The Yeast Heat Shock Response Is Induced by Conversion of Cells to Spheroplasts and by Potent Transcriptional Inhibitors

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We report here that procedures commonly used to measure transcription and mRNA decay rates in *Saccharomyces cerevisiae* induce the heat shock response. First, conversion of cells to spheroplasts with lyticase, a prerequisite for nuclear runoff transcription, induces the expression of HSP70 and HSP90 heat shock genes. The transcript levels of the non-heat-shock gene *ACT1* are slightly depressed, consistent with the general yeast stress response. Second, the DNA intercalator, 1,10-phenanthroline, widely employed as a general transcriptional inhibitor in *S. cerevisiae*, enhances the mRNA abundance of certain heat shock genes (HSP82, SSA1-SSA2) although not of others (HSC82, SSA4, HSP26). Third, the antibiotic thiolutin, previously demonstrated to inhibit all three yeast RNA polymerases both in vivo and in vitro, increases the RNA levels of HSP82 5- to 10-fold, those of SSA4 >25-fold, and those of HSP26 >50-fold under conditions in which transcription of non-heat-shock genes is blocked. By using an episomal *HSP82-lacZ* fusion gene, we present evidence that lyticase and thiolutin induce heat shock gene expression at the level of transcription, whereas phenanthroline acts at a subsequent step, likely through message stabilization. We conclude that, because of the exquisite sensitivity of the yeast heat shock response, procedures designed to measure the rate of gene transcription or mRNA turnover can themselves impact upon each process.

The principal determinants of mRNA concentration in the yeast *Saccharomyces cerevisiae* are transcriptional initiation and mRNA decay (reviewed in references 6 and 42). Sensitive and specific procedures for quantitating cellular steady-state message levels exist and include Northern (RNA) blot-hybridization (44), nuclease protection (2), and primer extension (10). Although widely employed, such procedures are of limited value in assessing promoter function, since they cannot distinguish between the relative contributions of transcription and mRNA turnover. Although less severely affected, pulse-labeling experiments suffer from the same complications (22). A common method for obviating this problem is to fuse the promoter DNA to a reporter gene such as *Escherichia coli lacZ* (8-galactosidase) (15). However, the activity of episomal promoter fusions can be influenced by the presence of flanking foreign DNA sequences, as well as by altered gene copy number, resulting in potentially misleading outcomes (4, 32). In this regard, nuclear runoff transcription, which measures the density of preinitiated RNA polymerase molecules along a given gene (30), is theoretically the most straightforward method for assessing promoter function.

Similarly, procedures for determining the half-lives of individual yeast mRNAs exist and include kinetic analysis of precursor incorporation into mRNA (approach to steady-state labelling) (11, 16), thermal inactivation of temperature-sensitive RNA polymerase II (16, 33), and inhibition of transcription by using specific drugs (reviewed in references 6 and 35). However, thus far there has been no study which has attempted to separate the roles of transcriptional activation and mRNA stability in the regulation of the yeast heat shock response. In our efforts to do so, we discovered that several of these procedures per se induce the stress response. Specifically, we employed nuclear runoff transcription to measure heat shock gene promoter activity and treated cells with 1,10-phenanthroline and thiolutin in an attempt to determine the relative stabilities of their RNA products. Surprisingly, in spite of the disparate nature of these treatments and mode of action of the inhibitors, the abundance of *HSP82* transcripts increased to nearly heat-shock-induced levels. Similar induction of other yeast heat shock genes, including *HSP26* and several members of the yeast *HSP70* gene family, was seen as well. We present evidence suggesting that spheroplasting and thiolutin induce *HSP82* at the level of transcription. In contrast, phenanthroline appears to enhance *HSP82* expression at a posttranscriptional step, likely by stabilizing its message.

