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# Gene Expression Analysis of Cold and Freeze Stress in Baker's Yeast

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**We used mRNA differential display to assess yeast gene expression under cold or freeze shock stress conditions. We found both up- and down-regulation of genes, although repression was more common. We identified and sequenced several cold-induced genes exhibiting the largest differences. We confirmed, by Northern blotting, the specificity of the response for *TPI1*, which encodes triose-phosphate isomerase; *ERG10*, the gene for acetoacetyl coenzyme A thiolase; and *IMH1*, which encodes a protein implicated in protein transport. These genes also were induced under other stress conditions, suggesting that this cold response is mediated by a general stress mechanism. We determined the physiological significance of the cold-induced expression change of these genes in two baker's yeast strains with different sensitivities to freeze stress. The mRNA level of *TPI1* and *ERG10* genes was higher in freeze-stressed than in control samples of the tolerant strain. In contrast, both genes were repressed in frozen cells of the sensitive strain. Next, we examined the effects of *ERG10* overexpression on cold and freeze-thaw tolerance. Growth of wild-type cells at 10°C was not affected by high *ERG10* expression. However, YEpERG10 transformant cells exhibited increased freezing tolerance. Consistent with this, cells of an *erg10* mutant strain showed a clear phenotype of cold and freeze sensitivity. These results give support to the idea that a cause-and-effect relationship between differentially expressed genes and cryoresistance exists in *Saccharomyces cerevisiae* and open up the possibility of design strategies to improve the freeze tolerance of baker's yeast.**

Baker's yeast (*Saccharomyces cerevisiae*) responds to various stresses during its propagation and industrial application (3, 30). For example, extreme environmental conditions arise during freezing, frozen storage, and thawing of bread dough, resulting in yeast cells with reduced viability and dough-leavening capacity (11, 14). These effects have a great technological and economic impact because the yeast gassing rate is critical in baking. Consequently, the improvement of the freeze tolerance in baker's yeast is of significant commercial importance.

The response of *S. cerevisiae* to cold shock stress has not been characterized in detail, but it is generally accepted that freeze-thaw tolerance correlates with cellular factors including growth phase (39), respiratory metabolism (22), lipid composition of the membrane (10), and accumulation of trehalose (23, 27, 37). Cultural conditions that result in yeast cells with these characteristics, especially high trehalose content, are commonly employed in the production of baker's yeast (3, 30), even though they provide stress resistance only in the absence of fermentable sugars (37). Thus, an additional mechanism(s) is thought to be triggered in response to sharp downshift changes in temperature and to be required for the maintenance of freeze tolerance.

Regulatory systems that control the stress response in *S. cerevisiae* act primarily at the transcriptional level. Adaptation of yeast cells to downshift in temperature also involves control of gene expression. By differential hybridization, a cold-shock-induced gene named *TIP1* (temperature-inducible protein) (18) and two homologues, *TIR1* and *TIR2*, have been identified in *S. cerevisiae* (19). Up-regulation of these genes in response to low temperature has been confirmed elsewhere by comparison of microarrays of mRNA from control and cold-shocked cultures (21). The significance of the up-regulation of these genes is uncertain, however, since triple disruption mutants (Δ*tip1* Δ*tir1* Δ*tir2*) do not show any obvious phenotype (19). The existence of a specific gene expression pattern in *S. cerevisiae* in response to cold and freeze stress and the relationship between this regulation and freeze tolerance are also unclear.

In this work, we have examined differential gene expression induced by cold or freeze shock stress, demonstrating that baker's yeast shows a specific pattern of gene expression in response to a shift to low temperature. Specifically, our approach has revealed a set of genes whose expression was up-regulated, providing clear evidence that some of the identified genes have a functional role in cold and freeze-thaw tolerance of both industrial and laboratory strains of *S. cerevisiae*.

## MATERIALS AND METHODS

**Strains and culture conditions.** Two commercial baker's yeast strains, designated Cinta Roja (Burns Philp) and Plus Vital (Lesaffre); the baker's yeast strain 13bxV4 (29); and the *S. cerevisiae* laboratory strains W303-1A (*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 GAL mal SUC2*), JRY4145 (*MAT*α *ade2 his3-11,15 leu2-3,112 ura3-1*), and JRY4274 (*MAT*α *ade2 his3-11,15 leu2-3,112 ura3-1 erg10*Δ*::LEU2*) (15) were used in this study. Cells were grown at 30°C on an orbital shaker (200 rpm) in medium (1% yeast extract, 2% Bacto Peptone) containing 2% glucose (YPD) or 3% ethanol (YPE) as carbon source. In some experiments, cells were incubated in YNB defined medium (0.17% yeast nitrogen base without amino acids and ammonium sulfate [Difco, Detroit, Mich.] plus 2% glucose and 0.5% ammonium sulfate) supplemented with essential nutrients as needed (32). To examine the effects of different stress conditions, cells were grown in YPD to exponential phase (optical density at 600 nm [$OD_{600}$] = 0.5 to 1.5 or 3.0 to 8.0 for laboratory and industrial yeast strains, respectively), harvested, and transferred to fresh YPD with 0.7 M NaCl (osmotic stress), YPE,

