A Heat Shock-resistant Mutant of *Saccharomyces cerevisiae* Shows Constitutive Synthesis of Two Heat Shock Proteins and Altered Growth

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**ABSTRACT** A heat shock-resistant mutant of the budding yeast *Saccharomyces cerevisiae* was isolated at the mutation frequency of $10^{-7}$ from a culture treated with ethyl methane sulfonate. Cells of the mutant are approximately 1,000-fold more resistant to lethal heat shock than those of the parental strain. Tetrad analysis indicates that phenotypes revealed by this mutant segregated together in the ratio $2^+:2^-$ from heterozygotes constructed with the wild-type strain of the opposite mating type, and are, therefore, attributed to a single nuclear mutation. The mutated gene in the mutant was herein designated *hsr1* (heat shock response). The *hsr1* allele is recessive to the *HSR1* + allele of the wild-type strain.

Exponentially growing cells of *hsr1* mutant were found to constitutively synthesize six proteins that are not synthesized or are synthesized at reduced rates in *HSR1* + cells unless appropriately induced. These proteins include one hsp/C0-protein (hsp48A), one hsp (hsp48B), and two G0-proteins (p73, p56). Heterozygous diploid (*hsr1/HSR1* +) cells do not synthesize the proteins constitutively induced in *hsr1* cells, which suggests that the product of the *HSR1* gene might negatively regulate the synthesis of these proteins. The *hsr1* mutation also led to altered growth of the mutant cells. The mutation elongated the duration of G1 period in the cell cycle and affected both growth arrest by sulfur starvation and growth recovery from it. We discuss the problem of which protein(s) among those constitutively expressed in growing cells of the *hsr1* mutant is responsible for heat shock resistance and alterations in the growth control.

Various organisms and cells are induced to synthesize a particular set of proteins, termed heat shock proteins (hsps), in response to an elevation in temperature (1, 19). These proteins are also induced as a result of cellular response to stress other than heat shock (1, 19). Evidence has been provided that the response protects cells from the stress (10, 11, 13, 14, 23), although the mechanism by which an accumulation of hsps within cells makes these cells resistant to further stress remains unclear.

We have recently made a new finding that eucaryotic cells specifically synthesize hsps when they enter the resting state, G0 (8). The induction of hsps in G0 cells is distinct in two respects from that in heat-shocked cells. First the former is durable whereas the latter is transient. Second, G0 cells synthesized mostly high molecular weight hsps and not low molecular weight hsps. These observations led us to hypothesize that high molecular weight hsps might function in the cellular transition from the proliferating state to G0 and/or in the maintenance of the G0 state. In the present study, we have intended to test this hypothesis by isolating heat shock-resistant mutants of the budding yeast, *Saccharomyces cerevisiae*, in which the hsp genes are expected to be constitutively expressed, and by examining the mutants for their properties with respect to growth control.

The above finding (8) may indicate the alternative possibility that a particular class of hsps synthesized in G0 cells do not function in the growth control whereas the synthesis of these hsps and that of functional proteins involved in the transition to G0 are coordinatively regulated under the same mechanism. The isolation of hsp-constitutive mutants would...
also provide an opportunity to disclose the nature of the induction mechanism of hsps and G0 proteins.

Here, we demonstrate that a single mutation within a gene, termed hsr1, (heat shock response) resulted in both heat shock resistance and altered growth with an elongated G1 period. The mutant cells were thoroughly arrested in G1, and subsequently entered G0 when the culture reached the stationary phase. However, only an incomplete G1 arrest was achieved with cells of the wild-type (HSR1*) strain under the same condition. Furthermore, the growth recovery from sulfur starvation required a longer lag period with the hsr1 strain than with the HSR1* strain. Cells of the hsr1 mutant were found to constitutively synthesize particular hsps and G0-induced proteins, suggesting that the expression of these proteins might be responsible for heat shock resistance and altered growth of the mutant. These results as well as those described in the preceding papers (7, 8) are discussed particularly with regard to the hypothesis described above and the induction mechanism of the hsp genes.

MATERIALS AND METHODS

Strains and Media: Genotypes and sources of S. cerevisiae strains used in this study are listed in Table I. Cells of these strains were grown overnight at 23°C to the mid-log phase before use.