**MATERIALS AND METHODS**

Yeast strains, media, and cell cultivation. The *S. cerevisiae* strain used in all experiments reported here was W303-1B (*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*). W303-1B cells were cultivated at 30°C in YPD broth containing 10 g of yeast extract per liter, 20 g of peptone per liter, and 20 g of d-glucose per liter to mid-log phase (~3.5 × 10⁷ cells per ml) on the basis of optical density at 600 nm prior to experimental manipulation.

Construction of episomal fusion genes and their transformation into *S. cerevisiae*. To construct an *HSP82-lacZ* fusion gene, an EcoRI-BclI fragment, containing *HSP82* sequence from position −1300 to +112 relative to the primary transcription start point (9), was isolated from the plasmid pUTX20 (a gift of D. B. Finkelstein, Panlabs Inc.). This fragment was then cloned into the polylinker of pSEVC102 (containing *lacZ*, *CEN4*, *ARS1*, and *URA3* sequences) (kindly provided by M. G. Douglas, University of North Carolina), generating an in-frame fusion between the 18th codon of *HSP82* and the 10th codon of *lacZ*. The resultant construct, termed PZ102, was transformed into W303-1B as previously described (7). A second heat shock element-driven *lacZ* fusion was generated by transforming the 31st and 20th codons of *HSP82* with a *lacZ* fragment from pSEVC102 (containing *lacZ*, *CEN4*, *ARS1*, and *URA3* sequences) (kindly provided by M. G. Douglas, University of North Carolina), generating an in-frame fusion between the 18th codon of *HSP82* and the 10th codon of *lacZ*. The resultant construct, termed PZ102, was transformed into W303-1B as previously described (7). A second heat shock element-driven *lacZ* fusion was generated by transforming the 31st and 20th codons of *HSP82* with a *lacZ* fragment from pSEVC102.
fusion, pBF101, was created by replacement of the Xhol fragment in pLG669-Z (15), with a fragment spanning the HSE2 heat shock element of SSA1 from position −203 to −182 (34) (a gift of B. R. Fishel and W. T. Garrard, University of Texas Southwestern Medical Center at Dallas).

Conversion of cells to spheroplasts and subsequent heat shock treatment. Cells were converted to spheroplasts as described by Jerome and Jaehning (19) with the following modifications. Cells from a 250-ml culture were harvested in mid-log phase by centrifugation for 10 min at 6,000 × g, washed in 1 M sorbitol, and resuspended in 15 ml of spheroplast buffer (1.4 M sorbitol, 40 mM HEPES (pH 7.5), 0.5 mM MgCl2). After the addition of 2-mercaptoethanol to a final concentration of 0.5%, the cells were preincubated at 30°C with mild shaking for 15 min. Oxalyticase (2,500 U) (Enzogenetics, Corvallis, Oreg.) was then added; typically, 2,500 U of oxalyticase was sufficient to convert 1.25 × 109 cells to spheroplasts in 30 to 40 min. Spheroplasts were harvested at 1,000 × g for 10 min, resuspended in 20 ml of YPD plus 1 M sorbitol, and incubated at 30°C for 35 min. The sample was then split into two 10-ml aliquots. One was subjected to a 10-min temperature upshift from 30 to 39°C (heat-shocked sample); the other was placed on ice and maintained at 0°C for the same period of time (non-heat-shocked sample). Spheroplasts were harvested and metabolically poisoned with 20 mM sodium azide (25), and RNA was isolated as described below.

Treatment of cells with thiolutin and 1,10-phenanthroline. Thiolutin (a generous gift of N. Belcher, Pfizer, Inc., Groton, Conn.), dissolved at a concentration of 2 mg/ml in dimethyl sulfoxide, and 1,10-phenanthroline (Sigma), dissolved at a concentration of 100 mg/ml in 95% ethanol, were added to 100-ml yeast cultures, maintained at 25°C, to final concentrations of 3 μg/ml and 500 μg/ml, respectively. At various times after the addition of either drug, 10-ml aliquots were removed, metabolically poisoned with 20 mM sodium azide, and centrifuged, and RNA was isolated as described below.

