Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 5853–5857, June 1993
Biochemistry

Proline isomerases function during heat shock

(cyclophilin/immunophilin/stress protein/yeast)

KATHRYN SYKES*†, MARY-JANE GETHING*‡, AND JOSEPH SAMBROOK*

*Department of Biochemistry and ‡Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235-9050

Communicated by Ronald W. Estabrook, March 24, 1993 (received for review January 27, 1993)

ABSTRACT The cyclophilins (CYPs) and FK506 binding proteins (FKBPs) are two families of distinct proline isomerases that are targets for a number of clinically important immunosuppressive drugs. Members of both families catalyze cis/trans isomerization of peptidyl-prolyl bonds, which can be a rate-limiting step during protein folding *in vitro* and *in vivo*. We demonstrate in *Saccharomyces cerevisiae* that heat shock causes a 2- to 3-fold increase in the level of mRNA encoded by the major cytoplasmic CYP gene, *CYP1*. The cloned *CYP1* promoter confers heat-inducible expression upon a reporter gene, and transcriptional induction is mediated through sequences similar to the consensus heat shock response element. Disruption of *CYP1* decreases survival of cells following exposure to high temperatures, indicating that CYP1 plays a role in the stress response. A second CYP gene, *CYP2*, encodes a cyclophilin that is located within the secretory pathway. Its expression is also stimulated by heat shock, and cells containing a disrupted *CYP2* allele are more sensitive than wild-type cells to heat. By contrast, expression of the *FKB1* gene, which encodes a cytoplasmic member of the yeast FKBP family, is neither heat responsive nor necessary for survival after exposure to heat stress.

Folding and assembly of newly synthesized proteins is assisted and perhaps directed by molecular chaperones and catalytic isomerases that are present in all organisms, cell types, and cellular compartments (1). The chaperones apparently stabilize folding intermediates, while the isomerases increase the rate of slow conformational steps. The peptidyl-prolyl isomerases (PPIases) catalyze conversions between the cis and trans rotamers of Xaa-Pro amide bonds of both peptide and protein substrates (2–4). The mammalian PPIases are also intracellular receptors for a group of immunosuppressant drugs that have dramatically advanced organ transplantation by reducing donor graft rejection (5). The cyclophilins (CYPs) are high-affinity receptors for cyclosporin A (CsA), while the FK506 binding proteins (FKBPs) bind FK506 and the related drug rapamycin. In both cases, binding of the drug inhibits isomerase activity of the enzyme (6).

CsA, FK506, and rapamycin are believed to prevent normal activation of T lymphocytes by forming toxic complexes with their cognate PPIases. These complexes interfere with transduction of signals from the cell surface to the nucleus of T cells and induce cell-cycle arrest in both mammalian and fungal cells (7, 8). Several of these complexes bind and inhibit the phosphatase calcineurin A, and apparently calcineurin inactivation is responsible for the effects of the drugs *in vivo* (9, 10). However, the phosphatase does not associate with all drug-bound PPIases and not to any drug-free PPIases (11, 12), leaving the biological partners of the PPIases in the absence of drugs unknown.

The abundance of PPIases, their high degree of sequence conservation, and the multitude of cellular isoforms (6) imply that these enzymes are required for physiological processes other than T-cell signaling. However, mutant strains of yeast that lack one or more proline isomerase genes divide, mate, and sporulate normally (13, 14). In view of their postulated role in protein folding, we reasoned that one or more of the PPIases might be involved in the cellular response to stress. In this paper, we show that expression of two CYP genes, *CYP1* and *CYP2*, is induced in cells exposed to heat and that the cloned *CYP1* promoter contains a functional heat shock element. Furthermore, disruption of either *CYP1* or *CYP2* reduces cell survival following transient exposure to high temperature. These data show that at least some of the proline isomerases are involved in the heat shock response.

MATERIALS AND METHODS

Disruption Strains and Microbiological Methods. The coding regions of the yeast *CYP1*, *CYP2*, and *FKB1* genes were amplified by PCR from yeast genomic DNA with oligonucleotide primers designed from the published coding sequences (15–17) and cloned into bacterial vectors. The *CYP1* coding region was disrupted at nucleotide position +252 with the *URA3* gene. A *CYP2* disruption allele was produced by inserting the *LEU2* gene between positions +243 and +709. The cloned *FKB1* coding region was disrupted by inserting the *HIS3* gene at position +147. All nucleotide positions are numbered relative to the translational start site.