SYE medium has been described (7). For sulfur starvation, a sulfur-free liquid medium was used which was the same in its composition as SYE medium, except that the salts containing sulfate were substituted by those containing chloride and yeast extract and three amino acids, methionine, tryptophane, and histidine, were omitted. A synthetic complete medium used for growth of the mutant. These results as well as those described elsewhere (20). A solid medium, SG, contained 6.7 g/liter Bacto-

Genetic Procedures: The methods described by Mortimer and Hawthorne (16) for mating, sporulation, dissection of asc, and tetrad analysis were generally followed in the present study.

Isolation of Heat Shock-resistant Mutants: A364A cells were treated with 1% ethyl methane sulfonate (Sigma Chemical Co., St. Louis, MO) at 23°C according to the method described elsewhere (2), so that the survival fraction of these cells decreased to 22%. The mutagenized cells were divided into aliquots, each of which contained 1.5 × 10^7 viable cells in 4 ml SYE medium, and incubated at 23°C for 9 h. They were then exposed to 57°C for 5 min in a water bath with shaking, spread onto YPD plates, and incubated at 23°C overnight. The plates were further heated at 57°C for 5 min in a water bath, and incubated at 23°C for another 5 d. The surviving cells that formed colonies were picked up and grown in SYE medium. Five heat-shock-resistant colonies were isolated from a batch. One of them, mutant strain H204, is 1,000-fold more resistant to lethal heat shock than A364A, whereas the others are, at most, 25-fold more resistant.

Determination of Heat Shock Resistance: 4 ml of culture, which were in the exponentially growing phase unless otherwise noted, were exposed to 52°C for 5 min in a water bath with shaking. The culture was then cooled by being transferred to an ice-water bath. The heated and unheated cultures were briefly sonicated, appropriately diluted with distilled water, and spread onto YPD plates.

Analysis of Cellular Proteins by Two-dimensional Polyacrylamide Gel Electrophoresis: Cells were pulse-labeled with 10 μCi/ml [35S]methionine (1,200 Ci/mmol, Amersham Japan, Tokyo, Japan) for 10 min and chased for 3 min by the addition of nonradioactive methionine to 0.5 mg/ml. The total proteins were solubilized and analyzed by two-dimensional nonequilibrium pH gradient electrophoresis/SDS PAGE (2D-NEPHGE/SDS PAGE) according to the methods described previously (8). The gels were stained with Coomassie Brilliant Blue, dried, and exposed to Kodak X-Omat AR film (XAR-5). Molecular weight markers (Bio-Rad Laboratories, Richmond, CA) were myoglobin (200,000), β-galactosidase (116,250), phosphorylase b (92,500), BSA (66,600), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and l-lysine (14,400). Isoelectric point markers (BDH Chemicals Ltd., Poole, England) were C-phytocyanin (A. nidulans, 4.65 and 4.85), Azurin (P. aeruginosa, 5.65), trifluoroacetylated myoglobin met (porcine, 5.92), myoglobin met (porcine, 6.45), myoglobin met (equine, 7.3), myoglobin met (sperm whale, 8.3), and cytochrome c (horse heart, 10.6).

Determination of Budded and Unbudded Cells: Small aliquots of cultures were mixed with formaldehyde solution in PBS to give a final concentration of 3.7%. The mixtures were briefly sonicated to dissociate cell aggregates, and examined under a microscope. To determine the proportion of unbudded cells in the total cells, we examined at least 600 cells.

Determination of Protein Content and Protein Synthesis: To determine the total protein content of cells, we cooled small aliquots of cultures, washed them with distilled water by centrifugation, and subjected the precipitated cells to the protein assay described by Stewart (25). The rate of protein synthesis was determined by incorporation of [35S]methionine into 5% trichloroacetic acid-insoluble fractions.

RESULTS

Isolation and Characterization of Heat Shock-resistant Mutants

A heat shock-resistant mutant, H204, was isolated at the mutation frequency of 10^{-7} from a culture of strain A364A treated with ethyl methane sulfonate. It was approximately 1,000-fold more resistant than the parental strain when exposed to 52°C for 5 min under which condition the survival frequency of A364A cells were 3 × 10^{-3} (Table II).