RNA isolation, Northern blotting, and hybridization. Total cellular RNA was prepared from yeast cells by using the glass bead lysis method of Lindquist (27). RNA samples (∼20 μg each) were fractionated on a 1.1% agarose–2 M formaldehyde gel (38), transferred to a nylon membrane (63/3 millipore) by using an LKB vacuum blotting unit (model 1626), and covered with the nylon membrane by using the Stratagene Cloning Systems, LaJolla, Calif., set at 120,000 μJ.

Antisense RNA probes were synthesized by using a pGEM-3 expression vector into which the following DNA fragments were subcloned: (i) an HSC82 fragment spanning positions +1256 to +1380 relative to the transcription start site (5), derived from plasmid pUTX203 (kindly provided by D. B. Finkeinstein); and (ii) a 1.6-kb BamHI-HindIII fragment bearing the ACT1 gene (16) (gift of M. Hampsey of Louisiana State University Medical Center). In vitro transcription was catalyzed by using either SP6 or T7 RNA polymerase (New England Nuclear and New England Biolabs, respectively) in the presence of 50 μCi of [α-32P]UTP (3,000 Ci/mmol) as previously described (12). DNA hybridization probes were synthesized from cloned templates by using primer extension (38) with either specific or random primers in the presence of 50 μCi of [α-32P]dATP (3,000 Ci/mmol). The following templates were used: (i) a synthetic oligonucleotide homologous to the HSP82 transcription unit between positions +2226 and +2287 relative to the transcription start site (9); (ii) a 651-bp HindIII-BglII fragment spanning 421 bp of the E. coli lacZ 5' coding region, isolated from M13mp18; (iii) a 3.6-kb PvuII-BamHI fragment encompassing the SSA1 gene (41); (iv) an 8.3-kb HindIII fragment encompassing the SSA4 transcription unit and 435 bp of a gene homologous to the rat S8 ribosomal protein gene (3, 4), hereafter termed RPS8; (v) a 2.7-kb BamHI fragment containing the HSP26 gene (43); and (vi) an 8.0-kb HindIII-ScaI fragment spanning the STE3 gene (16). The latter four clones were generously provided by E. A. Craig (University of Wisconsin), R. H. Morse (National Institutes of Health), and R. Parker (University of Arizona).

Hybridizations were conducted essentially as previously described (12), except that blots probed with the HSP82-specific oligonucleotide were hybridized and washed at 45°C. Despite the high stringency employed in the other hybridizations (65°C), the SSA1 probe extensively cross-hybridized with SSA2 transcripts because of the 97% sequence homology that exists between the two transcriptional units (41). In contrast, no detectable cross-hybridization was observed between SSA4 and SSA1-SSA2 or between HSP82 and HSC82, despite 80% (4, 41) and 93% (5, 9) sequence homologies, respectively. Nylon membranes were sequentially stripped and rehybridized up to five times. Radioactive probes were eluted by two 45-min incubation periods at 90°C in stripping buffer (0.1 × SSC [15 mM NaCl, 1.5 mM sodium citrate], 0.5% sodium dodecyl sulfate).

Autoradiography was achieved by exposing presensitized Kodak XAR film with intensifying screens at −70°C (38). Appropriate autoradiograms were scanned by using an LKB Bromma Ultrascan laser densitometer. Hybridization signals were quantified only within the linear response range of the film; typically, multiple exposures were taken of each hybridized blot. Normalization of mRNA-specific hybridization signals to that of 25S rRNA was attempted (to account for variation in load); however, hybridization to rRNA proved unreliable. Therefore, all quantitations cited in the text are based on direct measurements.

