments [28]. Thus, it is suggested that this enzyme may function as a mediator of the stress-induced metabolic responses and other integrated metabolic changes during biotic stress. In addition to biotic and abiotic stresses, Abscisic acid (ABA) also increased mRNA levels and enzymatic activity of GPD in the resurrection of plants [30]. Under stress conditions, cellular adjustment requires additional energy for growth, and expression is known of several genes involved in mitochondrial ATP formation. The increased GPD activity mobilizes carbon away from glycerol and into the pathway leading to glycolysis and ATP formation.

The *GPD* gene has a different expression pattern under different stress conditions. It reached its maximum expression level within 2 h under cold or heat, while mRNA levels increased proportionally with increasing treatment time under salt or dry conditions (Fig. 3). These results indicate that the *GPD* gene might use different pathways in different stress situations. This study demonstrated the role of *GPD* in abiotic stress resistance such as heat, salt, dehydration and cold using *GPD* transgenic yeast. A remarkable

increase of survival rate of the *GPD* transgenic yeast can be seen against salt, cold, heat, and dehydration stresses compared with the yeast that was transformed only by the pYES2 vector (Fig. 4). These observations suggest that the *GPD* gene plays an important role in tolerance to various biotic and abiotic stresses. The result with the GPD transgenic yeast experiment is very significant in that it maybe possible to use this gene to make transgenic crop plants having high abiotic stress resistance.

