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Heat Shock-Regulated Production of *Escherichia coli* β-Galactosidase in *Saccharomyces cerevisiae*

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The *HSP90* gene of the yeast *Saccharomyces cerevisiae* encodes a heat shock-inducible protein with an M_r of 90,000 (hsp90) and unknown function. We fused DNA fragments of a known sequence (namely, either end of a 1.4-kilobase *Eco*RI fragment which contains the *S. cerevisiae TRP1* gene) to an *Eco*RI site within the coding sequence of the *HSP90* gene. When these fusions are introduced into *S. cerevisiae* they direct the synthesis of unique truncated hsp90 proteins. By determining the size and charge of these proteins we were able to deduce the translational reading frame at the (*Eco*RI) fusion site. This information allowed us to design and construct a well-defined in-frame fusion between the *S. cerevisiae HSP90* gene and the *Escherichia coli lacZ* gene. When this fused gene is introduced into *S. cerevisiae* on a multicopy plasmid vector, it directs the heat shock-inducible synthesis of a fused protein, which is an enzymatically active β-galactosidase. Thus, for the first time, it is possible to quantitate the heat shock response in a eucaryotic organism with a simple enzyme assay.

The yeast *Saccharomyces cerevisiae* undergoes a heat shock response which is similar to that observed in other eucaryotes (1). Upon a rapid shift in cultivation temperature from 23 to 36°C, a coordinate increase is observed in the rate of synthesis of a small subset of proteins which have been termed heat shock proteins (12). This altered pattern of protein synthesis is regulated at the level of transcription (10), although the mechanism of this coordinate transcriptional regulation remains unknown.

We have recently isolated the *S. cerevisiae HSP90* gene which encodes the 90-kilodalton (kdal) yeast heat shock protein (hsp90). The dosage of this single-copy gene may be elevated by introducing the cloned *HSP90* gene back into *S. cerevisiae* on a multicopy plasmid vector with no apparent phenotypic consequence other than the overproduction of hsp90 (9). By modification of the plasmid-borne *HSP90* gene (which allows one to distinguish its gene product from the chromosomal *HSP90* gene product), it has been possible to demonstrate that the overproduction of hsp90 is a direct consequence of transcription of the plasmid-borne gene which is under the same control as its chromosomal counterpart (9).

To examine the mechanisms regulating the *S. cerevisiae* heat shock response it would be desirable to isolate cells harboring mutations which alter this response. As is true for any such genetic approach, these studies would be greatly aided by the availability of a readily scorable mutant phenotype. To provide such a phenotype, we report here the construction of a number of gene fusions between the *S. cerevisiae HSP90* gene and the *Escherichia coli lacZ* gene (which encodes β-galactosidase [β-D-galactoside galactohydrolase, EC 3.2.1.23]) (13). We show that when these fused genes are introduced into *S. cerevisiae*, their expression increases upon heat shock. Furthermore, when this gene fusion has the correct translational reading frame, the resultant gene product has a readily scorable β-galactosidase activity.

MATERIALS AND METHODS

***S. cerevisiae* transformation, growth, labeling, and protein analysis.** *S. cerevisiae* strains SC3 (*MATα trp1-1 Δhis3 ura3-52 gal2 gal10*) and DC5 (*MATa leu2-3 leu2-112 his3 can1-11*) were used as transformation recipients for *TRP1*- and *LEU2*-bearing plasmids, respectively. Yeast transformation was performed by the procedure of Beggs (2). Transformants were selected and maintained on a defined nutritionally selective medium with 2% glucose as a carbon source (9). Growth, heat shocking, pulse labeling with [^{35}S]methionine, preparation of sodium dodecyl sulfate (SDS)-soluble proteins, gel electrophoresis, staining, and autoradiography have all been previously described (12). For the isolation of SDS-soluble proteins from cells grown on plates, colonies were transferred to water with a toothpick to give a concentration of 2×10^7 cells per ml, and protein isolation was carried out

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TABLE 1. Plasmids used

Plasmid	Relevant characteristics[a]	Reference or source
pUTX1	Subclone of 1.85-kb *Hin*dIII-*Eco*RI fragment containing the 5′ portion of *HSP90*	(9)
YRp7	Ap^r Tc^r, source of *TRP1* DNA	(15, 16)
pUTX1/*TRP1*	*TRP1* counterclockwise	This work
pUTX1/*TRP1′*	*TRP1* clockwise	This work
pUTX8	Deletion of 220-bp *Kpn*I fragment from pUTX1	This work
pUTX8/*TRP1*	*TRP1* counterclockwise	This work
pUTX8/*TRP1′*	*TRP1* colckwise	This work
pUTX9	Deletion of 710-bp *Xba*I fragment from pUTX1	This work
pUTX9/*TRP1*	*TRP1* counterclockwise	This work
pUTC37	$2\mu^+$, *LEU2*	This work[b]
pMC1403	Source of 5′-deleted *lacZ* gene	(6)
pUTX40	Out-of-frame *HSP90-lacZ* fusion	This work
pUTX41	In-frame *HSP90-lacZ* fusion	This work

[a] Unless otherwise noted, all plasmids confer ampicillin resistance (Ap^r) but not tetracycline resistance (Tc^r).
[b] This plasmid is our equivalent construction of plasmid CV7 of Broach and Hicks (4).

exactly as described previously for liquid cultures (12). Stained gels were scanned at 625 nm with a Transidyne 2955 scanning densitometer.