The genomic copy of *CYP1* was disrupted by using a DNA fragment containing the *cyp1*::*URA3* mutated allele to transform yeast strain SC293 (provided by S. A. Johnston, University of Texas Southwestern Medical Center), which generated strain SC293/C1Δ. SC293/C1Δ was mated to strain SeY6210 (provided by S. Emr, University of California, San Diego) to produce the *CYP1*-disrupted strain C1Δ-1 (or KS1/CUa). The *CYP2* gene was genomically disrupted by transforming diploid strain SeY62/D (provided by S. Emr) with a fragment containing the *cyp2*::*LEU2* allele and $LEU2^+$ segregants were selected (C2Δ). The genomic *FKB1* gene was disrupted in strain CC104 (provided by S. A. Johnston) with an *fkb1*::*HIS3* allele (F1Δ). To genomically revert the *CYP1*-disrupted strain to a *CYP1*-expressing one (C1ΔR), pRS-TCYP1 was used to transform strain C1Δ-1. Strain genotypes are summarized in Table 1. Medium preparation and procedures for mating, sporulation, and transformation have been described (18, 19). Recombinant DNA techniques were carried out as described (20). Sites of chromosomal integration were confirmed by PCR. No isomerase gene expression was detectable in the disrupted strains. Wild-type strains SC293, SEy6210, and CC104 were found to express *CYP1*, *CYP2*,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CYP, cyclophilin; FKBP, FK506 binding protein; PPIase, peptidyl-prolyl *cis/trans*-isomerase; HSE, heat shock response element.

†To whom reprint requests should be addressed.

Table 1. Yeast strains and plasmids

Strain	Genotype
SC293	*MAT***a** *ade2-1 his3-Δ200 leu2-3,112 trp1-Δ901 ura3-52*
SEy6210	*MAT***a** *his3-Δ300 leu2,3-112 lys2-801 suc2-Δ9 trp1-Δ901 ura3-52*
C1Δ-1	*MAT***a** *ade2-1 his3-Δ200 leu2-3,112 trp1-Δ901 ura3-52 cyp1::URA3*
C1ΔR	C1Δ-1 *TRP1 CYP1*
C1Δ-2	C1Δ-1 *TRP1*
CC104	*MAT***a** *ade2-1 his3-Δ200 leu2-3,112 ura3-52*
F1Δ	*MAT***a** *ade2-1 his3-Δ200 leu2-3,112 ura3-52 fkb1::HIS3*
C2Δ	*MAT***a** *ade2-1 his3-Δ300 cyp2::LEU2 LYS2 trp1-Δ901 ura3-52*

Plasmid	Parent	Description
pRS-TCYP1	pRS304	*TRP1 CYP1*
pC1PR-WT	pJLB	*CYC1* TATA *URA3* 2-μm ori, wild-type *CYP1* promoter
pC1PR-MUT	pJLB	*CYC1* TATA, *URA3* 2-μm ori, mutated *CYP1* promoter
pC1-HSE	pJLB	*CYC1* TATA *URA3* 2-μm ori *CYP1* HSE
pRS-CYP1	pRS313	*HIS3 CEN6/ARSH4 CYP1*
pMTL-CYP1	pMTL-1	*LEU2 CEN4/ARS1 GAL1* promoter, *CYP1* coding sequence

and *FKB1* by RNA blot analysis and shown to produce CYP1 protein by immunoblot analysis (data not shown).

***CYP1* Promoter and Gene Expression Plasmids.** The 5′ flanking region of *CYP1* was amplified from a *CYP1* genomic clone (7A) with primers, containing *Xho* I sites, spanning nucleotide positions −948 to −923 and −121 to −96. The *Xho* I fragment was inserted into the complementary site of the reporter gene expression plasmid pJLB (21), generating pC1PR-WT. Site-directed mutagenesis (22) of the heat shock response element (HSE)-like sequence generated pC1PR-MUT (see Fig. 3). Oligonucleotides corresponding to the putative HSE region from positions −548 to −522 were synthesized with *Xho* I ends, annealed, and inserted into the *Xho* I site of pJLB (pC1-HSE).

A 4.5-kb *Sal* I fragment containing the *CYP1* gene was isolated from genomic clone 7A. Ligation of the fragment into the integrating vector pRS304 (23) produced pRS-TCYP1, and ligation into the yeast replicating vector pRS313 (23) generated pRS-CYP1. Plasmid MTL-CYP1 was generated by inserting *CYP1* (−30 to +597) 3′ from the *GAL1* promoter in pMTL-1 (a gift of C. Giroux, Wayne State University). CYP1 expression in cells carrying these vectors was determined by immunoblot analysis. Plasmid descriptions are summarized in Table 1.