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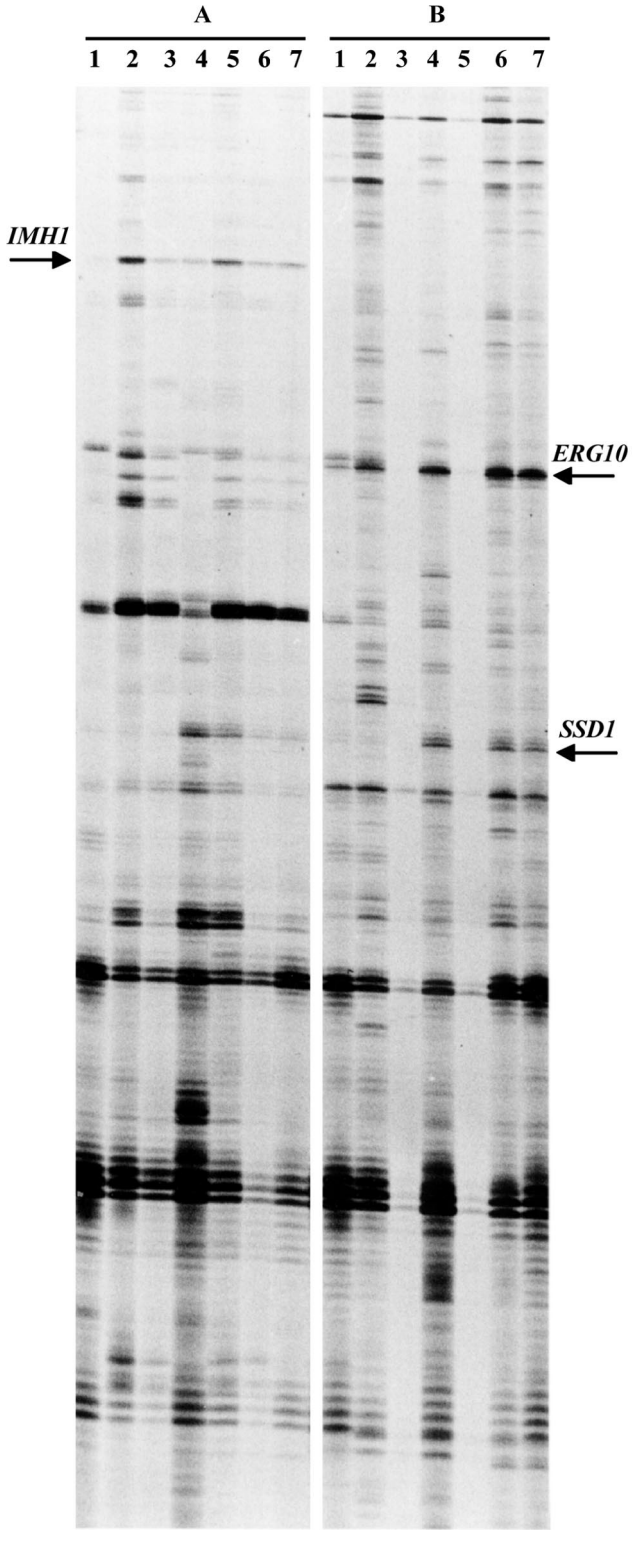


FIG. 1. Cold-induced gene expression analysis with differential display. The figure shows a section of the polyacrylamide gel used to separate two different cDNA pools (A and B) generated by reverse transcription and PCR from RNA samples of cells grown under different physiological conditions. Lanes 1, control cells of the baker's yeast strain 13bxV4 grown in YPD medium to exponential phase ($OD_{600}$ = 3.0 to 8.0); lanes 2, heat-shocked cells (42°C, 30 min); lanes 3 and 4, heat-shocked cells stored at −20°C (overnight) and thawed for 2 h on ice (lanes 3) or at 30°C (lanes 4); lanes 5 and 6, control cells stored at −20°C (overnight) and thawed for 2 h on ice (lanes 5) or at 30°C (lanes 6); lane 7, cold-shocked cells (10°C, overnight). cDNA bands corresponding to some identified cold- and/or freeze-induced genes are indicated by arrows. Results of a representative experiment are shown. Independent experiments revealed a similar pattern of differential display.

or minimal medium lacking ammonium sulfate (nitrogen starvation) or glucose (carbon starvation). Cultures were incubated for 1 or 6 h (YPE) at 30°C. Cold, freeze, and heat shocks were carried out by shifting an aliquot of the YPD cell culture to 10, −20, and 39 (W303-1A) or 42°C, respectively. For freeze stress experiments, the cells were thawed on ice or at 30°C for 1 h. The viability of freeze-stressed cells was determined after dilution by plating cells on YPD agar and incubating them at 30°C for 2 days. Survival rates are expressed as percentages of the numbers of colonies grown in the stressed culture relative to those grown in the nonstressed control.