***E. coli* growth, transformation, and plasmid isolation.** Growth and transformation of *E. coli* K-12 strain MC1066 (*leuB600* Δ[*lacIPOZY*]*X74 trpC9830 rpsL pyrF74*::Tn*5*) have been previously described. Plasmids were isolated as described (9).

β-Galactosidase. For detection of β-galactosidase on plates, the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-galactose (XGal) was employed (13). For detection in *E. coli*, XGal (40 μg/ml) was included in M9 plates supplemented with 0.4% Casamino Acids, tryptophan (50 μg/ml), leucine (30 μg/ml), uracil (30 μg/ml), and ampicillin (25 μg/ml). For detection in *S. cerevisiae*, the formulation of XGal plates was that of Rose et al. (14).

For the assay of β-galactosidase in liquid cultures, 50- to 100-μl samples of actively growing cells were diluted directly into 1 ml of Z buffer (13) and permeabilized by vortexing after the addition of 0.05 ml of $CHCl_3$ and 0.02 ml of 0.1% SDS (11), and *o*-nitrophenol-β-D-galactoside–hydrolyzing activity was measured as described (13). Activity is expressed as nanomoles of *o*-nitrophenol β-D-galactoside hydrolyzed per minute per milligram of protein (where an absorbance at 420 nm of 0.0045 represents 1 nmol of *o*-nitrophenol [13]). Protein content was related to cell turbidity by using as conversion factors 1.4 units of absorbency at 600 nm of *E. coli* = 150 μg of protein (13) and 1 Klett unit (Klett-Summerson colorimeter [660-nm filter]) of *S. cerevisiae* = 2 μg of protein (8).

Plasmids. The various plasmids used in the experiments reported here are listed in Table 1. Plasmids pUTX8 and pUTX9 were produced by the ligation of 1 μg of *Kpn*I-digested or *Xba*I-digested pUTX1, respectively, in a volume of 100 μl of 50 mM Tris-hydrochloride (pH 7.4)–10 mM $MgCl_2$–10 mM dithiothreitol–1 mM ATP in the presence of 1 μl of T4 DNA ligase (New England Biolabs) for 16 h at 14°C. The ligation mixtures were used to transform *E. coli* MC1066, and transformants were screened for the appropriate deletion by restriction enzyme analysis with *Eco*RI and *Hin*dIII. The 1,453-base-pair (bp) *S. cerevisiae TRP1* DNA segment was transferred from the Tc^r plasmid YRp7 to the Tc^s plasmids pUTX1, pUTX8, and pUTX9 by ligating 2 μg of *Eco*RI-cut YRp7 and 2 μg of the desired *Eco*RI-cut recipient plasmid in a volume of 60 μl. Trp^+ transformants of *E. coli* MC1066 were selected and screened for sensitivity to tetracycline. The orientation of the *TRP1* DNA insert was determined by restriction analysis with *Hin*dIII (*TRP1* DNA is cut asymmetrically by *Hin*dIII [15]). The orientations are designated as *TRP1*, for insertion in the same direction (relative to the pBR322-derived sequences) as that in YRp7 (corresponding to counterclockwise transcription of the gene [16]), or as *TRP1′*, for insertion in the opposite orientation.

For the construction of the out-of-frame *HSP90-lacZ* gene fusion (pUTX40), which involved a three-fragment ligation, the appropriate fragments were purified by agarose gel electrophoresis before ligation. Samples of 2.5 μg of pUTX1 (containing the 3′-deleted *HSP90* gene), pMC1403 (containing the 5′-deleted *lacZ* gene), and pUTX37 (containing both *S. cerevisiae* and *E. coli* selectable markers and replication origins) were digested with *Hin*dIII plus *Eco*RI, *Eco*RI plus *Sal*I, and *Hin*dIII plus *Sal*I, respectively. The appropriate purified fragments (see Fig. 5) were mixed and ligated in a volume of 60 μl, as described above. *E. coli* MC1066 was transformed with 3 μl of the ligation mixture and spread on XGal plates containing ampicillin. After 5 days of incubation at 37°C, ca. 50% of the colonies showed a very faint greenish color. The plasmids isolated from most of these colored colonies were identical, and one was designated pUTX40 (Fig. 5).

Plasmid pUTX41 was constructed by filling in the cohesive termini of 1 μg of *Bam*HI-cleaved pUTX40 in an incubation buffer (50 μl of 50 mM Tris-hydrochloride [pH 7.8]–5 mM $MgCl_2$–10 mM β-mercaptoethanol–20 mM each of dATP, dGTP, dCTP, and dTTP) containing 5 U of *E. coli* DNA polymerase I (large fragment; New England Biolabs) for 30 min at room temperature. After ethanol precipitation, the DNA was recircularized by incubation for 16 h at 14°C in 60 μl of the buffer used for cohesive end ligation (see above) in the presence of 3 μl of T4 DNA ligase. After

a further digestion with *Bam*HI (to reduce the background of plasmids with an unfilled *Bam*HI site) the entire mixture was used to transform *E. coli* MC1066. Approximately 10% of the transformants (plated on XGal plates containing ampicillin) showed a blue color within 24 to 36 h of growth at 37°C. Plasmids isolated from a number of these blue colonies had a restriction map identical to that of pUTX40, with the exception of the absence of a *Bam*HI cleavage site. One of these plasmids, designated pUTX41, was subjected to a further analysis to confirm that the *Bam*HI site had been correctly filled. Filling of a *Bam*HI site is expected to generate the sequence GGATCGATCC, which contains the recognition sequence for the restriction enzyme *Cla*I (underlined). This sequence is not cleaved by *Cla*I when isolated from a *dam*$^+$ host such as *E. coli* MC1066 (owing to methylation of adenines which occurs in the sequence GATC), but is rendered susceptible to *Cla*I cleavage when isolated from a *dam* host such as *E. coli* GM33. Plasmid pUTX41 exhibited the expected *dam*-dependent *Cla*I cleavage site, which mapped to the same location as the absent *Bam*HI site, thus confirming that the *Bam*HI site had been properly filled.