Cloning and Sequencing of the *CYP1* Promoter. A *Saccharomyces cerevisiae* genomic DNA library (provided by S. Falço, M. Carlson, and D. Botstein, Stanford University) was probed with a radiolabeled fragment containing the *CYP1* coding sequence by colony hybridization techniques (20). A 5.5-kb *CYP1* clone (7A) was sequenced by both the PCR fmol sequencing (Promega) and dideoxynucleotide (Sequenase; United States Biochemical) methods.

Northern Blot Analyses and β-Galactosidase Assays. Total yeast RNA was isolated (19), electrophoresed, and hybridized to radiolabeled DNA as described (20). The filter was exposed to x-ray film and then analyzed on a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) to quantitate band intensities. Protein extracts were prepared and β-galactosidase activity assays were conducted as described (19). Specific activities of the extracts are expressed as nmol of *o*-nitrophenyl β-D-galactoside hydrolyzed per min per mg of extract as described (24).

High-Temperature Heat-Sensitivity Assays. Stationary cultures of yeast were diluted 1:1000 into selective medium and grown at 23°C to early/midlogarithmic phase (OD_{600} = 0.6). An aliquot of cells was removed, diluted with medium, and plated onto selective plates to measure the total number of cells. A second aliquot was removed and diluted into a flask with medium prewarmed in a 48°C waterbath shaker. Cells were removed from the shaker at the time points indicated in the figures, spread onto selective plates, and then grown at 30°C for 2–3 days. The number of colonies that developed was used as a measure of cell survival.

RESULTS

The *CYP1* Gene Transcript Level Is Induced by Heat Shock. The *CYP1* gene encodes the yeast cyclophilin A protein (15). The size and abundance of *CYP1* mRNA before and after heat shock are shown in Fig. 1. The 750-base *CYP1* message is induced in response to a shift in temperature from 23°C to 37°C. After 15 min, *CYP1* RNA levels increase significantly relative to those of unshocked cells and then begin to decline, returning to basal level 60 min after the temperature upshift. Fig. 1 also shows that the yeast stress 70 gene *SSA1* undergoes a classic heat shock response (25), while the transcript level of the yeast actin gene *ACT* is not altered. The time course of *CYP1* RNA induction closely resembles that of *SSA1*. The amounts of *SSA1* and *CYP1* RNA were determined by densitometry to increase 2.8-fold and 2.3-fold, respectively, 15 min following the shift to 37°C (data not shown).

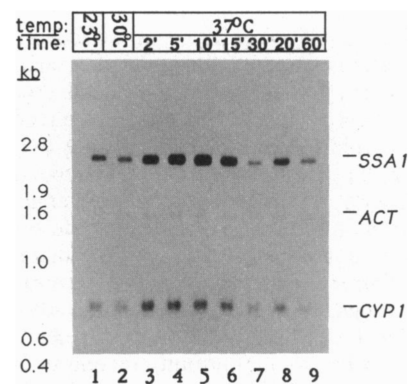

FIG. 1. The *CYP1* gene transcript level is compared to that of *SSA1* before and after stress by Northern blot analysis. CC104 cells were grown at 23°C or 30°C to early/midlogarithmic phase (OD_{600} = 0.5) in YPD liquid medium (2% peptone/1.0% yeast extract/2% glucose). The culture grown at 23°C was heat shocked in a 37°C water bath and eight aliquots were removed during a 1-h time course. Total RNA was isolated from each, electrophoresed through a 1.8% denaturing gel, and transferred to a membrane. The filter was simultaneously hybridized to radiolabeled DNA fragments containing the coding sequences of the *SSA1*, *CYP1*, and *ACT* genes. Previously conducted hybridizations with separated probes identified the 2400-base *SSA1* transcript, the 1600-base *ACT* transcript, and the 750-base *CYP1* message. The identity of the *CYP1* message was confirmed by RNA analysis of a *CYP1*-disrupted strain. The amount of *CYP1* and *SSA1* mRNA was measured by densitometry, normalized to the amount of *ACT* mRNA in each lane, and then given a value relative to the amount isolated from unshocked cells. Lanes contain RNA from cells as follows: 1, grown at 23°C; 2, grown at 30°C; 3–9, grown at 23°C, shifted to 37°C, and harvested after time points from 2 to 60 min as indicated.