Yeast cells were transformed by the lithium acetate method (17). The *Escherichia coli* DH10β host strain was transformed by electroporation according to the manufacturer's instructions (Eppendorf). *E. coli* was grown in Luria-Bertani medium (1% peptone, 0.5% yeast extract, 0.5% NaCl) supplemented with ampicillin (50 mg/liter).

**RNA extraction.** Total RNA was isolated from cells grown into early exponential phase or post-diauxic phase or from stressed cells under appropriate conditions. The cells were harvested and washed with ice-cold water, and the RNA was extracted as previously described (32).

**cDNA synthesis and differential display.** Reverse transcription of total RNA from control and cold-shocked cells was performed with four arbitrary 3′ oligo(dT) primers (T1, 5′TTTTTTTTTTT[AC]G3′; T2, 5′TTTTTTTTTTT[AC]A3′; T3, 5′TTTTTTTTTTT[AC]C3′; and T4, 5′TTTTTTTTTTT[AC]T3′; RNAmap Kit; GenHunter, Brookline, Mass.) and Superscript reverse transcriptase (Gibco BRL, San Diego, Calif.) according to the protocol of Liang and Pardee (24). PCR amplification of the cDNA generated in each first-strand synthesis reaction was carried out with the corresponding 3′ oligo(dT) primer combined with five arbitrary 5′ primers (AP1, 5′AGCCAGCGAA3′; AP2, 5′GACCGCTTGT3′; AP3, 5′AGGTGACCGT3′; AP4, 5′CGTACTCCAC3′; and AP5, 5′GTTGCGATCC3′; GenHunter) and [α-$^{35}$S]dATP as radiolabel nucleotide. Twenty cDNA pools were thus obtained for each total RNA sample. cDNAs were resolved by electrophoresis on a denaturing 6% polyacrylamide gel and visualized by autoradiography with Kodak BioMax MR film. The relative abundance of RNAs in cells grown under different physiological conditions was deduced from visual comparison of the intensities of the corresponding cDNA bands.

**Cloning and sequencing of cDNA fragments.** cDNA bands with differential expression patterns were excised from the dry gel, and the cDNA fragments were eluted and used as templates for a second round of PCR with the same pair of primers. The PCR products were cloned into the pGEM-T Easy Vector (Promega, Madison, Wis.) and sequenced with a sequencing kit (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, United Kingdom).

**Construction of the YEpERG10 plasmid.** A DNA fragment containing the coding region and the promoter of *ERG10* was amplified by PCR from *S. cerevisiae* genomic DNA with the specific synthetic oligonucleotides ERG10-1, 5′ACGATTAATGgATccGAGAGGT3′ (*Bam*HI site underlined), and ERG10-2, 5′gAgAAGAgCATGcGAAAATCG3′ (*Sph*I site underlined). The amplified fragment was treated with *Bam*HI and *Sph*I and cloned into the YEplac195 plasmid (12), previously digested with the same set of enzymes, to obtain the plasmid YEpERG10. This plasmid was used to overexpress *ERG10* in the W303-1A and JRY4145 yeast strains. The plasmid YEplac195 was used to generate control transformants.

**Probe labeling and Northern blot analysis.** Probes were generated from cloned DNAs by labeling with [α-$^{32}$P]dCTP (Amersham Pharmacia Biotech) by using the Ready To Go DNA labeling kit (Amersham Pharmacia Biotech). Total RNA samples (10 μg per lane) were separated on formaldehyde-agarose gels, transferred to a Hybond-N nylon membrane (Amersham Pharmacia Biotech), and hybridized with the radioactive probes.

**Enzyme assay.** Cells were harvested, rinsed once with chilled water, resuspended in 0.3 ml of cold homogenization buffer (50 mM Tris-HCl, 1 mM phenylmethylsulfonyl fluoride, pH 8.2), and transferred into a tube containing 1.0 g of glass beads (acid washed, 0.4-mm diameter). The mixture was vortexed at maximum speed for 3 min and then centrifuged at 3,800 × *g* (4°C) for 10 min, and the supernatant was used for further analysis.

Acetoacetyl coenzyme A (CoA) thiolase activity was measured spectrophotometrically as described by Hiser et al. (15). One unit of activity is defined as the amount of enzyme that is required for the conversion of 1 μmol of acetoacetyl-CoA per min at 25°C. The protein content was determined by the method of Lowry et al. (25) with immunoglobulin G as standard.