RESULTS

Converting yeast cells to spheroplasts induces both HSP90 and HSP70 heat shock genes. To determine the degree to which the yeast heat shock response is regulated at the level of promoter activation, we isolated nuclei from control and heat-shocked cells and performed an in vitro transcription under conditions optimized for S. cerevisiae (19). A prerequisite for isolation of intact yeast nuclei is the conversion of cells to spheroplasts, typically accomplished by removing the cell wall with a lytic enzyme preparation free of contaminating nuclease and proteases (40). Logarithmically growing cells, concentrated by centrifugation, were therefore converted to spheroplasts by treatment with lyticase for 30 to 45 min at 30°C. The resultant spheroplasts were harvested and allowed to reestablish metabolic activity for 35 min at 30°C in a rich, osmotically stabilized medium. A similar recovery incubation period has been shown to increase the transcriptional activity of non-heat-shock genes in isolated yeast nuclei (19).

Because this procedure seemed potentially stressful, we tested the possibility that heat shock genes were being transcriptionally induced during the conversion of cells to spheroplasts. Indeed, as shown in the Northern blot-hybridization depicted in Fig. 1, spheroplasting resulted in about a fivefold induction in the level of HSP82 RNA (Fig. 1, lanes 1 and 2), comparable to that seen after a brief heat shock of
were mildly induced during a 30- to 45-min incubation period in spheroplasting buffer at 30°C (<10% the level seen when the enzyme is present); induction of HSP82 was essentially obviated when 2-mercaptoethanol was withheld (data not shown). Furthermore, neither HSC82 nor SSA1-SSA2 were detectably induced. Therefore, we conclude that converting cells to spheroplasts per se induces the yeast heat shock response.

The transcriptional inhibitors 1,10-phenanthroline and thiolutin induce certain yeast heat shock genes but not others. Since basal promoter function of yeast heat shock genes could not be assessed by using nuclear transcription, we attempted to distinguish the relative contributions of transcription and turnover by quantitating the disappearance of each heat shock message following the inhibition of transcription. For these experiments, we employed two well-characterized transcriptional inhibitors with widely different modes of action, 1,10-phenanthroline, a Zn++ chelator (21) and a DNA intercalator (6), and thiolutin, an antibiotic and antifungal agent previously shown to be effective as an inhibitor of yeast RNA polymerases I, II, and III in vivo and in vitro (20, 45). Surprisingly, we found that both drugs strongly induced HSP82, even when added at concentrations previously reported to quantitatively inhibit transcription of class II genes.

For example, 10 min after the addition of phenanthroline to mid-log-phase cells, HSP82 showed a three- to fourfold increase in steady-state mRNA levels (Fig. 2). To determine whether phenanthroline had a similar effect on the expression of other yeast heat shock genes, RNA blots were stripped and sequentially rehybridized with probes specific for HSC82, SSA1-SSA2, SSA4, and HSP26. While SSA1 and SSA2 showed a combined twofold induction 20 min after addition of the drug, HSC82, SSA4, and HSP26, in notable contrast, were not detectably induced during a 60-min exposure (Fig. 2 and data not shown). As positive controls, we assessed the effect of phenanthroline on two non-heat-shock genes, ACT1 and RPS8, within the same samples. We found that ACT1 decayed with a half-life of ~85 min, consistent with previous observations (16, 39), and that RPS8 decayed with a half-life of ~21 min, similar to other ribosomal protein gene transcripts (16). We conclude that concentrations of phenanthroline previously shown to inhibit total cellular transcription to <5% of wild-type levels (250 to 500 μg/ml) (39) induce the yeast stress response, albeit not as strongly, or pervasively, as thermal shock.

Similarly, as revealed in Fig. 3 (lanes 1 to 4), treatment of cells with thiolutin, at a level (3 μg/ml) previously shown to strongly inhibit yeast RNA polymerases I, II, and III (20, 45), induced the heat shock response. The RNA levels of HSP82 were increased 5- to 10-fold, a level comparable to that elicited by thermal shock; likewise, the RNA levels of SSA4 and HSP26 were elevated at least 25- and 50-fold, respectively. The combined expression of SSA1 and SSA2 was also enhanced, albeit less than that of the other heat shock genes (~35%), while HSC82 was not induced. In these experiments, the increase in heat shock RNA was not due to the vehicle used to deliver the inhibitor (0.15% [vol/vol] dimethyl sulfoxide; data not shown). Furthermore, three non-heat-shock genes, STE3, RPS8, and ACT1, were transcriptionally inhibited, and their mRNAs exhibited half-lives at 25°C of ~8, 20, and 100 min, respectively (Fig. 3), similar to those recently reported by Herrick et al. (16).