The *CYP1* Promoter Contains an HSE-like Sequence. Transcription of heat shock genes in eukaryotes is induced by interactions between the heat shock transcription factor and a cis-acting HSE, located within the promoters of heat-inducible genes (26). To establish whether the promoter of the heat-inducible *CYP1* gene contains this type of transcriptional control element, two independent genomic clones, of 5.5 and 6.5 kb, containing the *CYP1* promoter and coding region were isolated from a yeast library by colony hybridization. The gene coding region and some promoter sequence have been determined (15). The nucleotide sequence of an extended upstream promoter region is shown in Fig. 2. A stretch of 16 nucleotides from position −541 to −526 shows similarity to the HSE consensus, which consists of three or more adjacent 5-bp modular units of NGAAN in alternating orientations (27). The *CYP1* sequence differs from the consensus by only 1 nucleotide insertion between the second and third modular unit. If spacing is maintained, then the *CYP1* sequence differs from the HSE consensus in only 1 of 15 positions.

The *CYP1* Promoter Confers Heat Inducibility upon a Linked Reporter Gene. A fragment containing the putative *CYP1* promoter sequences from position −937 to −111 (see Fig. 2 and ref. 15) was linked 5′ to the *CYC1* TATA box and the *LacZ* reporter gene in a yeast expression vector. The resulting plasmid, pC1PR-WT, was used to transform yeast, and β-galactosidase activity produced by the cells was measured before and after heat shock. Fig. 3 shows that the cloned *CYP1* upstream sequences direct a heat shock response. The magnitude of the increase in β-galactosidase activity (2.4-fold) corresponds closely to the heat-induced increase in *CYP1* RNA (Fig. 1). To establish whether the HSE-like element in the *CYP1* promoter was necessary for heat inducibility, base substitutions were made in positions corresponding to the second and third modular units. Similar mutations have been shown to reduce, but not eliminate, HSE activity in the classic heat shock gene promoter *SSA1* (28). Cells transformed with the mutated *CYP1* promoter-reporter construct, pC1PR-MUT, showed no change in basal β-galactosidase activity but the magnitude of induction after heat shock was reduced from 2.4- to 1.5-fold. To verify that the HSE-like sequence from the *CYP1* promoter was sufficient for heat-induced activity, the putative element was directly linked to the reporter sequences within the expression vector. β-Galactosidase activity increased 2.9-fold after heat shock in yeast strains transformed with the resulting plasmid, pC1-HSE. Cells transformed with the parental plasmid, pJLB, showed no response to heat.

CYP Expression Is Required for Cell Survival Following Temperature Stress. The heat-induced expression of *CYP1* suggested that the viability of cells lacking CYP1 might be altered after exposure to heat. Preliminary experiments

```
   -937                                          -900
ctcgagTCTC ACTCATCGCG AATGTAGTTT TCTTGCCGCC GGTGGCACTA
                                                 -850
ACACCTATGC TTGTCTCCTT CTTCTCCGTC GTGGAGACCT GAATGGGCGC
                                                 -800
GGCGGAAAAG TCTTGGGCGG GTGGCAGACC CTTGAAGTCT CTCTGAATCC
                                                 -750
TCTTTAATTG CGATATGGAG CTGGAAAGGT TGGTGTCGAT GTTGAGGCTG
                                                 -700
GTGTTAGCCA CAGCGGCAGC GTCCGCGTCA CGCGTTGCCG CTGATGGTAT
                                                 -650
TCATGGTACT ACTATCAATA ATGTTCTCCT TCTCATCGAT AAAGCTCTCC
                                                 -600
AGAAATTGAA GAACCTCGTC AGTAGAGACA TGTTGGGGCA GCTGCGTGGC
                                                 -550
ATGAATGACC ACAGGCGTGT TTAGCGTGGT AGCAGTGTTA TTACCTGTTC
                                                 -500
TTCTAGAACC TTTCATCATC TTGTATTATC GAAATCTTAC CTCAGACGTC
```

FIG. 2. Nucleotide sequence of the yeast *CYP1* gene upstream region. Sequences with similarity to the HSE consensus are underlined; repeated trimers are italicized. Nucleotide positions are numbered relative to the translational start site.

Promoter Constructs	β-Galactosidase Activity			
	23°C	37°C	Δ	R
WT ········HSE·········· (tcTTC ta GAA cct TTC at)	75	181	106	2.4
MUT·········//········· (tc TTC ta AAC cgt TTT at)	74	115	41	1.5
HSE ———	10	29	19	2.9
pJLB	8.5	8.6	-	1.0

FIG. 3. β-Galactosidase activities of wild-type and mutated *CYP1* promoter–*LacZ* fusion vectors before and after heat shock. Yeast cells transformed with the reporter plasmids were grown at 23°C to OD_{600} = 0.6 and then split into two cultures; one was maintained at 23°C and one was shifted to 37°C for 1 h. Promoter constructs: WT, pC1PR-WT; MUT, pC1PR-MUT; HSE, pC1HSE. *CYP1* promoter HSE-like region is indicated by a short solid line. Double-hatched lines indicate mutated region. Δ is the unit difference between induced and uninduced (37°C–23°C) activities. R is the ratio of heat shock to basal expression (23°C/37°C). β-Galactosidase units represent average of at least three independent experiments. Activity values obtained from the promoter-reporter plasmids varied <10%; values from double-stranded oligonucleotide reporter plasmid varied <15%.

showed that *CYP1* expression is not required for cell viability after heat shock at temperatures up to 45°C. However, strains carrying the *CYP1* null allele exhibit a lower survival rate than wild-type strains following heat shock at 48°C (Fig. 4).