## RESULTS

**Differential display of cold-induced mRNA.** The differential display technique allows identification of individual mRNAs expressed differentially due to physiological conditions. We evaluated total RNA samples from cells incubated at 10°C (cold shock) or −20°C (freeze stress) and thawed at 0 or 30°C. For cold response, we also analyzed heat-shocked cultures to determine if the specificity of the cold response and the magnitude of the cold induction could be increased by a heat shock pretreatment.

We found 40 to 140 cDNA bands per lane in a differential display gel (Fig. 1). There was a specific cold expression pattern (lanes 5 to 7) that differed from the control (lanes 1) and the heat-shocked (lanes 2) cells. Heat shock (lanes 2) resulted in new cDNA fragments and in changes in intensity for others with respect to the control (lanes 1). In response to cold shock the general intensity of most of the cDNA fragments decreased (lanes 5 to 7), suggesting a general reduction of the transcriptional rate, probably of housekeeping genes. Consistent with this, the reduction was more pronounced for freeze-shocked (lanes 5 and 6) than for cold-shocked (lanes 7) samples.

Between 40 and 60 cDNA bands from the 20 cDNA pools were induced by cold stress (Fig. 1, lanes 5 to 7). The majority of these overamplified PCR products changed similarly in response to cold shock and to heat shock (Fig. 1), suggesting that the cold-induced up-regulation of these cDNAs is mediated by a general stress response mechanism (34). Nevertheless, cells subjected to both stress conditions, heat and then freeze shock (lanes 3 and 4), did not have a higher increase in these cDNAs than did cells that were subjected to only one of the stresses.

**Identification of cold-induced genes and Northern blot analysis.** From the cDNA bands showing both cold- and freeze-induced up-regulation, we selected 18 cDNA fragments for further study. Once sequenced, several of the cloned cDNAs were found to correspond to partial fragments of the same open reading frame. Hence, a total of nine genes, *TPI1*, *MMS2*, *PAK1*, *ERG10*, *SEC11*, *SSD1*, *IMH1*, *YNL278w*, and *YFL030w*, with different functions were identified from the set of 18 cDNAs. Two of the nine genes, *MMS2* and *PAK1*, have roles in DNA repair. *MMS2* encodes a ubiquitin enzyme-like protein and is a member of the yeast error-free postreplication repair pathway (6). *PAK1* encodes a protein kinase that suppresses yeast DNA polymerase mutations (16). Two additional genes, *IMH1* (36) and *SEC11* (5, 40), encode proteins involved in protein transport in yeast. *TPI1*, which encodes triose-phosphate isomerase (1); *ERG10*, the gene for acetoacetyl-CoA thiolase (15); and *YFL030w*, an open reading frame with a protein predicted to be similar to several transaminases, are all probably essential metabolic functions. *SSD1*, an RNase II (RNA-binding) family member, is involved in the tolerance of high concentrations of $Ca^{2+}$ (35). Finally, *YNL278w* (*CAF120*) encodes a protein of unknown function.

We used Northern blots to confirm the expression pattern of the differential display-identified genes (Fig. 2). Transcript levels for *TPI1*, *ERG10*, and *IMH1* were higher in cold- and freeze-shocked baker's yeast cells of strain 13bxV4 than in cells of other strains. Up-regulation of *TPI1* (Fig. 3, second and third lanes from left), *ERG10*, and *IMH1* (data not shown) was also found in cells of laboratory strain W303-1A. This appeared not to be the case for *MMS2*, *SEC11*, and *YFL030w*, which showed no detectable change in their expression under cold or freeze stress conditions (data not shown). Furthermore, no detectable hybridization occurred with the *PAK1*, *SSD1*, and *YNL278w* probes (data not shown), indicating that their level of expression was rather low under any of the conditions tested. These results suggest that the differential display method is able to identify differentially expressed mRNA independently of its prevalence in the mRNA population, as previously reported (38).

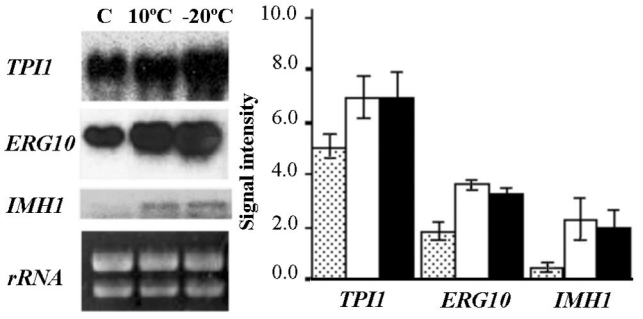


FIG. 2. Verification by Northern blot analysis of cold-induced genes identified by differential display. Cells of the 13bxV4 baker's yeast strain were grown and subjected to cold or freeze-thaw stress (−20 and 0°C, respectively) as described in the Fig. 1 legend. Probes for *TPI1*, *ERG10*, and *IMH1* were generated from cloned DNAs. Total RNA samples (10 μg per lane) from control (lane C and stippled bars), cold-shocked (lane 10°C and open bars), or freeze-shocked (lane −20°C and solid bars) cells were analyzed. The bar graph represents the quantification of the mRNA levels relative to those of rRNA. Values are given as means and standard deviations of three independent experiments.