RESULTS

To place the *E. coli lacZ* gene under the control of the *S. cerevisiae HSP90* gene it is necessary to fuse an appropriately 5′-deleted *lacZ* gene into the coding region of the *HSP90* gene. If the translational reading frame established by the proximal (*HSP90*) gene fragment is the same as the open reading frame of the distal (*lacZ*) gene fragment, it might be expected that the product of such a fused gene would have β-galactosidase activity.

Figure 1 presents a restriction map of the 5.6-kilobase (kb) *Hin*dIII fragment of *S. cerevisiae* DNA which we have shown to contain the *HSP90* gene (9). On the basis of previous studies it has been deduced that the transcription of the *HSP90* gene proceeds from left to right and that the coding region of this gene spans the leftmost *Eco*RI site of this DNA. Thus, the 1.85-kb *Hin*dIII-*Eco*RI fragment of DNA provides a possible 3′-deleted *HSP90* gene to be used for gene fusion. As a 5′-deleted *lacZ* gene containing an *Eco*RI site suitable for gene fusion has been constructed by Casadaban et al. (6), it was necessary to determine whether the translational

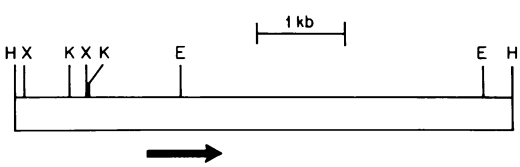


FIG. 1. Restriction map of the *S. cerevisiae HSP90* gene. Restriction sites: H, *Hin*dIII; E, *Eco*RI; X, *Xba*I; and K, *Kpn*I. Arrow indicates the direction of transcription of this gene.

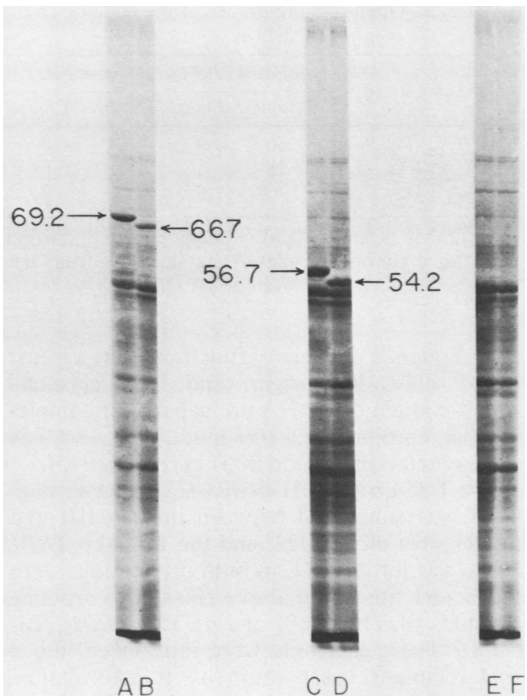


FIG. 2. Electrophoretic analysis of fused proteins. *S. cerevisiae* SC3 transformed with various *TRP1*-bearing plasmids was grown for 48 h on agar plates containing nutritionally selective growth media. Cells were removed from the plates, and SDS-soluble proteins were extracted as described in the text and subjected to electrophoresis on an SDS-10% polyacrylamide slab gel. The gel was stained with Coomassie brilliant blue. The resident plasmids are pUTX1/*TRP1* (lane A), pUTX1/*TRP1*′ (lane B), pUTX8/*TRP1* (lane C), pUTX8/*TRP1*′ (lane D), pUTX9/*TRP1* (lane E), and YRp7 (lane F). Arrows indicate the position of plasmid-encoded proteins.

reading frame at the *HSP90 Eco*RI site was compatible with that of the 5′-deleted *lacZ* gene.

Translational reading frame in *HSP90*. To determine the translational reading frame at this *Eco*RI site, we have exploited the observation that, when introduced into *S. cerevisiae* on a multicopy plasmid, the 1.85-kb *Hin*dIII-*Eco*RI fragment of the *HSP90* gene produces a readily detectable truncated hsp90 protein (9). It was reasoned that if two different, defined DNA sequences were joined to this *Eco*RI site, then a comparison of the differences in the resulting truncated hsp90 proteins would allow us to deduce the translational reading frame at the *Eco*RI site. The two DNA sequences employed for these experiments were the opposite ends of the 1,453-bp *Eco*RI-generated *S. cerevisiae* DNA insert of plasmid YRp7 (16). In addition to providing a defined sequence, this fragment of DNA carries the *S. cerevisiae TRP1* gene as well

TABLE 2. Open reading frames at the ends of *TRP1* DNA

Reading frame	Forward (*TRP1*)		Reverse (*TRP1'*)	
	Residues[a]	Charge	Residues	Charge
1 (G AAT TC . . .)	16	0	4	0
2 (GA ATT C . . .)	18	5^+	41	6^+
3 (GAA TTC . . .)	26	6^+	6	0

[a] Number of amino acid residues encoded starting with the underlined triplet. Data deduced from the published sequence of *TRP1* DNA (16).

as a sequence capable of functioning as a yeast DNA replication origin, and thus plasmids which contain this DNA are capable of complementing chromosomal *trp1* mutations as well as autonomous replication in *S. cerevisiae* (15).