| Strain       | Genotype         | Source                                      |
|--------------|------------------|---------------------------------------------|
| A364A        | MATa ade1 ade2 ural1 his7 lys2 tyr1 gag1 | YGSC*                                       |
| X2180-1A     | MATa SUC2 mal gal2 CUP1                  | YGSC                                        |
| X2180-1B     | MATa SUC2 mal gal2 CUP1                  | YGSC                                        |
| H204*        | MATa hsr1 ade1 ade2 ural1 his7 lys2 tyr1 | Mutant from A364A                           |
| H204-7B      | MATa hsr1 his7 lys2 gag1                  | Segregant from H204 × X2180-1B cross       |
| H204-7B-2B   | MATa hsr1                  | Segregant from H204-7B-8-1A cross           |
| H204-7B-8D   | MATa hsr1                  | Haploid from the same family of H204-7B-8   |
| H204-7B-5D   | MATa                    | Haploid from the same family of H204-7B-8   |
| H204-7B-6D   | MATa                    | Haploid from the same family of H204-7B-8   |
| H204-7B × X2180-1A | MATa[MATa +hsr1 +his7 +lys2 +gal1 gag2] | Haploid from the same family of H204-7B-8   |
| H204-7B-5D × X2180-1B | MATa               | Haploid from the same family of H204-7B-8   |
| H204-7B-2B × X2180-1B | MATa[MATa hsr1]+ | Haploid from the same family of H204-7B-8   |
| H204-7B-8D × X2180-1B | MATa[MATa hsr1]+ | Haploid from the same family of H204-7B-8   |

* YGSC, Yeast Genetic Stock Center, University of California, Berkeley, California.

\* Strain H204 is a heat shock-resistant mutant, which was originally isolated from strain A364A mutagenized with ethyl methane sulfonate, and is found to contain a mutation(s) that causes the low viability of spores, besides the hsr1 mutation (see the text).
A resultant diploid strain from a cross between H204 (α mating type) and X2180-1B (α mating type), a wild-type strain, was sporulated. The viability of spores was unusually low (79%), whereas the viability of spores yielded by a diploid strain resulting from the parental strain A364A and X2180-1B was 95%. The strain H204 was thus suspected to have mutation(s), which might account for the low viability of spores, in addition to a mutation that caused the heat shock-resistant phenotype. H204 was therefore out-crossed and dissected, after which cells derived from spores were examined for degrees of heat shock resistance. H204-7B, a segregant from H204 × X2180-1B, was found to be as much resistant to heat shock as H204 (Table II), and gave a high viability (97.5%) of spores when crossed with X2180-1B. H204-7B-2B (α mating type) and H204-7B-8D (α mating type) are segregants from H204-7B × X2180-1B, and are similar to H204-7B in their heat shock resistance and spore viability (97.5–100%). H204-7B-5D (α mating type) and H204-7B-6D (α mating type) are also segregants from the above cross, but are similar to A364A, not to H204-7B, in their heat shock sensitivity. The spore viabilities of these two strains were high, when crossed with X2180-1B and X2180-1A, respectively. Heterozygous diploids obtained by crossing H204-7B-2B and X2180-1B showed similar heat shock sensitivity to that of diploids formed from H204-7B-5D × X2180-1B (Table II). Thus, the mutant allele is recessive to the wild-type allele. We tentatively designated the gene, originally mutated in H204 and segregated into H204-7B, H204-7B-2B, and H204-7B-8D hsrl.

Both homozygous and heterozygous diploids for the hsrl locus were sporulated and dissected. The phenotypes regarding heat shock resistance segregated 2:2:2 in all four-spored tetrads from the heterozygous diploid strain (Fig. 1), indicating that it could be attributed to a single nuclear mutation. The results also showed that all of the segregants with the phenotype of heat shock resistance formed small colonies compared with those with wild type (Fig. 1, B and F). This seems to suggest that a defect in the HSR1 gene might affect colony size in addition to thermal resistance (Fig. 1). No segregant showing heat shock resistance was derived

### Table II

| Strain | Constitution | Survival Fractions* |
|--------|--------------|---------------------|
| A364A  | HSR1*        | 0.03 ± 0.01         |
| A364A pretreated# | HSR1*        | 28 ± 2              |
| A364A pretreated$ | HSR1*        | 63 ± 3              |
| A364A pretreatedD | HSR1*        | 22 ± 2              |
| X2180-1A | HSR1*        | 0.03 ± 0.02         |
| X2180-1B | HSR1*        | 0.04 ± 0.01         |
| H204   | hsr1         | 24 ± 4              |
| H204-7B | hsr1         | 20 ± 2              |
| H204-7B-2B | hsr1 | 22 ± 1          |
| H204-7B-2B pretreated$ | hsr1 | 70 ± 3          |
| H204-7B-8D | hsr1 | 25 ± 2          |
| H204-7B-5D | HSR1* | 0.03 ± 0.01      |
| H204-7B-8D | HSR1* | 0.03 ± 0.02      |
| H204-7B-5D × X2180-1B | HSR1/HSR1* | 0.04 ± 0.01      |
| H204-7B-2B × X2180-1B | hsr1/HSR1* | 0.05 ± 0.00      |
| H204-7B-2B × H204-7B-8D | hsr1/hsrl | 50 ± 4         |

* Survival fractions (%) as determined by colony formation (mean ± SEM).
# Preincubated at 36°C for 1.5 h before the heat shock.
$ Preincubated at 23°C for 2 d so that the cells entered the stationary phase.
D Preincubated at 23°C for 46 h in sulfur-free medium.