We also analyzed the expression pattern of *TPI1* in response to heat shock, nitrogen starvation, carbon starvation, ethanol growth, post-diauxic-growth phase, or osmotic stress (Fig. 3). According to the differential display data shown above, the expression of *TPI1* was increased in response to all of the stresses, except for nitrogen starvation (Fig. 3, fifth lane from left). These results further support a general stress response mechanism underlying the cold-shock-induced up-regulation in *S. cerevisiae*.

**The up-regulation of *TPI1* and *ERG10* genes correlates positively with cryoresistance in baker's yeast.** The changes reported above appeared to reflect the requirement for an adjustment of metabolic pathways at low temperatures. Of special interest was the up-regulation of *TPI1* and *ERG10*, since these genes could control key cellular factors in the adaptive response to cold stress. Therefore, we further investigated the significance of the *TPI1* and *ERG10* regulation by analyzing their level of expression in two commercial baker's yeast strains, Cinta Roja and Plus Vital, used for fresh and frozen dough, respectively. As shown in Table 1, the two strains differed in their sensitivities to freeze stress. RNA samples from control and cold- and freeze-shocked cultures of

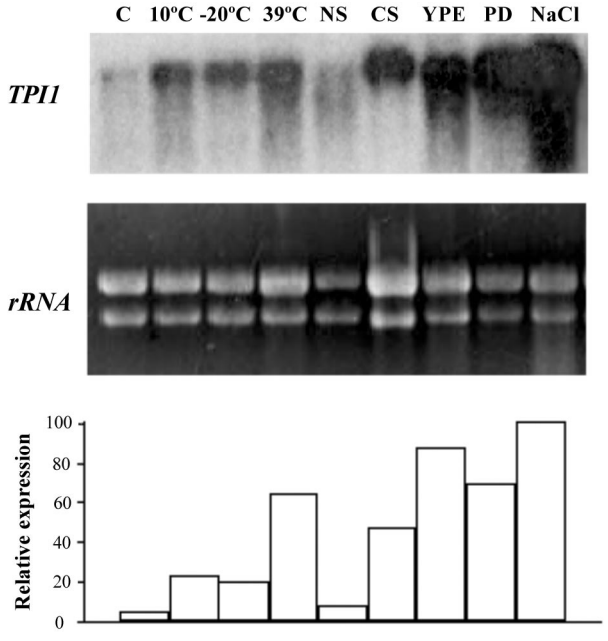


FIG. 3. Northern blot analysis of the expression pattern of *TPI1* under different stress conditions. Total RNA samples (10 μg per lane) from the *S. cerevisiae* laboratory strain W303-1A (C) were grown in YPD to exponential phase ($OD_{600}$ = 0.5 to 1.5) and subjected to cold stress (10°C), freeze-thaw stress (−20 and 0°C, respectively), or heat shock (39°C) or transferred to minimal medium lacking ammonium sulfate (nitrogen starvation [NS]) or glucose (carbon starvation [CS]) or to YP medium with 3% ethanol (YPE) or YPD with 0.7 M NaCl (NaCl). Cells grown in YPD medium to the post-diauxic (PD) phase of growth ($OD_{600}$ = 9.0) were also analyzed. RNA extraction and Northern blot analysis were performed as described in Materials and Methods. The bar graph represents the quantification of the mRNA levels relative to those of rRNA. The highest relative level was set at 100. Results of a representative experiment are shown. Independent experiments revealed identical expression profiles of *TPI1* under the stress and growth conditions tested.