After 10 min at 48°C, the viability of a culture of the *CYP1*-expressing strain C1ΔR is reduced by 76% (Fig. 4*A Upper*). Viability decreases further as the length of exposure to high temperature increases. After 60 min at 48°C, only 1.1% of the cells survive. However, the congenic strain C1Δ-2 carrying a disrupted *CYP1* gene shows 4- to 5-fold enhanced sensitivity to heat; only 10% of the cells survive exposure to 48°C for 10 min, while only 0.26% are viable after 60 min.

The increased sensitivity to heat of the strain carrying a disrupted *CYP1* allele is abrogated if a wild-type copy of the *CYP1* gene is provided in a replicating vector (pRS-CYP1). Fig. 4*A* (*Lower*) shows (*i*) that 0.1% of the cells harboring the *CYP1* expression plasmid survive exposure to 48°C for 60 min, and (*ii*) that the survival rate of these cells is 4–5 times higher than congenic cells carrying only plasmid sequences (pRS313). The protective effect of the *CYP1* gene is therefore similar whether the gene is carried episomally or in an integrated state.

To dissect the role of CYP1 in the heat shock response, the *CYP1* gene was linked to the regulatable *GAL1* promoter in the yeast expression vector pMTL-1, producing pMTL-CYP1. The ability to control expression of *CYP1* by altering the carbon source of the cells enabled us to test whether CYP1 activity was advantageous during stress or during recovery from stress. The carbon source-dependent activity of the *GAL1* promoter is induced in galactose-, uninduced in glycerol/lactic acid-, and repressed in glucose-containing medium. Fig. 4*B* shows the viability, after exposure to 48°C, of *CYP1*-disrupted cells that have been transformed with either pMTL-CYP1 or the parental vector pMTL-1. Fig. 4*B* (*Upper*) presents the survival of cells induced to express *CYP1* during growth and heat shock and then plated onto either inducing or repressing medium for recovery. Galactose-induced expression of *CYP1* during heat treatment abrogates heat sensitivity; however, continued *CYP1* expression during the subsequent recovery period does not further improve survival (compare Fig. 4*B Upper Left* and *Upper Right*). Fig. 4*B* (*Lower*) shows results obtained from cells