REFERENCES

1. Hirano, T., Sato, T., Okawa, K., and Kanda, K. (1999) Isolation and characterization of the glyceraldehyde-3-phosphate dehydrogenase gene of *Lentinus edodes. Bioscie. Biotechnol. Biochem.* **63**(7), 1223–1227.
2. Niu, X., Wang, H., Bressan, R. A., and Hasegawa, P. M. (1994) Molecular cloning and expression of a glyceraldehyde-3-phosphate dehydrogenase gene in a desert halophyte, *Atriplex nummularia* L. *Plant Physiol.* **104,** 1105–1106.
3. Redkar, R. J., Herzog, R. W., and Singh, N. K. (1998) Transcriptional activation of the *Aspergillus nidulans gpdA* promoter by osmotic signals. *Appl. Environ. Microbiol.* **64,** 2229–2231.
4. Manjunath, S., and Sachs, M. M. (1997) Molecular characterization and promoter analysis of the maize cytosolic glyceraldehyde 3-phosphate dehydrogenase gene family and its expression during anoxia. *Plant Mol. Biol.* **3,** 97–112.
5. Punt, P. J., Dingemanse, M. A., Jacobs-Meijsing, J. M., Pouwels, P. H., and van de Hondel, C. A. M. J. J. (1988) Isolation and characterization of glyceraldehyde-3-phosphate dehydrogenase gene of *Aspergillus nidulans. Gene* **69,** 49–57.
6. McAlister, L., and Holland, M. J. (1985) Differential expression of the three yeast glyceraldehyde-3-phosphate dehydrogenase genes. *J. Biol. Chem.* **260**(28), 15019–15027.
7. Fort, P., Marty, L., Piechaczyk, M., El Sabouty, S., Dani, C., Jeanteur, P., and Blanchard, J. M. (1985) Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate dehydrogenase multi-gene family. *Nucleic Acids Res.* **13,** 1431–1442.
8. Hanauer, A., and Mandel, J. L. (1984) The glyceraldehyde-3-phosphate dehydrogenase gene family: Structure of the human cDNA and of an X-chromosome linked pseudogene: Amazing complexity of the gene family in mouse. *EMBO J.* **3,** 2627–2633.
9. Kreuzinger, N., Podeu, R., Gruber, F., Gobl, F., and Kubicek, C. P. (1996) Identification of some ectomycorrhizal Basidiomycetes by PCR amplification of their *gpd* (Glyceraldehyde-3-phosphate dehydrogenase) genes. *Applied Environ. Microbiol.* **62,** 3432–3438.
10. Lindquist, S., and Craig, E. A. (1988) The heat-shock proteins. *Annu. Rev. Genet.* **22,** 631–677.
11. Nickells, R. W., and Browder, L. W. (1988) A role for glyceraldehyde-3-phosphate dehydrogenase in the development of thermotolerance in *Xenopus laevis* embryos. *J. Cell Biol.* **107,** 1901–1909.
12. Russell, D. A., and Sachs, M. M. (1989) Differential expression and sequence analysis of the maize glyceraldehyde-3-phosphate dehydrogenase gene family. *Plant Cell* **1,** 793–803.
13. Webster, K. A. (1987) Regulation of glycolytic enzyme RNA transcriptional rates by oxygen availability in skeletal muscle cells. *Mol. Cell. Biochem.* **77,** 19–28.
14. Martinez, P., Martin, W., and 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.
15. Ricard, B., Rival, J., and Pradet, A. (1989) Rice cytosolic glyceraldehyde-3-phosphate dehydrogenase contains two subunit differentially regulated by anaerobiosis. *Plant Mol. Biol.* **12,** 131–139.
16. Yang, Y., Kwon, H. B., Peng, H-P., and Shih, M-C. (1993) Stress responses and metabolic regulation of glyceraldehyde-3-phosphate dehydrogenase genes in *Arabidopsis. Plant Physiol.* **102,** 209–216.
17. Ito, Y., Pagano, P. J., Tornheim, K., Brecher, P., and Cohen, R. A. (1996) Oxidative stress increases glyceraldehyde-3-phosphate dehydrogenase mRNA levels in isolated rabbit aorta. *Am. Physiol. Soc.* **279**(2), H81–H87.
18. Schaeffer, H. J., Forstheoefel, N. R., and Cushman, J. C. (1995) Identification of enchander and silencer regions involved in salt-responsive expression of Crassulacean acid metabolism (CAM) genes in the facultative halophyte *Mesembryanthemum crystallinum. Plant Mol. Biol.* **28,** 205–218.
19. Vernon, D. M., Ostrem, J. A., and Bohnert, H. J. (1993) Stress perception and response in a facultative halophyte: The regulation of salinity-induced genes in *Mesembryanthemum crystallinum. Plant Cell Environ.* **16,** 437–444.
20. Graham, G. C. (1994) A simplified method for the preparation of fungal genomic DNA for PCR and RAPD analysis. *Biotechniques* **16**(1), 49–50.
21. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
22. Schiestl, R. H., and Gietz, R. D. D. (1989) High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. *Curr. Genet.* **16,** 339–346.
23. Christine, G. (1991) Guide to yeast genetics and molecular biology. *Methods Enzymol.*
24. Ishitani, M., Xiong, L., Lee, H., Stevenson, B., and Zhu, J-K (1998) *HOS1,* a genetic locus involved in cold-responsive gene expression in Arabidopsis. *Plant Cell* **10,** 1151–1161.
25. Prandle, R., Hinderhofer, K., Eggers-Schumacher, G., and Schoffl, F. (1998) HSF3, a new heat shock factor from *Arabidopsis thaliana,* derepresses the heat shock response and confers thermotolerance when overexpressed in transgenic planta. *Mol. Gen. Genet.* **258,** 269–278.
26. Shinwari, Z. K., Nakashim, K., Miura, S., Kasuga, M., Seki, M., Yamaguchi-Shinozaki, K., and Shinozaki, K. (1998) An *Arabidopsis* gene family encoding DRE/CRT binding proteins involved in low-temperature-responsive gene expression. *Biochem. Biophys. Res. Commun.* **250,** 161–170.
27. Shinozaki, K., and Yamaguchi-Shinozaki, K. (1997) Gene expression and signal transduction in water-stress response. *Plant Physiol.* **115,** 327–334.
28. Laxalt, A. M., Cassia, R. O., Sanllorenti, P. M., Madrid, E. A., Andreu, A. B., Daleo, G. R., Conde, R. D., and Lamattina, L. (1996) Accumulation of cytosolic glyceraldehyde-3-phosphate dehydrogenase RNA under biological stress conditions and elicitor treatments in potato. *Plant Mol. Biol.* **30,** 961–972.
29. Dewdney, J., Conley, T. R., Shih, M-C., and Goodman, H. M. (1993) Effects of blue and red light on expression of nuclear genes encoding chloroplast glyceraldehyde-3-phosphate dehydrogenase of *Arabidopsis thaliana. Plant Physiol.* **103,** 1115–1121.
30. Velasco, R., Salamini, F., and Bartels, D. (1994) Dehydration and ABA increase mRNA levels and enzyme activity of cytosolic GAPDH in the resurrection plant *Craterostigma plantagineum. Plant Mol. Biol.* **26**(1), 541–546.