The 1.85-kb *Hin*dIII-*Eco*RI *HSP90* gene fragment was subcloned between the *Hin*dIII and *Eco*RI sites of pBR322, and the 1,453-bp *TRP1* DNA was introduced, in both orientations, into the *Eco*RI site of the above plasmid to produce plasmids pUTX1/*TRP1* and pUTX1/*TRP1'* (Table 1). These plasmids were introduced into a *trp1* recipient yeast strain by transformation, and transformants were selected by the phenotype of tryptophan-independent growth. Transformants were grown on plates, and SDS-soluble proteins, isolated from whole-cell lysates, were displayed by SDS-polyacrylamide slab gel electrophoresis (Fig. 2). An examination of this stained gel reveals that whereas the pUTX1/*TRP1*-transformed cells contained an abundant protein of 69.2 kdal (Fig. 2, lane A), this protein was absent in the pUTX1/*TRP1'* transformants, in which, instead, a protein of 66.7 kdal was observed (Fig. 2, lane B). Neither of these proteins was observed in cells carrying the plasmid YRp7 (Fig. 2, lane F). We deduce that these protein species are plasmid-related gene products, as the loss of plasmid (by selection of *trp1* revertants) results in the disappearance of these proteins (data not shown). The *TRP1* orientation-dependent size difference of 2.5 kdal suggests that the 1,453-bp *TRP1*-containing DNA segment of these plasmids is encoding a portion of these unique protein species.

When the above experiment was repeated with plasmids which contained a deletion of a 220-bp *Kpn*I-bounded segment of DNA from within the *HSP90* DNA sequence (plasmids pUTX8/*TRP1* and pUTX8/*TRP1'*), the plasmid-derived gene products were each 12.5 kdal smaller than those produced by cells carrying their nondeleted counterparts (compare Fig. 2, lanes C and D with lanes A and B, respectively). This result demonstrates that the unique proteins observed are in part encoded by the *HSP90* sequences. The conservation of the 2.5-kdal *TRP1* orientation-dependent size difference demonstrates that the direction of transcription is 5'–*HSP90-TRP1* (or *TRP1'*)–3' and, furthermore, that the *Kpn*I deletion must be an in-frame deletion.

When the above experiment was repeated with a plasmid which contained a deletion of a 710-bp *Xba*I fragment from within the *HSP90* segment (plasmid pUTX9/*TRP1*), no plasmid-derived gene product was observed (Fig. 2, lane E); this is consistent with the direction of transcription deduced above.

We thus conclude that the differences in size between the proteins encoded by pUTX1/*TRP1* (pUTX8/*TRP1*) and pUTX1/*TRP1'* (pUTX8/*TRP1'*) are a consequence of the translational read-through into the RNA encoded by either end of the *TRP1*-bearing DNA segment. From a comparison of the size of these various truncated hsp90 proteins with the size predicted for the C-terminal extensions synthesized due to read-through into either end of the *TRP1*-bearing DNA in each possible reading frame (Table 2), it is clear that the translational reading frame at the *HSP90-TRP1 Eco*RI fusion site cannot be frame 2, for if this were the case, one would have expected that the gene products of pUTX1/*TRP1* and pUTX8/*TRP1* would have been smaller than the products of pUTX1/*TRP1'* and pUTX8/*TRP1'*, respectively. Indeed, the relative differences in size of these truncated hsp90 proteins are close to those expected if the translational reading frame at the *Eco*RI fusion site were frame 3. As an independent confirmation of this point, protein samples isolated from the various transformants were subjected to two-dimensional gel electrophoresis to determine the relative charge of the truncated hsp90 proteins. The truncated hsp90 proteins produced by fusion to *TRP1* (Fig. 3B and D) are considerably more basic than those produced by fusion to *TRP1'* (Fig. 3C and E), as expected if the reading frame at the *HSP90-TRP1 Eco*RI fusion site is frame 3 (Table 2).

Fusion strategy. Having deduced that the translational reading frame at the *Eco*RI site of the 3'-deleted *HSP90* gene fragment is frame 3, it was necessary to design an appropriate strategy to achieve an in-frame fusion with a 5'-deleted *lacZ* gene. The plasmid pMC1403 contains such a deleted gene (6). In this plasmid, the first seven and two-thirds codons of the gene have been replaced with a linker which is cleavable by a number of restriction enzymes, including *Eco*RI (6).