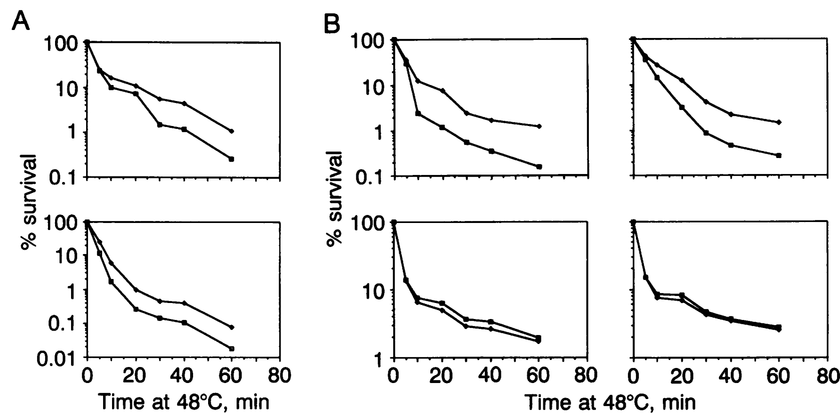

FIG. 4. Strains expressing *CYP1* are more resistant to heat. (*A*) Either chromosomal or episomal expression of *CYP1* can rescue a *CYP1*-disrupted strain. *CYP1*-disrupted vs. wild-type strains: Survival of cells containing a genomically integrated copy of the *CYP1* gene (◆, C1ΔR) is compared to that of cells containing a *CYP1* null allele (▫, C1Δ-2) after exposure to a 48°C heat shock (*Upper*). Plasmid rescue of *CYP1*-disrupted strain: Survival of *CYP1*-disrupted strain C1Δ-1 carrying a plasmid-borne copy of the *CYP1* gene (◆, pRS-CYP1) is compared to that of C1Δ-1 carrying only the parental plasmid (▫, pRS313) after exposure to high temperature (*Lower*). (*B*) Regulated expression of *CYP1* establishes that CYP1 functions before or during heat shock. Survival of *CYP1*-disrupted strain C1Δ-1 carrying either the *CYP1* expression plasmid pMTL-CYP1 (◆) or the parental plasmid pMTL-1 (▫) was compared after exposure to a 48°C heat shock. Expression of *GAL1* promoter-linked *CYP1* was controlled during the period of growth and heat shock and the period of recovery. (*Upper Left*) Cultures were grown and shocked in galactose and then permitted to recover in medium containing galactose. (*Upper Right*) Cultures were grown and shocked in galactose and then shifted to glucose-containing medium for recovery. (*Lower Left*) Cultures were grown and shocked in glycerol/lactic acid and then permitted to recover in medium containing galactose. (*Lower Right*) Cultures were grown and shocked in glycerol/lactic acid and then plated on glucose-containing medium for recovery. Plotted values are an average of two or three independent experiments. Survival rates differed 15–25% between experiments.

grown and shocked in the noninducing, glycerol/lactic acid-containing medium and then permitted to recover on either *CYP1*-inducing or -repressing medium. Cell survival is similar regardless of CYP1 activity during recovery, suggesting that *CYP1* expression only during recovery is not sufficient to improve survival. Alternatively, the glycerol/lactic acid-grown cells may be stress protected and consequently independent of CYP1 activity.

***CYP2*, but Not *FKB1*, Is Involved in the Heat Shock Response.** To determine whether the heat-induced expression and heat-sensitive phenotype of *CYP1* were characteristics common to other PPIases, two other proline isomerases were analyzed. *CYP2* encodes a CYP believed to reside within the secretory pathway (16), while *FKB1* encodes a cytosolic FKBP (13, 17). The effect of heat shock on the expression of these genes is shown in Fig. 5*A*. The 800-base *CYP2* message was determined by densitometry to increase 1.5-fold in abundance 15 min after the temperature upshift and then to return to basal level. The abundance of the 550-base *FKB1* RNA is unaffected by heat. The *FKB1* probe also hybridizes to a 650-base transcript that may be encoded by another member of the FKBP gene family—perhaps the recently isolated *FKB2* (29). This cross-hybridizing mRNA increases in abundance 10 min after the temperature upshift and diminishes during continued exposure to heat. The 650-base transcript is therefore heat inducible and less stable than that of *FKB1*.

Cells carrying a disrupted copy of *CYP2* show the same degree of hypersensitivity to heat as cells lacking *CYP1* (Fig. 5*B Upper*). However, inactivation of *FKB1* has no effect on cell survival following exposure to high temperature (Fig. 5*B Lower*). Strains lacking both (*i*) *CYP1* and *CYP2* and (*ii*) *CYP1* and *FKB1* are no more sensitive to heat than congenic strains lacking either *CYP1* or *CYP2* gene expression (data not shown).

DISCUSSION

The experiments reported here show that (*i*) transcription of two yeast genes coding for CYPs *CYP1* and *CYP2* is induced by heat, while transcription of another proline isomerase gene, *FKB1*, is not. However, the *FKB1* probe detected an additional RNA (perhaps originating from the *FKB2* gene) that is heat inducible. (*ii*) The magnitude of the induction of the CYPs (and the unknown FKB transcript) is approximately the same as that of an authentic heat shock gene (*SSA1*). (*iii*) Induction of *CYP1* is mediated through a cis-acting sequence similar to the well-characterized heat shock element HSE (27). (*iv*) *CYP1* and *CYP2*, but not *FKB1*, encode proteins that facilitate the survival of cells exposed to high temperature. The *CYP1* gene product functions during cellular stress.

Previous work demonstrating that the proline isomerases serve as ligands for immunosuppressant drugs has greatly advanced studies of the mechanisms of immunosuppression and drug action but has given few clues about the normal function of these proteins. Their ubiquity suggests that they must play an essential role in natural cellular processes in the absence of exogenous drugs. In view of their ability to catalyze cis/trans isomerization of peptidyl-prolyl bonds, it seems reasonable to believe that the CYPs and FKBPs are involved in some stage of protein folding. This hypothesis is in agreement with the observation that *ninaA*, a member of the CYP family expressed in the photoreceptor cells of the *Drosophila* eye, is required for proper synthesis and transport of a subset of rhodopsins (30, 31). Furthermore, *CYP3*, a minor mitochondrial isoform of yeast CYP is essential for growth on L-lactate at 37°C (32). Perhaps *CYP3* is required for proper folding of proteins involved in the transport or catabolism of lactate. Finally, one member of the FKBP mammalian family, FKBP59, is known to be heat inducible (33), and it, in addition to cyclophilin A, has been shown to physically associate with chaperone proteins *in vitro* (34, 35).