Lyticase and thiolutin induce an HSP82-lacZ fusion gene. In an attempt to determine the point at which lyticase, phenanthroline, and thiolutin induce heat shock gene expression,

To test whether HSP82 and HSC82 RNA induction was a part of the general yeast stress response, we rehybridized the RNA blots with probes specific for HSP70 transcripts. As shown in Fig. 1 (lanes 1 and 2), the combined SSA1-SSA2 signal was moderately induced (approximately twofold) by spheroplasting, whereas the SSA4 signal was very strongly induced (50-fold), paralleling the induction seen during heat shock of intact cells (Fig. 1, lanes 1 and 3) (4, 48). HSP26 is also induced by such a procedure (36). Thus, spheroplasting leads to a general induction of the yeast stress response. The moderate decline of ACT1 mRNA levels upon conversion of cells to spheroplasts (Fig. 1, lanes 1 and 2) is consistent with this notion, since heat shock is known to transiently reduce the mRNA abundance of non-heat-shock genes in S. cerevisiae (16, 17, 22, 24, 27, 31, 33).

To address the possibility that a component of the buffer used in spheroplasting accounts, in whole or in part, for the stress response, we performed a mock spheroplasting (in the absence of lyticase). We found that both HSP82 and SSA4

**FIG. 1**. Steady-state mRNA levels in yeast cells before and after conversion to spheroplasts. Total RNA was prepared from log-phase cells either before (lanes 1, 3) or after (lane 2) conversion to spheroplasts. Cells were grown at 30°C to ~3.5 × 10⁶ cells/ml, harvested by centrifugation, resuspended in spheroplasting buffer, and converted to spheroplasts with lyticase as described in Materials and Methods. Spheroplasts were allowed to recover at 30°C to YPD plus 1 M sorbitol for 35 min before isolation of RNA. Samples of intact cells were subjected to heat shock (30 to 39°C shift) for 10 min before the addition of sodium azide to 20 mM and subsequent isolation of RNA (lane 3). Twenty micrograms of each RNA sample (quantitated by optical density at 260 nm) were electrophoresed on a 1.1% agarose-2.2 M formaldehyde gel, vacuum blotted to GeneScreen, and sequentially hybridized, stripped, and rehybridized with the five gene-specific probes indicated. Data for each transcript are derived from a single autoradiographic exposure; a similarly constructed composite is shown in Fig. 3. Quantitations cited in the text are based on two independent experiments. C, intact cells; S, spheroplasts; −, non-heat-shocked sample; +, heat-shocked sample.
FIG. 2. Time course effect of 500 μg of 1,10-phenanthroline per ml on mRNA levels of heat-shocked and non-heat-shocked samples. Aliquots (10 ml) of cells were removed at the indicated times after drug addition, RNA was isolated, and 5% of the total was electrophoresed, blotted, hybridized, stripped, and rehybridized as indicated in the legend to Fig. 1. Similar results were obtained when 1,10-phenanthroline was added to 250 μg/ml. Control experiments ruled out a measurable role for the vehicle used to deliver the drug (0.5% [vol/vol] ethanol; data not shown).