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**Figure 1** Segregation of pheno-
types regarding heat shock resis-
tance. Strains, H204-7B-5D (MATa) and H204-7B-2B (MATa hsr1), were individually mated with a strain, X2180-1B (MATa). Diploids were selected and sporulated. For a cross H204-7B-5D × X2180-1B (A) and a cross H204-7B-2B × X2180-1B (B), tetrads from each ascus were plated in rows onto slabs of YPD dissec-
tion agar. The slabs were placed on YPD agar plates and incubated at 23°C for 4 d. Colonies of segregants from asci indicated by numerals (A, 1–10) were picked up and suspended in H2O, after which they were replica-
plated onto two YPD plates (C and D) with a 48-rod inoculator in an ordered pattern. These two plates were incubated at 23°C for 12 h to allow the plated cells to grow in the exponential phase. One (D) of the plates was then heated at 57°C for 13 min in a water bath, and chilled in the ice. Both the heated plate (D) and control plate (C) were incubated at 23°C for 4 d. Segregants from asci (B, 1–10) were also analyzed in the same way. F was heated at 57°C for 13 min and E was not heated.
Identification of Proteins Specifically Associated with the hsr1 Mutation

HSR1* yeast cells became heat shock-resistant to a considerable degree when preincubated at 36°C for 1.5 h or forced to enter the resting state by an increase in the cell density or by sulfur starvation so that these cells were induced to synthesize hsp8s in sufficient amounts (reference 14 and Table II). We have examined the possibility that some, if not all, hsp8s might be constitutively synthesized in hsr1 cells.

Exponentially growing cells of H204-7B-5D (HSR1*) and H204-7B-2B (hsr1) strains were separately pulse-labeled with [35S]methionine at 23°C for 10 min and chased for 3 min in the presence of an excess amount of unlabeled methionine. The total proteins were extracted from the cells, and analyzed by 2D-NEPHGE/SDS PAGE (17). We found that proteins with molecular weight 73,000, 63,000, 56,000, and 48,000 (acidic form) (p75, p63, p56, and p48A) were specifically synthesized in hsr1 cells when compared with HSR1* cells (Fig. 2, A and B). The synthesis of p60 and p48B (basic form of protein with molecular weight of 48,000) was significantly and reproducibly enhanced in hsr1 cells although these proteins were also synthesized to some extents in HSR1* cells (Fig. 2).

The results are summarized in Table III together with the relationship of the proteins described above to hsp8s and G0 proteins based upon our previous observations (8). p48, which is identical to hsp48, exists in two isoforms, an acidic minor component, hps48A, and a basic major one, hsp48B. hsp48A was identified as a G0 protein (8). One-dimensional peptide mapping with Staphylococcus aureus V8 protease showed that hsp48A is not distinguishable in its polypeptide composition from hsp48B (Fig. 3). In addition, the results show that these two proteins induced in heat-shocked HSR1* cells, sulfur-starved HSR1* cells, or growing hsr1 cells are not distinguishable in their peptide composition. hps48B that is synthesized in a small amount in growing HSR1* cells appears to be identical to the corresponding protein induced in sulfur-starved cells (Fig. 3).

We found that the altered pattern of protein synthesis seen with hsr1 cells always co-segregated with phenotypes of heat shock resistance and of small colony formation. For instance, cells from spores a and c of ascus No. 1 in Fig. 1 B, were heat shock resistant (Fig. 1 F), formed small colonies (Fig. 1 B), and constitutively synthesized the above six proteins (Fig. 4), whereas those from spores b and d were heat shock sensitive (Fig. 1 F), formed large colonies, and did not show the altered protein synthesis (Fig. 4).