The parallels between the physiology of proline isomerases and the proteins of the stress 70 family are striking. Both families are highly conserved and universally expressed. The isomerases and stress 70 proteins are found in all cellular compartments with some, such as the cytosol, containing several different family members. Both families contain proteins that are stress inducible and others that are expressed constitutively. Members of the two families facilitate the survival of cells exposed to high temperature. In addition, many stress 70 proteins function as chaperones during protein folding and assembly *in vivo* and at least one CYP not

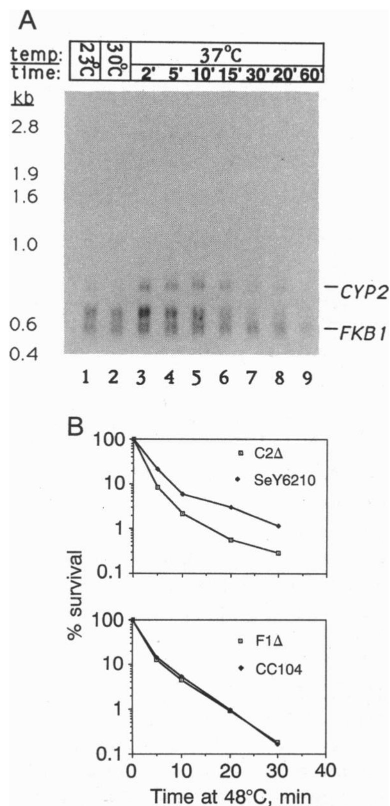

FIG. 5. Expression of *CYP2* is heat-inducible and its gene product is involved in high-temperature stress. (*A*) Northern blot analysis of *CYP2* and *FKB1* messages shows that the transcript level of *CYP2*, but not *FKB1*, is heat responsive. Lanes contain RNA from cells as follows: 1, grown at 23°C; 2, grown at 30°C; 3–9, grown at 23°C, shifted to 37°C, and harvested at time points from 2 to 60 min as indicated. Blot shown in Fig. 1 was stripped and rehybridized with probes corresponding to the *FKB1* and *CYP2* coding regions. Both transcripts were identified, confirmed by analysis of disrupted strains, and quantitated as described in Fig. 1. (*B*) Genomic disruption of *CYP2*, but not *FKB1*, increases heat sensitivity of cells. Survival of cells carrying a normal chromosomal copy (◆) of *CYP2* (*Upper*) and *FKB1* (*Lower*) are compared to cells harboring disrupted alleles (▫) after exposure to 48°C heat shock.

only increases the rate of folding but also prevents aggregation of a protein (carbonic anhydrase) *in vitro* (36). Finally, in the case of the stress 70 proteins, there is strong genetic evidence that the family members expressed in the same compartment carry out overlapping functions. In yeast, for example, it is necessary to disrupt the genes coding for three of four cytosolic hsp70 proteins before a phenotype is unveiled, and expression of other *hsp70* genes are required for growth only at stress temperatures (37). A similar situation seems likely with the CYP family. Several of the genes encoding the cytosolic PPIases of yeast have been disrupted, singly and in combination, with no effect on cell growth and viability under normal conditions. However, until the entire gene family has been cloned and their activities analyzed, the only available assay for *in vivo* function may be to expose cells to extreme stress. This paper shows that at least two of the CYPs carry out a function(s) essential for survival in marginal conditions and opens the way to *in vivo* analysis of mutants of *CYP1*, *CYP2*, and other proline isomerases.

This work was supported by a National Institutes of Health grant to J.S. K.S. was supported by a National Institutes of Health Cardiology training grant. M.-J.G. is a Howard Hughes Investigator.