FIG. 3. Time course effect of 3 μg of thiolutin per ml on mRNA levels of heat-shocked and non-heat-shocked samples. Lanes 1 to 4, 10-ml aliquots of cells were removed at the indicated times after drug addition, and RNA was isolated from each sample and analyzed as described in the legend to Fig. 2. Lane 5, a 10-ml aliquot of cells from the control yeast culture was subjected to a thermal shift from 30 to 39°C for 15 min. Quantitations cited in the text represent mean values of two independent experiments.

we constructed a chimeric gene composed of the promoter and 5' end of HSP82 (spanning positions from -1300 to +112 relative to the transcription start site) fused to the coding region of E. coli lacZ (Fig. 4A). The level of expression of the fusion gene was monitored by measurement of its transcript by using a lacZ-specific hybridization probe. As shown in Fig. 4B and C, the response of HSP82-lacZ to either lyticase or thiolutin closely parallels that of HSP82 itself. In particular, conversion of cells to spheroplasts caused approximately a fivefold increase in HSP82-lacZ RNA levels, while 25 min of incubation in the presence of 3 μg of thiolutin per ml resulted in a 15-fold induction, comparable to that seen after a 15-min heat shock (Fig. 4B, lanes 1 to 4 and 9 to 12; Fig. 4C, lanes 7, 8, and 10). In contrast, phenanthroline, added to a final concentration of 500 μg/ml, had no detectable effect on HSP82-lacZ expression (Fig. 4B, lanes 5 to 8; Fig. 4C, lanes 7 and 9). In addition, no external agent, including thermal stress, increased lacZ expression of the parental vector pSEYCl02 (Fig. 4C, lanes 1 to 6), consistent with the idea that lyticase and thiolutin mediate their inductive effect through the HSP82 sequence.

Finally, to test whether thiolutin is acting through a heat shock upstream activation sequence, we examined the inducibility of an episomal CYCI-lacZ gene in which the CYCI upstream activation sequence was replaced with the principal heat shock element of SSA1, termed HSE2 (34). We found that lacZ transcript levels were indeed elevated by thiolutin, although to a lesser extent than by heat shock; neither agent induced a CYCI-lacZ fusion gene lacking a heat shock element (data not shown). Therefore, we conclude that thiolutin induces heat shock gene expression in yeast cells at the level of transcriptional initiation.

**DISCUSSION**

The heat shock response, characterized by the vigorous induction of a subset of heat shock genes in response to thermal, chemical, or anoxic stress, is a universal phenomenon; it has been found in every organism in which it has been sought, prokaryotic or eukaryotic (28). In experiments reported here, we have shown that the yeast heat shock response is exquisitely sensitive to procedures designed to measure the rate of transcription of a gene or the decay of its message. In particular, we have found that the conversion of cells to spheroplasts, an essential prerequisite for nuclear transcription, strongly induces both HSP70 and HSP90 steady-state mRNA levels while reducing those of ACT1 (Fig. 1). Our observations are thus consistent with those
In yeast, the induction of heat shock genes is a complex process that involves both spheroplasting and phenanthroline but only minimally by thiolutin. HSC82 is weakly induced by spheroplasting but shows no detectable induction by either drug. Similar differential expression of yeast heat shock genes has been observed during ascospore formation (23, 48) and upon the diauxic shift (1, 48).

How might lyticase, phenanthroline, and thiolutin trigger the stress response? Recent experiments implicate polypeptide-associated ribosomes as initiators of the heat shock response in prokaryotes (46). Moreover, a precipitous drop in the synthesis of ribosomal proteins accompanies the yeast heat shock response (22, 24). However, since prior treatment with cycloheximide has no effect on HSP82 RNA levels in either control or heat-shocked cells (26), translational inhibition is unlikely to be the underlying mechanism.