Synthesis of the particular proteins that are constitutively expressed in growing H204-7B-2B (hsr1) cells (Fig. 2 and 3) was found to be suppressed in cells of a heterozygous diploid strain for the hsr1 locus (Fig. 5). All the heat shock-resistant segregants from this heterozygous diploid strain constitutively synthesized the proteins listed in Table III (Fig. 4).
Unusually Long $G_1$ Period in the hsr1 Mutant

As seen in Fig. 1, segregants in tetrads, which showed heat shock resistance, formed smaller colonies than those of heat shock-sensitive segregants. The result was attributed to a relatively slow growth rate of $hsr1$ cells at 23°C compared with $HSR1^+$ cells. A mean doubling time was determined to be 2.5 h for H204-7B-2B ($hsr1$) at 23°C while it was 2.1 h for H204-7B-5D ($HSR1^+$) (Table IV). We determined the proportions of unbudded ($G_1$) and budded ($S + G_2 + M$) cells for exponentially growing cultures of strains H204-7B-2B and H204-7B-5D, after which we estimated the length of the $G_1$ period in the total cell cycle-time according to the equation of Rivin and Fangman (18) (Table IV). The $G_1$ period of exponentially growing H204-7B-2B cells was approximately twice as long as that of H204-7B-5D cells, whereas periods of $S + G_2 + M$ did not appear to be different between these strains. We noted that unbudded cells of H204-7B-2B were not smaller in size than those of H204-7B-5D (data not shown).

Cells of H204-7B-5D ($HSR1^+$) and H204-7B-2B ($hsr1$) were allowed to grow to the stationary phase, after which they were incubated for an additional 24 h in the same cultures. We found that both the cell density and the proportion of budded cells are higher in the arrested $HSR1^+$ cells than in the arrested $hsr1$ cells (Table IV).

Growth Recovery from Sulfur Starvation

When H204-7B-5D ($HSR1^+$) and H204-7B-2B ($hsr1$) cells were starved for sulfur, a majority of cells divided twice within 12 h and rested mostly in the unbudded phase of the cell cycle. Proportions of unbudded cells in the total cell populations of the sulfur-starved cultures were 96% for H204-7B-5D and 98% for H204-7B-2B (Fig. 6). When the starved cells were shifted to the complete medium, they re-entered $S$ phase after a time lag. The duration of the lag appeared to be a function of the time that yeast cells were starved for sulfur (reference 7 and Fig. 6). The lengths of the lag observed with H204-7B-2B cells are significantly longer than those observed with H204-7B-5D cells when both of the cultures were starved for the same period (Fig. 6). In addition, the transition probability originally defined by Smith and Martin (21), the rate constant with which these cells entered $S$ phase, was found to be lowered by the $hsr1$ mutation (Fig. 6). Furthermore, the transition probability of H204-7B-2B ($hsr1$) was reduced as the starvation period was elongated in contrast with the observation that the transition probability of H204-7B-5D

**Figure 4** Co-segregation of the heat shock sensitivity and the pattern of protein synthesis. A tetrad from an ascus, No. 1 of Fig. 1B, were examined for the protein synthesis. Cells from each spore were separately grown to the exponentially growing phase and labeled with [35S]methionine as described in the legend to Fig. 2. The total proteins (1 x 10^6 dpm for each gel) extracted from the labeled cells were analyzed by 2D-NEPHGE/SDS PAGE and autoradiography. Arrows indicate hsp48A and hsp48B and arrowheads indicate p73, p63, p60, and p56. (A) spore 1a; (B) spore 1b; (C) spore 1c; (D) spore 1d.
and H204-7B-2B (hsrl) cells, which had been (a) grown in an exponentially growing phase at 23°C, (b) starved for sulfur for 37 h, and (c) incubated in the complete medium for 2 h after the sulfur starvation (Fig. 7).

As described above, the synthesis of the six proteins listed in Table III was observed in exponentially growing H204-7B-2B (hsrl) cells but was not detected, or only slightly, if at all, in exponentially growing H204-7B-5D (HSRI+) cells. These proteins except p63 were induced by sulfur starvation in H204-7B-5D cells (Fig. 7, A and B). The relative synthesizing rate of these five proteins increased upon sulfur starvation also in H204-7B-2B cells (Fig. 7, D and E). Especially, the induction of hsp48A and hsp48B was remarkable. When sulfur was readded to the above-starved H204-7B-5D cells, the induced synthesis of hsp48A, hsp48B, p73, p60, and p56 ceased to the uninduced levels by 2 h after the readdition of sulfur. It was observed that bud emergence recovered in these cells. Although the synthesizing rate of hsp48A and hsp48B in starved H204-7B-2B cells reduced to the levels in growing cells of the same strain 2 h after the readdition of sulfur, bud emergence had not resumed at that time (Fig. 7F).