A comparison of the translational reading frame at the proposed *Eco*RI fusion site of the 3'-deleted *HSP90* gene fragment with that at the *Eco*RI site of the 5'-deleted *lacZ* gene fragment of plasmid pMC1403 (Fig. 4A) reveals that they

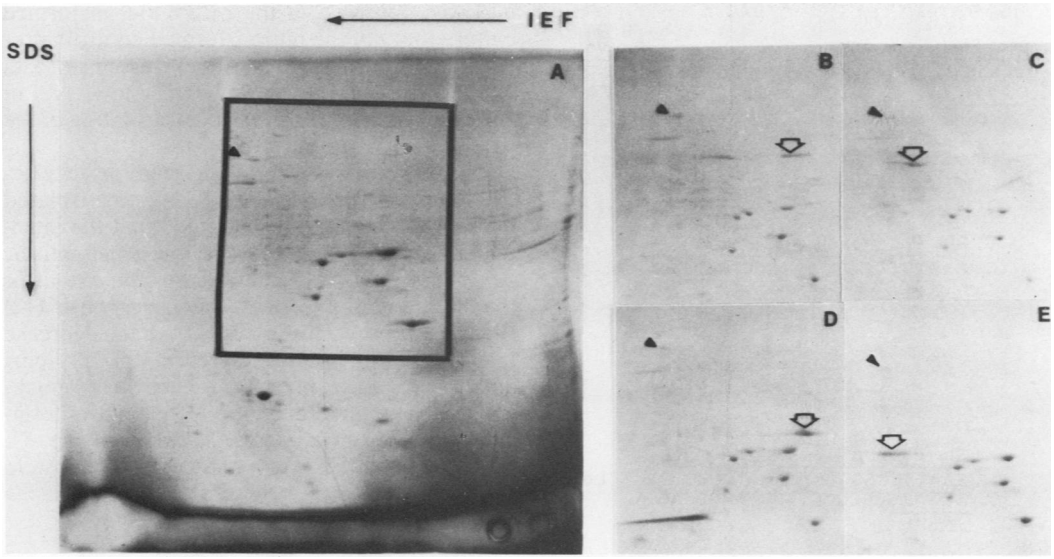


FIG. 3. Two-dimensional electrophoretic analysis of fused proteins. The SDS-soluble protein samples described in the legend to Fig. 2 were subjected to two-dimensional gel electrophoresis as previously described (12). Isoelectric focusing was performed from right (basic) to left (acidic), followed by SDS-polyacrylamide gel electrophoresis (top to bottom). A photograph of the entire gel is presented for strain SC3 transformed with YRp7 (A). The region corresponding to the enclosed area only is shown for strain SC3 transformed with pUTX1/*TRP1* (B), pUTX1/*TRP1'* (C), pUTX8/*TRP1* (D), and pUTX8/*TRP1'* (E). The filled arrows indicate the chromosomally encoded (intact) hsp90, and the open arrows mark the migration of the plasmid-encoded truncated hsp90 proteins.

are not the same (frame 3 and 2, respectively). Thus, a fusion of these two gene fragments at the *Eco*RI site would not be expected to yield an active β-galactosidase molecule owing to the dominance of the upstream *HSP90* translational reading frame (Fig. 4B). An appropriate frame shift, however, can be produced by cleaving the *lacZ* gene at the *Bam*HI site (G ↓ GATCC) located 11 bp downstream from the *Eco*RI site, filling the cohesive termini, and religating the blunt-ended fragments. The results of this frame shift should allow a gene fusion which would be expected to produce an active β-galactosidase molecule (Fig. 4C).

The actual construction used to achieve this gene fusion and frame shift is illustrated in Fig. 5. As the *HSP90* gene fragment and the *lacZ* gene fragment are both contained as inserts in opposite orientations in pBR322 (Fig. 5, top), it was necessary to purify the 1.85-kb *Hin*dIII-*Eco*RI fragment of pUTX1 (the 3'-deleted *HSP90* gene) and the 6.2-kb *Eco*RI-*Sal*I fragment of pMC1403 (the 5'-deleted *lacZ* gene) and join them together in the presence of an 8-kb *Hin*dIII-*Sal*I recipient fragment isolated from the vector pUTX37. This pUTX37-derived fragment is itself incapable of circularizing owing to the different cohesive termini at the ends of the molecule (*Hin*dIII and *Sal*I). However, upon joining with the *HSP90* gene fragment (at the *Hin*dIII site) and the *lacZ* gene fragment (at the *Sal*I site), the resulting molecule (which now contains *Eco*RI-generated cohesive termini at both ends) can circularize to generate a plasmid capable of transforming *E. coli*. This plasmid (designated pUTX40; Fig. 5) has thus fused *HSP90* to *lacZ* in the proper orientation. However, as noted above, this fusion is out of frame. As the only *Bam*HI site in plasmid pUTX40 is that contributed by the *lacZ*-bearing DNA, it is possible to bring the *lacZ* gene into the correct frame (relative to *HSP90*) by performing the frame shift protocol outlined in Fig. 4. The result of this manipulation is a plasmid (designated pUTX41) which is expected to contain an in-frame *HSP90-lacZ* fusion.