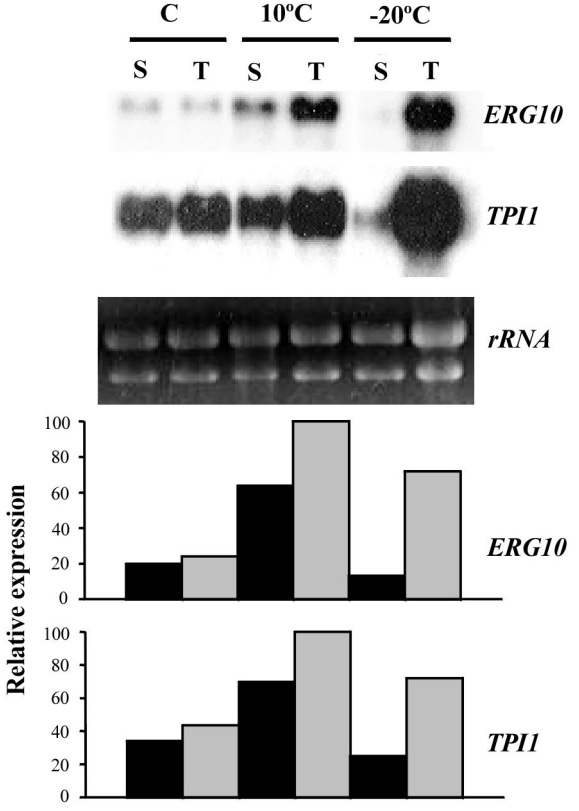


FIG. 4. The induction of *ERG10* and *TPI1* correlates positively with freeze tolerance in baker's yeast. Northern blot analysis of total RNA samples (10 μg per lane) from control (C), cold-stressed (10°C) or freeze-thaw-stressed (−20 and 0°C, respectively) cells of the sensitive (S, black bars) and tolerant (T, gray bars) baker's yeast strains was performed as described in Materials and Methods. The bar graph represents the quantification of the mRNA levels of *ERG10* and *TPI1* relative to those of rRNA. The highest relative level of each gene was set at 100. Results of a representative experiment are shown. Independent experiments revealed similar expression patterns of *ERG10* and *TPI1*.

these strains were analyzed by Northern blotting. Figure 4 shows a higher transcription level of *TPI1* and *ERG10* in cold-stressed than in control samples of both strains. The expression of these genes was also up-regulated in freeze-stressed samples of the tolerant strain. However, both genes were repressed under the same condition in sensitive cells (Fig. 4).

TABLE 1. Susceptibility of exponential and post-diauxic-phase baker's yeast cells to freeze stress

| Strain | Growth phase | Survival (%)[a] | |
|---|---|---|---|
| | | 7 days | 14 days |
| Sensitive | Exponential | 1.0 ± 0.5 | 0.03 ± 0.02 |
| | Post-diauxic | 30 ± 10 | 15 ± 7 |
| Tolerant | Exponential | 14 ± 9 | 0.5 ± 0.4 |
| | Post-diauxic | 88 ± 20 | 84 ± 23 |

[a] Cells of two commercial baker's yeast strains named Cinta Roja and Plus Vital, referred to as sensitive and tolerant strains, respectively, were grown in YPD medium to mid-exponential ($OD_{600}$ = 3.0) or post-diauxic ($OD_{600}$ = 16.0) phase, collected, resuspended in YPD, frozen (−20°C), and stored frozen at the same temperature. At the indicated times, samples were thawed (0°C), diluted, and plated on YPD agar to monitor cell viability.

**Overexpression of *ERG10* induces freeze tolerance.** The results above suggested a positive correlation between up-regulation of *TPI1* and *ERG10* and freeze tolerance in commercial baker's yeast strains. Therefore, we analyzed the effect of the overexpression of *ERG10* in two laboratory strains, W303-1A and JRY4145, on cold and freeze tolerance. Specific acetoacetyl-CoA thiolase activity for the YEpERG10 transformants was significantly higher, from five- to eightfold, than that found for control cells (43.5 ± 10.3 and 51.4 ± 1.0 U/mg of protein in the W303-1A and JRY4145 strains, respectively). As shown in Fig. 5A, this higher level of acetoacetyl-CoA thiolase activity had no effect on growth at 30 or 10°C. However, when cells were frozen at −20°C and stored frozen for 5 days, overexpression of *ERG10* enhanced cell survival, especially for the W303-1A strain (Fig. 5A).

We also examined the effects of a null allele of *ERG10*, *erg10*::*LEU2*, producing no detectable acetoacetyl-CoA thiolase (15). Since mutations in the *ERG10* gene produce mevalonate auxotrophy, the tolerance of the *erg10* mutant was tested in YPD medium supplemented with this compound