**DISCUSSION**

**Proteins Responsible for Heat Shock Resistance**

A positive correlation between the synthesis of hsps and the acquisition of resistance to lethal temperature has been reported in *Escherichia coli* (23), *S. cerevisiae* (14), Dictyostelium (13), Drosophila (15), and Chinese hamster fibroblasts (11). These results suggest that an accumulation of hsps inside cell bodies might make cells resistant to heat shock.

Another approach to the same problem was conducted by Loomis and Wheeler (13), who have isolated a mutant of Dictyostelium that is defective in the acquisition of heat resistance and have shown that this mutant specifically fails to synthesize a set of low molecular weight hsps (26,000–32,000 mol wt). This finding suggests that these low molecular weight hsps function in the protection from lethal heat shock in this organism (13).

A thermal resistant mutant of the yeast, H204-7B-2B (hsrl), was shown in the present study to constitutively synthesize two hsps at the physiological temperature (Table III). The mutant strain also constitutively synthesized three G0-induced proteins and two unidentified proteins, p63 and p60. Among these proteins, p48A is simultaneously incorporated into the families of both hsps and G0-proteins (8). P73, p63, p60, or p56 are not significantly induced in HSRI+ cells by heat shock (8), which suggests that these proteins might not function in protection from thermal killing. Thus, it seems likely that both or either one of the two hsps among the six proteins listed in Table III may be responsible for heat resistance of this mutant. Growing cells of the hsrl mutant are resistant to heat shock to the same degree as preheated HSRI+ cells are (Table II), even though the mutant cells do not constitutively synthesize any other hsps than hsp48s. In addition, since an exposure-time to lethal temperature was only 5 min (Table II), the possibility seems unlikely that a set of hsps that were not expressed in hsrl cells might be readily induced by the exposure in the hsrl mutant but not in the HSRI+ strain and function in the protection. For these reasons, these hsps do not appear to directly participate in the acquisition of heat resistance in the hsrl strain.

Recently, Finkelstein and Strausberg (6) have reported that

![Figure 5](image_url)
TABLE IV  
Effect of the hsr1 Mutation on the Cell Cycle

| Strain      | hsr1 allele | Exponentially growing phase | Stationary phase |
|-------------|-------------|-----------------------------|-----------------|
|             |             | Unbudded cells* | $T_0^*$ | $G_1^*$ | $S + G_2 + M^*$ | Budded cells$^1$ | Cell density $^1$ |
| H204-7B-5D  | HSR1$^+$    | 37             | 2.1     | 0.6     | 1.5           | 18              | 10.5             |
| H204-7B-2B  | hsr1        | 53             | 2.5     | 1.1     | 1.4           | 18.5            | 8.9              |

* The population of unbudded cells were determined with cultures of the mid-log phase (~1 x 10$^6$ cells/ml) at 23°C in SYE medium.  

$^*$ The mean doubling time ($T_0$) was determined with mid-log phase cultures.  

$^*$ The length of the $G_1$ period was calculated from populations of unbudded cells in mid-log phase cultures according to the equation described by Rivin and Fangman (18), $T_0 = (1 - \log(2 - F_{unb}d)/\log 2)$, where $F_{unb}$ is the fraction of unbudded cells.  

$^*$ Cultures in the mid-log phase (~1 x 10$^6$ cells/ml) were incubated for 48 h at 23°C in SYE medium, after which the population of budded cells and the cell density were determined.

Figure 6 Growth recovery from sulfur starvation. Cells growing at 23°C in the exponential phase (1 x 10$^6$ cells/ml) in synthetic liquid medium were washed three times with sulfur-free synthetic medium, and resuspended in the same medium, and incubated for 14 h (a), 37 h (b), and 60 h (c). The cells were then shifted back to synthetic complete medium. Bud emergence was determined as a function of time after the shift. (A) Strain H204-7B-5D (HSR1$^+$); (B) strain H204-7B-2B (hsr1).

an increase in the level of synthesis of hsp89 (HSP90, according to their designation) in yeast cells, to which a cloned hps89 gene was introduced by using a multicopy plasmid vector, did not alter the sensitivity to heat shock. This is compatible with our present results because hsp89 is not constitutively expressed in cells of a heat shock-resistant mutant, strain H204-7B-2B.