1. Gething, M.-J. & Sambrook, J. (1992) *Nature (London)* **355,** 33–45.
2. Lang, K., Schmid, F. X. & Fischer, G. (1987) *Nature (London)* **329,** 268–270.
3. Takahashi, N., Hayano, T. & Suzuki, M. (1989) *Nature (London)* **337,** 473–475.
4. Schonbrunner, E. R., Mayer, S., Tropschug, M., Fischer, G., Takahashi, N. & Schmid, F. X. (1991) *J. Biol. Chem.* **266,** 3630–3635.
5. Shevach, E. M. (1985) *Annu. Rev. Immunol.* **3,** 397–423.
6. Schreiber, S. L. (1991) *Science* **251,** 283–287.
7. Schreiber, S. L. & Crabtree, G. R. (1992) *Immunol. Today* **13,** 136–142.
8. Stamnes, M. A., Rutherford, S. L. & Zuker, C. S. (1992) *Trends Cell Biol.* **2,** 272–276.
9. Clipstone, N. A. & Crabtree, G. R. (1992) *Nature (London)* **357,** 695–697.
10. Foor, F., Parent, S. A., Morin, N., Dahl, A. M., Ramadan, N., Chrebet, G., Bostian, K. A. & Nielsen, J. B. (1992) *Nature (London)* **360,** 682–684.
11. Liu, J., Farmer, J. D., Lane, W. S., Friedman, J., Weissman, I. & Schreiber, S. L. (1991) *Cell* **66,** 807–815.
12. Wiederrecht, G., Hung, S., Chan, K., Marcy, A., Martin, M., Calaycay, J., Boulton, D., Sigal, N., Kincaid, R. & Siekierka, J. (1992) *J. Biol. Chem.* **267,** 21753–21760.
13. Heitman, J., Movva, N. R., Hiestand, P. C. & Hall, M. N. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1948–1952.
14. McLaughlin, M. M., Bossard, M. J., Koser, P. L., Cafferkey, R., Morris, R. A., Miles, L. M., Strickler, J., Bergsma, D. J., Levy, M. A. & Livi, G. P. (1992) *Gene* **111,** 85–92.
15. Haendler, B., Keller, R., Hiestand, P. C., Kocher, H. P., Wegman, G. & Movva, N. R. (1989) *Gene* **83,** 39–46.
16. Koser, P. L., Sylvester, D., Livi, G. P. & Bergsma, D. J. (1990) *Nucleic Acids Res.* **18,** 1643.
17. Wiederrecht, G., Brizuela, L., Elliston, K., Sigal, N. H. & Siekierka, J. J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1029–1033.
18. Rothstein, R. J. (1983) *Methods Enzymol.* **101,** 202–211.
19. Rose, M. D., Winston, F. & Hieter, P. (1988) *Laboratory Course for Methods in Yeast Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
20. Sambrook, J. S., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
21. Finley, R. L., Chen, S., Ma, J., Byrne, P. & West, R. W. (1990) *Mol. Cell. Biol.* **10,** 5663–5670.
22. Sarkar, G. & Sommer, S. S. (1990) *BioTechniques* **8,** 404–407.
23. Sikorski, R. S. & Hieter, P. (1989) *Genetics* **122,** 19–27.
24. Rose, M. & Botstein, D. (1983) *Methods Enzymol.* **101,** 167–191.
25. Werner-Washburne, M., Stone, D. E. & Craig, E. A. (1987) *Mol. Cell. Biol.* **7,** 2568–2577.
26. Parker, C. S. & Topol, J. (1984) *Cell* **37,** 273–283.
27. Perisic, O., Xiao, H. & Lis, J. T. (1989) *Cell* **59,** 797–806.
28. Park, H.-O. & Craig, E. A. (1989) *Mol. Cell. Biol.* **9,** 2025–2033.
29. Nielsen, J. B., Foor, F., Siekierka, J. J., Hsu, M.-J., Ramadan, N., Morin, N., Shafiee, A., Dahl, A. M., Brizuela, L., Chrebet, G., Bostian, K. A. & Parent, S. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7471–7475.
30. Stamnes, M. A., Shieh, B.-H., Chuman, L., Harris, G. H. & Zuker, C. S. (1991) *Cell* **65,** 219–227.
31. Colley, N. J., Baker, E. K., Stamnes, M. A. & Zuker, C. S. (1991) *Cell* **67,** 255–263.
32. Davis, E. S., Becker, A., Heitman, J., Hall, M. N. & Brennan, M. B. (1991) *Proc. Natl. Acad. Sci. USA* **89,** 11169–11173.
33. Sanchez, E. K. (1990) *J. Biol. Chem.* **265,** 22067–22070.
34. Ku Tai, P.-K., Albers, M. W., Chang, H., Faber, L. E. & Schreiber, S. L. (1992) *Science* **256,** 1315–1318.
35. Nadeau, K., Das, A. & Walsh, C. T. (1992) *J. Biol. Chem.* **268,** 1479–1487.
36. Freskgard, P. O., Bergenhem, N., Jonsson, B.-H., Svensson, M. & Carlsson, U. (1992) *Science* **258,** 466–468.
37. Craig, E. A. (1990) in *Stress Proteins in Biology and Medicine,* eds. Morimoto, R. I., Tissieres, A. & Georgopoulos, C. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 301–321.