Rather, our experiments suggest that lyticase and thiolutin act at the level of promoter activation. Specifically, we find that an HSP82-lacZ hybrid gene exhibits a response to spheroplasting and thiolutin similar to that of wild-type HSP82 (compare Fig. 4 with Fig. 1 and 3). While this result does not rule out a potential role for the 5' end of HSP82 in message stabilization, it is noteworthy that determinants of HSP2 mRNA stability have been mapped to the 3' end of the transcript (47). In addition, we have observed that a 26 bp fragment containing the HSE2 sequence from SSA1 confers both thermal and thiolutin inducibility on a truncated CYC1-lacZ fusion gene (data not shown). The 25- to 50-fold induction of SSA4 and HSP26 by thiolutin (Fig. 3) is also consistent with the drug acting at the transcriptional level, since neither gene exhibits detectable basal activity (4, 43). In interesting contrast, phenanthroline does not appear to act as an HSP2 promoter function and may inhibit weakly with 50 mM phenanthroline (4). Given its well-known pleiotropic effects (6, 21), we speculate that this inhibitor may act by stabilizing HSP82 transcripts that are basally synthesized at relatively high levels but are unstable under nonstressed conditions.

The elevation of heat shock RNA levels by thiolutin is paradoxical given the inhibitory effect of this drug on general class II transcription in yeast cells (Fig. 3) (16, 20, 45). In particular, it is remarkable that HSP82, SSA4, and HSP26 transcript levels are increased 5- to 50-fold after a 50-min treatment with 3 µg of thiolutin per ml, conditions previously shown to result in greater than 95% inhibition of mRNA synthesis (16, 20). This, in combination with the HSP82-lacZ fusion gene results discussed above, suggests the possibility that yeast heat shock genes are transcriptionally regulated in a way that fundamentally differs from that of other class II genes. In this regard, it is pertinent to note that in E. coli, preferential transcription of heat shock genes requires substitution of σ70 by σ32, a subunit of RNA polymerase which specifically recognizes heat shock promoters (14).

In certain respects, the striking induction of these heat shock genes is also analogous to the induction of the SOS response genes of E. coli (29). In each case, the cell is faced with a potentially lethal condition, and in each case it selectively transcribes those genes that provide it with its best chance for survival. It is to be appreciated that the effects of phenanthroline and thiolutin, at the concentrations and durations employed in our experiments, are entirely reversible (1, 20), with cell viability ranging from 60 to 80% (1). In contrast to the induction seen with these drugs, sodium azide, a potent inhibitor of cellular ATP production, completely terminates heat-shock-induced HSP82 transcription within seconds of its addition (25). However, azide, when added at levels that irreversibly block all energy-dependent cellular reactions (20 mM), similarly appears recently reported by Pederson and Morse (36), who found that lyticase (zymolyase) treatment of yeast cells caused a slight, transient induction of HSP26 and a concomitant decrease of TRP1 RNA.

In addition, we have found that when cells are treated with either of two potent inhibitors of non-heat-shock gene transcription, 1,10-phenanthroline and thiolutin, HSP26, HSP70, and HSP90 RNA levels are likewise induced (Fig. 2 and 3). Nonetheless, we see differences in the responses of individual heat shock genes. For example, HSP82, SSA4, and HSP26 are strongly induced by spheroplasting and exposure to thiolutin; only HSP82, however, is induced by phenanthroline. In contrast, SSA1-SSA2 is moderately induced by
unsuitable for half-life determination of HSP82 mRNA, as it seems to stabilize preexisting transcripts (1, 25).

By using the conditionally lethal RNA polymerase II mutant rp61-l (16), Herrick et al. recently performed a comprehensive analysis of yeast mRNA decay rates (16). Their measurements were largely in agreement with those determined by using alternative approaches, including the two considered here. However, these workers did not examine the RNA products of any heat shock genes. Our results indicate that the RNA levels of these genes are increased upon treatment of log-phase yeast cells with potent transcriptional inhibitors. Strikingly, while this temperature-sensitive lesion has been reported to lead to the rapid cessation of RNA synthesis after a shift to 36°C (16, 33), HSP82 is nonetheless transcriptionally induced to a level comparable to that seen in experiments reported here (26). These observations underline the degree to which yeast heat shock genes are poised for transcriptional activation, consistent with constitutive binding of yeast heat shock factor to DNA in vivo (13, 18). They furthermore raise the possibility that yeast heat shock genes, like those in Droso-

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