Expression of fused genes in *S. cerevisiae*. The plasmids pUTX40 and pUTX41, which each contain the yeast 2μ plasmid replication origin (which allows a high transformation frequency as well as a high plasmid copy number in yeast [2, 4]) as well as the yeast *LEU2* gene, were introduced into an appropriate *leu2* recipient strain of *S. cerevisiae*, and transformants were obtained by selection for leucine-independent growth. Examination of the resulting transformants on indicator plates containing XGal (a chromogenic substrate which is cleaved by β-galactosidase) revealed that the pUTX41-transformed yeast cells produced intense blue colonies,

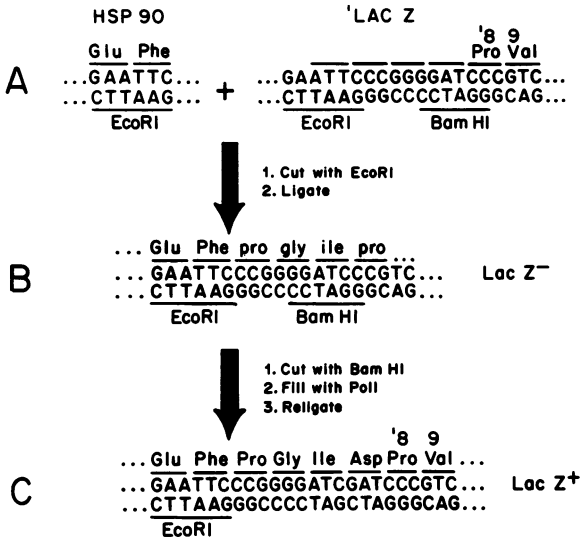
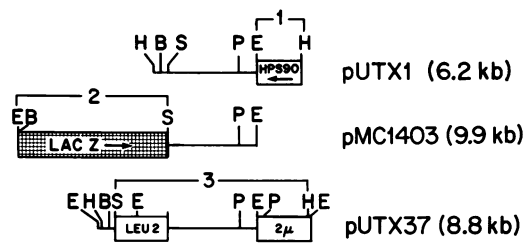


FIG. 4. *HSP90-lacZ* fusion strategy. (A) The translational reading frame at the *Eco*RI site of the *HSP90* gene (left) was deduced from the results depicted in Fig. 3. The sequence and reading frame required for the correct translation of the 5'-deleted *lacZ* fragment of pMC1403 (right) are taken from the data of Casadaban et al. (6). (B) Fusion of the *Eco*RI-cleaved *HP90* gene to the *Eco*RI-cleaved *lacZ* gene. (C) Shift of the reading frame. See text for details.

whereas the pUTX40-transformed yeasts showed virtually no detectable cleavage of XGal (data not shown). This result suggests the the pUTX41- (but not the pUTX40-) transformed colonies are producing an active β-galactosidase.

To evaluate the regulation of expression of the fused *HSP90-lacZ* gene products, the transformants (as well as an untransformed control) were grown to mid-log phase in liquid at 23°C, and a portion of each culture was subjected to a heat shock by transferring to 36°C. At various times, samples were removed from the various cultures and either assayed for β-galactosidase activity or pulse-labeled with [^{35}S]methionine for later gel electrophoretic analysis. The assay of β-galactosidase activity revealed (Fig. 6) that the pUTX41-transformed cells contain a readily detectable level of active enzyme when grown at 23°C, consistent with the fact that the *HSP90* gene is expressed even before the imposition of a heat shock (10, 12). When the pUTX41-transformed cultures were shifted to 36°C, the level of β-galactosidase began to increase by 20 min and reached its maximum level by 90 min after the imposition of a heat shock. Neither the untransformed control nor the pUTX40-transformed cells contained detectable enzyme activity (<5 U/mg) before heat shock (data not shown). However, a barely detectable activity (~10 U/mg) was observed in the pUTX40-transformed culture (but not the untransformed control) after the induction of the heat shock response (data not shown). We attribute this very low level of enzyme activity to errors in the translation of the pUTX40-encoded mRNA.

Examination by SDS-polyacrylamide gel electrophoresis of pulse-labeled proteins isolated from the above cultures revealed that the introduction of the fused *HSP90-lacZ* genes into *S. cerevisiae* in no way alters either the extent or the duration of the heat shock response (12) (Fig. 7, bottom). This includes both the decrease in synthesis of heat shock-repressible proteins and the increase in synthesis of heat shock-inducible proteins (examples of each are designated for the untransformed control in Fig. 7). The rate of synthesis of a unique 70-kdal protein



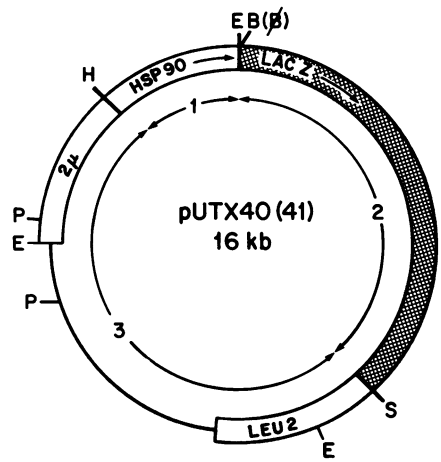


FIG. 5. Construction of *HSP90-lacZ* fusion. The various plasmids used to construct an *HSP90-lacZ* fusion suitable for *S. cerevisiae* transformation are diagrammed at the top. The fragments designated 1, 2, and 3 were purified and combined in the orientation shown to produce the plasmid pUTX40. The single *Bam*HI site of pUTX40 was eliminated as outlined in the lower portion of Fig. 4 to produce the plasmid designated pUTX41. Sequences of *S. cerevisiae* DNA are indicated with open bars. Sequences of plasmid pBR322 are indicated with a thin line. The *lacZ*-containing DNA sequence is depicted as a cross-hatched bar. Restriction sites: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; S, *Sal*I.