Induction Mechanism of hsps

Cytosol prepared from heat-shock Drosophila cells contains specific substances that induce heat shock puffs in isolated polytene nuclei, suggesting that the induction of hsps is positively regulated (4, 5). The positive control of heat shock response has been also suggested by hypersensitive mutants to a moderate heat shock that are defective in the induction of certain hsps (13, 23). By contrast, our results seem to suggest that yeast cells are also endowed with a system that negatively controls the expression of the hsps genes.

We have observed that (a) without heat shock or other stress, a mutation in the HSR1 gene resulted in the induction of a small set of proteins including hsp48A and hsp48B, and (b) hsr1/HSR1$^+$ heterozygous diploid cells, HSR1$^+$/HSR1$^+$ homozygous diploid cells, and HSR1$^+$ haploid cells are essentially the same in the sensitivity to heat shock (Table II) and also in the expression of these specific proteins (Figs. 2 and 5). Thus, the HSR1 gene would be a regulatory gene, whose product might repress the expression of the proteins listed in Table III.

It should be noted, however, that a mutation in the HSR1 gene resulted in the constitutive expression of only a small number of hsps and $G_0$-induced proteins (Table III), whereas at least 13 hsps are induced in response to heat shock and nine $G_0$ proteins are induced when arrested in $G_0$ (8). This result indicates that the synthesis of most hsps and $G_0$ proteins is not regulated by the HSR1 gene.

Differential expressions of hsp genes have been reported in various induction systems, although all the hsps are inducible, according to their identification, in response to appropriate heat shock. For instance, different inducing agents or conditions induce different puffs in Drosophila (reviewed in reference 1). Lindquist (12) has demonstrated with cultured Drosophila cells various patterns of hsp production that critically depend upon the degree of temperature elevation, the rate of temperature shift, and culture media. Furthermore, ecysoterone induces a set of low molecular weight hsps but not high molecular weight hsps in Drosophila cells (9).

Possible Involvement of hsps in Growth Control

In this study, the isolation of heat shock-resistant mutants was conducted in the hope that such mutants might also differ from the wild-type strain in properties regarding the growth control. This idea derived from the working hypothesis that hsps might function in the transition from the proliferating state to $G_0$ and/or in the maintenance of the $G_0$ state (7, 8). As has been seen above, the results met our expectations.

Cells of hsr1 mutant showed the following four distinctive properties regarding the growth control from those of the wild-type (HSR1$^+$) strain. (a) The duration of the $G_1$ period in the exponentially growing phase was elongated (Table IV). (b) The percentage of budded cells in the stationary phase was higher in HSR1$^+$ cells than in hsr1 cells (Table IV). This result indicates that hsr1 cells were much more strongly forced to cease from growing at the $G_1$ phase of the cell cycle than were HSR1$^+$ cells when the cultures reached the stationary phase. (c) The lag before the growth recovery of sulfur-starved cells was longer in hsr1 cells than in HSR1$^+$ cells when starved for sulfur for the same periods (Fig. 6). (d) The rate of decrease in the proportion of unbudded cells seen upon the growth recovery from sulfur starvation was reduced (Fig. 6). This may be related to a. The results c and d indicate that both the duration of lag and the rate of transition into $S$ phase observed for the growth recovery of yeast cells from sulfur starvation are affected by the hsr1 mutation. Brooks (2) has previously made a similar observation with mouse 3T3 cells that low concentration of cycloheximide caused elongation of the lag before the growth recovery from serum starvation and also the rate constant for entry into S phase. We have no evidence,
however, that the hsr1 mutation primarily affected the machinery of protein synthesis (unpublished results).

These results do not necessarily indicate, however, that the same protein(s) simultaneously functions both in the protection from heat shock and in the regulation of growth. Proteins preferentially synthesized in a mutant of the HSR1 gene are hsp48A, hsp48B, p73, p63, p60, and p56. hsp48s are among prominent proteins that specifically distinguish between HSR1+ and hsr1 strains (Fig. 2). It would be possible that hsp48B is responsible for heat shock resistance of the hsr1 strain, because the synthesis and accumulation of this protein are significant and specific with hsr1 cells. However, hsp48B is not a G0 protein (8) and, therefore, may not be involved in altered growth of those cells. In addition, we have found that p63 or p60 was not significantly induced by sulfur starvation (Fig. 7). For this reason, these two peptides may not be involved in the cessation of growth in G1 or G0. Three proteins, hsp48A, p73, and p56 have been shown to be
preferentially synthesized also in G0 cells of the yeast including sulfur-starved cells and thus were identified as G0 proteins in this microorganism (8). Furthermore, the initiation of growth recovery from sulfur-starvation appeared to coincide with the decrease in the synthesis of these three proteins to the unstimulated levels in both HSR1+ and hsr1 cells (Figs. 6 and 7). We suggest, therefore, that all or a part of the above three proteins might be eligible for the altered properties regarding cell growth associated with the hsr1 mutant.