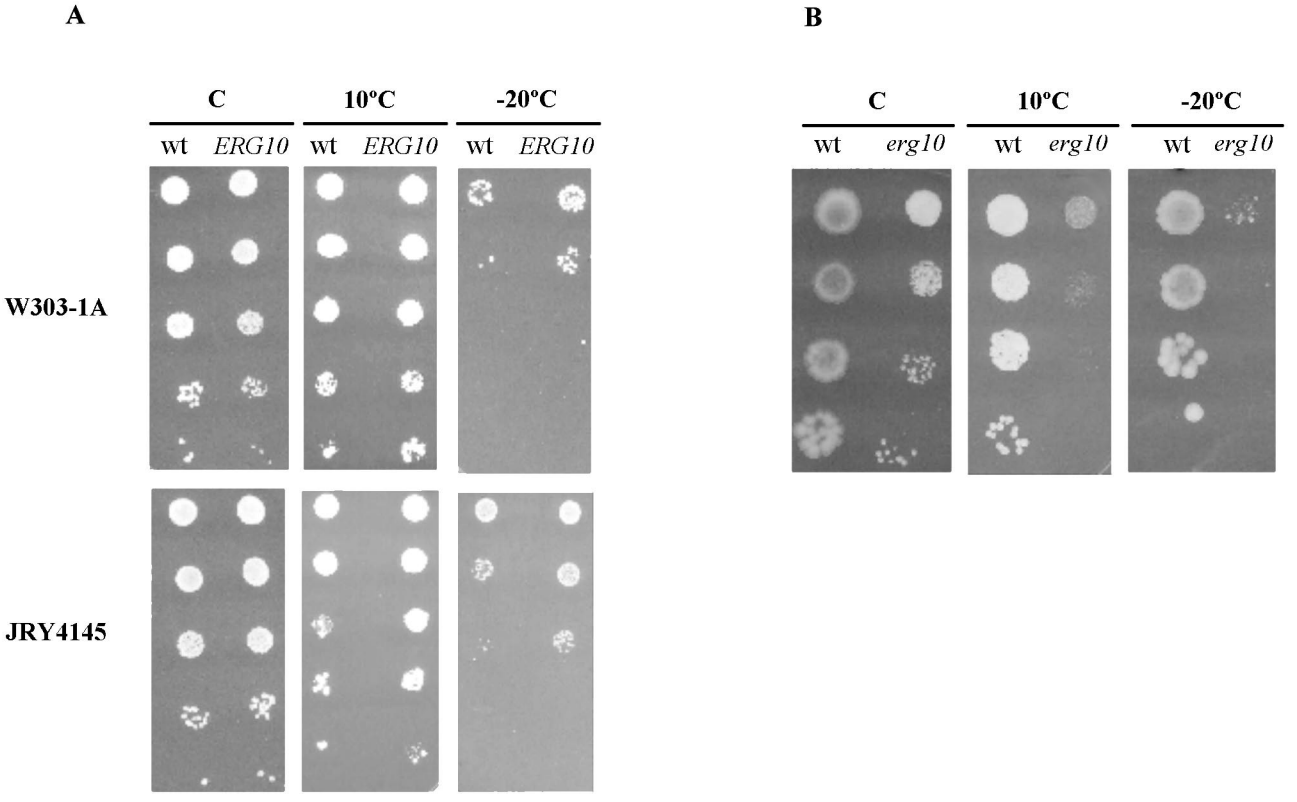


FIG. 5. The expression level of *ERG10* affects cold and freeze tolerance. (A) YEplac195 (wt) and YEpERG10 (ERG10) transformants of the W303-1A and JRY4145 wild-type strains. (B) JRY4145 (wt) and JRY4274 (*erg10*) yeast strains. Cells were grown in YNB medium plus 2% glucose to exponential phase ($OD_{600}$ = 0.3 to 0.5). Having been harvested, cells were resuspended in the same medium (1 $OD_{600}$ U/10 μl of medium), spotted directly onto YNB solid medium, and incubated at 30 (C) or 10°C for 2 or 15 days, respectively. For freeze stress experiments, an aliquot of the cell suspension was frozen at −20°C, stored for 5 days, and thawed at 0°C before cultivation on YNB plates at 30°C for 4 to 6 days. Cells were spotted (5 μl) in a 1:10 dilution series from $10^{-1}$ to $10^{-5}$. Results of a representative experiment are shown. Independent experiments revealed similar growth behavior.

(Fig. 5B). Although the *erg 10* mutant grows more slowly than does the wild type at 30°C (Fig. 5B, subpanel C), exposure to cold and freezing had a far greater killing effect on this mutant than on the wild type. Thus, growth of *erg10* mutant cells was almost abolished at 10°C. Under the same conditions, however, the wild-type strain showed significant growth (Fig. 5B, 10°C subpanel). Similarly, the *erg10* mutant showed a much greater sensitivity to freeze-thaw stress than did the wild-type strain (Fig. 5B, −20°C subpanel). Hence, our results indicate that *ERG10* is required for optimal growth at low temperature and that this function affects freeze sensitivity in yeast cells. High expression of *ERG10* confers freeze tolerance, suggesting that the cold-induced up-regulation of *ERG10* expression provides protection against freeze injury.

## DISCUSSION

In this work, we used the differential display technique to obtain an overview of the transcriptional response to cold and freeze stress in *S. cerevisiae* and to identify genes whose expression responded to these conditions. Our analysis revealed that most of the cold-regulated genes were repressed. These changes should reflect the decreased rate of growth associated with a shift to low temperatures. Indeed, diminished expression of genes involved in fundamental metabolic pathways as a response to stress conditions that result in a decreased rate or arrest of growth is well documented elsewhere (9, 13, 28).