(the expected product of the out-of-frame *HSP90-lacZ* fusion) in pUTX40-transformed cells, as well as that of a unique 180-kdal protein (the expected product of the in-frame *HSP90-lacZ* fusion) in pUTX41-transformed cells, is altered during heat shock and follows the same pattern as that observed for the 90-kdal product of the (intact) chromosomal *HSP90* gene.

A photograph of the stained gel which was used to generate the above autoradiograph reveals that the products of both the in-frame and out-of-frame *HSP90-lacZ* gene fusion are abundant cell proteins (Fig. 7, top). The fused gene product is in fact present at a higher level than the chromosomally encoded hsp90. Densitometric analysis of the putative in-frame hsp90–β-galactosidase of the pUTX41-transformed cells reveals that this protein represents 2% of the total SDS-soluble cell protein after heat-shock induction, the level expected from the data shown in Fig. 6 if the hsp90–β-galactosidase had the same specific activity as that reported for the purified *E. coli* enzyme (300,000 U/mg [13]).

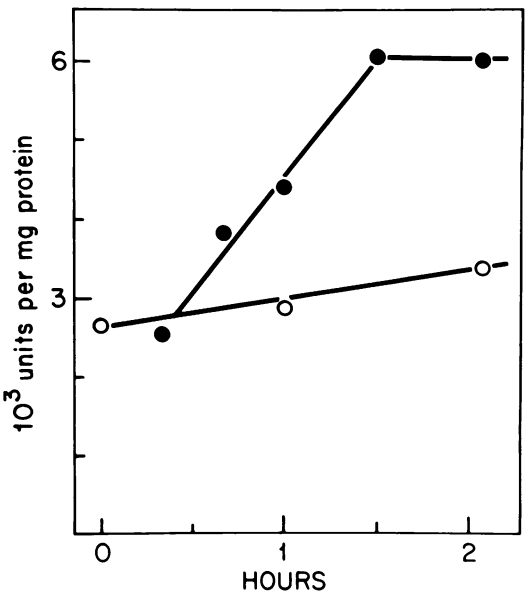


FIG. 6. Regulated expression of *HSP90-lacZ* gene fusion. Strain DC5 transformed with pUTX41 was grown to mid-log phase (2×10^7 cells per ml) at 23°C. At time zero the culture was split into two portions. One portion was shifted to 36°C while the other portion was maintained at 23°C. At various times, samples were removed from both cultures and assayed for β-galactosidase activity as outlined in the text. ○, Culture maintained at 23°C; ●, culture shifted to 36°C.

DISCUSSION

The fusion of genes to the gene encoding the *E. coli* β-galactosidase (*lacZ*) has allowed a very sophisticated examination of many questions about *E. coli*. By providing an easily scorable phenotype for gene expression, mutations may be readily analyzed. Indeed, the fusion of *S. cerevisiae* genes to *lacZ* has been successfully accomplished by a number of workers (11, 14).

Rather than follow an in vivo approach to the isolation of in-frame *lacZ* fusions (14), we created a well-defined fusion of *HSP90* to *lacZ*. The methods employed here do not require a complete gene sequence or a knowledge of the limits of the gene. In fact, the only requirement to enable one to design a successful gene fusion is the knowledge of the translational reading frame at the proposed fusion site. We found it possible to unambiguously determine the reading frame at the potential fusion site by examining the various products made when *HSP90* was fused to DNA fragments of a defined sequence. Although our experiments were simplified by the fact that the 3′-deleted *HSP90* gene produces an abundant cell protein, this strategy could be applied to a minor protein species by the use of Western blot analysis and detection with suitable antibodies (5).

One potential pitfall which might have been anticipated in these experiments did not occur. One might predict that for a gene fusion to work a suitable transcription termination signal must be introduced downstream from the gene fusion site (18). Although one might suppose that the normal *TRP1* transcription termination signal could be utilized in the plasmid pUTX1/*TRP1*, inversion of the *TRP1* DNA would preclude the utilization of this signal in pUTX1/*TRP1′*. In this regard, it is interesting that the level of truncated hsp90 proteins in the pUTX1/*TRP1* and pUTX1/*TRP1′* transformants varies by no more than a factor of two (Fig. 2). Thus, either strand of the *TRP1*-bearing DNA appears to contain a sequence(s) capable of transcription termination.

Having determined the translational reading frame at a single restriction site within *HSP90*, it has been a relatively straightforward task to devise an appropriate method to achieve an in-frame gene fusion with the *lacZ* gene of *E. coli*. When the fused *HSP90-lacZ* gene is introduced into *S. cerevisiae*, its expression (either in frame or out of frame) is regulated in the same fashion as that of the chromosomal *HSP90* gene (Fig. 7). Thus, by merely raising the cultivation temperature of a suitably transformed *S. cerevisiae* strain one can increase the hsp90–β-galactosidase content of the cell.

Either before or after the administration of a heat shock, the fused gene products were present at a higher level than the chromosomally encoded hsp90 (Fig. 7). This is not surprising given that the gene dosage of the fused genes is higher than that of the single chromosomal copy

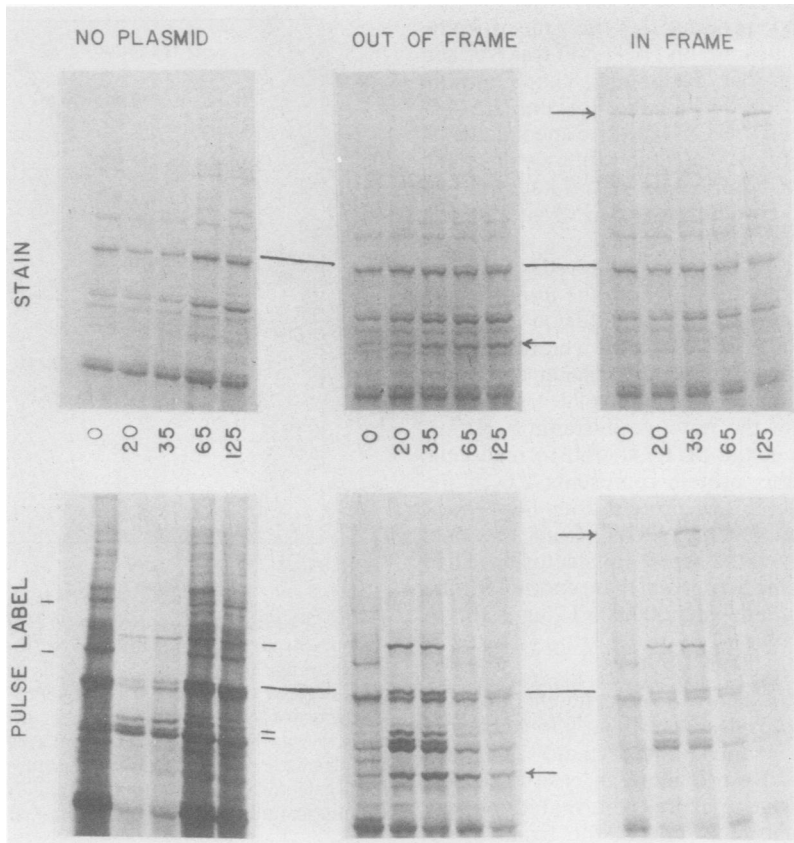


FIG. 7. Heat shock response of fused genes. Samples of the pUTX41 transformant used to generate the data in Fig. 6, as well as identically treated cultures of pUTX40-transformed cells and untransformed cells, were pulse-labeled for 5-min periods with [^{35}S]methionine (10 μCi/ml) at various times before or after the elevation of cultivation temperature from 23 to 36°C. SDS-soluble proteins were isolated from the samples and subjected to SDS-10% polyacrylamide gel electrophoresis. The gel was stained with Coomassie brilliant blue and subsequently dried and subjected to autoradiography. Only that portion of the stained gel or autoradiograph corresponding to proteins of >50 kdal is presented. Numbers between the upper and lower panels correspond to the time (minutes) of termination of labeling relative to temperature elevation. The line joining the panels indicates the location of the (intact) chromosomally encoded hsp90 protein. Short lines to the right and left of the autoradiograph of the untransformed culture (lower left panel) indicate examples of heat shock-inducible and heat shock-repressible proteins, respectively. Arrows indicate the putative products of the fused *HSP90-lacZ* gene.

of *HSP90*. It is interesting that both the in-frame and out-of-frame fusions of *HSP90* to *lacZ* resulted in the production of approximately the same level of protein (Fig. 7, top). This result is somewhat unexpected insofar as the in-frame fusion product was approximately 2.5 times the size of the out-of-frame fusion product. As we have not determined the plasmid copy number in pUTX40- and pUTX41-transformed *S. cerevisiae* we cannot rule out the possibility that differences in the abundance of the fused proteins could be due to variation in plasmid copy number. However, given that the plasmids which encode these products differ only in the addition of 4 bp within the transcription unit (namely, a filled *Bam*HI site), one would expect that plasmid copy number, gene transcription, RNA transport, and initiation of translation should be identical. Whether the underexpression of the in-frame *HSP90-lacZ* fusion is due to inefficient translation of a long mRNA or perhaps to a relatively poor codon bias of the *lacZ* gene (3) remains to be determined.

The level of expression of the fused *HSP90-lacZ* gene is significantly higher in *S. cerevisiae* than that reported for *lacZ* fusions in which other yeast genes are employed (11, 14). This is not surprising when one considers that, after heat shock, hsp90 becomes one of the most abundant proteins in *S. cerevisiae* (12). We have demonstrated here (by measurement of active enzyme as well as by protein staining) that the

hsp90–β-galactosidase fusion product represents ca. 2% of the protein of our transformant after induction by heat shock (Fig. 6 and 7), and we have, in fact, on numerous occasions been able to induce active enzyme to nearly twice this level (data not shown).

In light of the recent report which demonstrated the heat shock-regulated transcription of the *Drosophila HSP70* gene by mouse L cells (7), we note in passing that the *S. cerevisiae HSP90-lacZ* fusion gene is poorly expressed in *E. coli*, yielding a level of active enzyme which is less than one twentieth of that observed in *S. cerevisiae* before heat shock (data not shown). Furthermore, the level of expression of this fused gene is not enhanced when *E. coli* is subjected to a heat shock by shifting the cultivation temperature of this organism from 30 to 42°C (17; data not shown).

The fact that an in-frame *HSP90-lacZ* fusion product is an easily assayable enzyme will allow a more precise quantitation of the *S. cerevisiae* heat shock response as well as a rapid evaluation of alternative conditions of stress which induce this response. The availability of a readily scorable phenotype for heat shock induction should now allow the isolation of *S. cerevisiae* chromosomal mutants exhibiting an altered heat shock response. Experiments along this line are currently under way.

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