Out of this general pattern, several genes appeared to be induced during the process of adaptation to cold shock. Up-regulation of such genes was also observed upon heat shock, although the magnitude of the cold induction was not altered by a heat shock pretreatment. This overlap probably reflects a general stress response (34) mediated by the transcriptional factors Msn2p and Msn4p (26), as extensively reported elsewhere for other stresses (8). Hence, although repression was the most common component of the transcriptional program of cold adaptation, enhanced production of several proteins could be required to cope with cold and freeze stress.

A total of nine genes were identified by sequencing as cold and freeze induced. However, verification by Northern blot analysis confirmed up-regulation in only three of them: *TPI1*, which encodes triose-phosphate isomerase (1); *IMH1*, encoding a protein involved in protein transport (36); and *ERG10*, the gene for acetoacetyl-CoA thiolase (15). This result could be the consequence of a high false-positive rate associated with the differential display technique, as previously reported (38). Indeed, this situation was confirmed for three genes, *MMS2*, *SEC11*, and *YFL030w*. However, the remaining genes, *PAK1*,

*SSD1*, and *YNL278w*, showed a lack of detection by Northern blotting, suggesting that these genes could fit into the category of differentially expressed rare mRNAs. In this way, it is worth noting that cDNAs identified in differential display are dependent upon the primer used and not on mRNA prevalence (38). Nevertheless, further investigation is required to confirm this point and to understand the physiological role of differentially expressed rare mRNAs in the response to cold shock.

Cold-shock-induced expression of *TPI1* and *ERG10* appeared to confirm the requirement for an adjustment of metabolic pathways in response to a shift to low temperatures. Tpi1p is a key metabolic enzyme, which controls the triosephosphate interconversion, directing the carbon flux from the upper part to the lower part of glycolysis. The level of dihydroxyacetone phosphate is, in turn, key in the formation of glycerol, which is required for osmotic and redox regulation (2, 4). As we show in this work, expression of *TPI1* was induced by different stress conditions. It is difficult, then, to try to rationalize the changes of *TPI1* expression in terms of osmotic adjustment, which might be advantageous in some of the conditions tested, like osmotic stress. In addition, disruption of *TPI1* enhances glycerol production (7) but not glycerol overexpression (S. Rodriguez-Vargas, F. Estruch, and F. Randez-Gil, unpublished data). It has been also reported previously that iron affects *TPI1* expression in *S. cerevisiae* positively by stabilization of the *TPI1* transcript and that this regulation stimulates glycerol production in iron-starved cells (20). Moreover, all the stresses that induce *TPI1* have in common a temporary arrest of growth. Therefore, the induction of *TPI1* could function as a mechanism to avoid overaccumulation of $NAD^+$ under situations, like cold shock, of low glycolytic flux demand.

On the other hand, the up-regulation of *ERG10* may reflect the requirement at low temperatures of higher sterol levels in the cell membrane. Earlier studies with a yeast sterol auxotroph reported a positive correlation between ergosterol and tolerance to heat and ethanol (33). Changes in the expression of genes encoding enzymes in lipid metabolism have been also reported previously for *S. cerevisiae* cells subjected to different stress conditions (9, 31).

An important support for the physiological role of the cold-shock-induced up-regulation of *TPI1* and *ERG10* was the finding that expression of both genes was activated in freeze-stressed samples of a tolerant baker's yeast strain but not in a sensitive strain. This result suggested that the product of these genes is probably required in higher amounts for optimal adaptation to low temperatures. Nevertheless, since the strains tested were not of the same genetic background, the failure of the cold-sensitive cells to increase expression of these genes may show only that the cells have not detected or responded to freeze stress, rather than that Tpi1p and Erg10p confer cold tolerance.

We tried to clarify this point by studying the effects of overexpression of *ERG10*. As we show in this work, high expression of *ERG10* resulted in enhanced freeze tolerance in two *S. cerevisiae* laboratory strains. Moreover, survival of the JRY4145 wild-type strain after freezing and frozen storage dropped dramatically in an *erg10*-null mutant. The growth capacity of mutant cells at 10°C was also sharply diminished. However, no evident phenotype was found at 30°C. Our results, then, clearly indicate that any product of the mevalonate pathway could play an important role in cold and freeze tolerance in *S. cerevisiae* and that cold-induced expression of *ERG10* is required for optimizing sterol content in the membrane.

In our study we show a specific pattern of gene expression upon a shift to low temperatures in *S. cerevisiae*. As with other, better-known stress responses, changes in the expression of some genes seem to be important factors for cold and freeze tolerance in baker's yeast. The finding that high expression of *ERG10* reduces freeze sensitivity in *S. cerevisiae* suggests that overexpression of cold-induced genes could be a useful tool to improve the adaptive response to these stress conditions in industrial strains. However, altered expression levels of multiple genes, not a single gene, are probably required for obtaining fully adapted cells. Further identification of new cold- and freeze-responsive genes will provide valuable information for grouping these genes into response classes and for selecting genes with similar patterns of expression for comparative promoter studies.

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