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REFERENCES

1. Ashburner, M., and J. J. Bonner. 1979. The induction of gene activity in Drosophila by heat shock. Cell. 17:241-254.
2. Brooks, R. F. 1977. Continuous protein synthesis is required to maintain the probability of entry into S phase. Cell. 12:311-317.
3. Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli. 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. 252:1102-1106.
4. Compton, J. L., and J. J. Bonner. 1978. An in vitro assay for the specific induction and regression of puffs in isolated polytene nuclei of Drosophila melanogaster. Cold Spring Harbor Symp. Quant. Biol. 42:835-838.
5. Compton, J. L., and B. J. McCarthy. 1978. Induction of Drosophila heat shock response in isolated polytene nuclei. Cell. 14:191-201.
6. Finkelnberg, D. B., and S. Strausberg. 1983. Identification and expression of a cloned yeast heat shock gene. J. Biol. Chem. 258:1908-1913.
7. Iida, H., and I. Yahara. 1984. Specific early-G1 blocks accompanied with stringent response in Saccharomyces cerevisiae lead to growth arrest in resting state similar to the G0 of higher eucaryotes. J. Cell Biol. 98:1185-1193.
8. Iida, H., and I. Yahara. 1984. Durable synthesis of high molecular weight heat shock proteins in G0 cells of the yeast and other eucaryotes. J. Cell Biol. 99:199-207.
9. Ireland, R. C., and E. M. Berger. 1982. Synthesis of low molecular weight heat shock peptides stimulated by osmolyte in a cultured Drosophila cell line. Proc. Natl. Acad. Sci. USA. 79:855-859.
10. Li, G. C., and G. M. Hahn. 1978. Ethanol-induced tolerance to heat and to adriamycin. Nature (Lond.). 274:699-701.
11. Li, G. C. and Z. Werb. 1982. Correlation between synthesis of heat shock proteins and development of thermotolerance in Chinese hamster fibroblasts. Proc. Natl. Acad. Sci. USA. 79:3216-3222.
12. Lindquist, S. 1980. Varying patterns of protein synthesis in Drosophila during heat shock: implications for regulation. Dev. Biol. 77:463-479.
13. Lootens, W. F., and S. A. Wheeler. 1982. Chromatin-associated heat shock proteins of Drosophila. Dev. Biol. 90:412-418.
14. McAlister, L., and D. B. Finkelman. 1980. Heat shock proteins and thermal resistance in yeast. Biochem. Biophys. Res. Commun. 93:819-824.
15. Mitchell, H. K., G. Mollier, N. S. Petersen, and L. Lips-Sarmiento. 1979. Specific protection from phenocopy induction by heat shock. Dev. Genet. 1:181-192.
16. Mortimer, R. K., and D. C. Hawthorne. 1969. Yeast genetics. In The Yeasts, Vol. 1. A. H. Rose and J. S. Harrison, editors. Academic Press, Inc., New York. 385-460.
17. O'Farrell, P. Z., H. M. Goodman, and P. H. O'Farrell. 1977. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell. 12:1133-1142.
18. Rivin, C. J., and W. L. Faigman. 1980. Cell cycle phase expansion in nitrogen-limited cultures of Saccharomyces cerevisiae. J. Cell Biol. 85:96-107.
19. Schaefer, S. M., M. Ashburner, and A. Thomsen, editors. 1982. Heat Shock from Bacteria to Man. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 440 pp.
20. Sherman, F., G. R. Fink, and C. W. Lawrence. 1979. Methods in Yeast Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 98 pp.
21. Smith, J. A., and L. Martin. 1973. De cella cycle? Proc. Natl. Acad. Sci. USA. 70:1263-1267.
22. Stewart, P. R. 1975. Analytical methods for yeasts. Methods Cell Biol. 12:111-147.
23. Yamamoto, T., and T. Yura. 1982. Genetic control of heat-shock protein synthesis and its bearing on growth and thermal resistance in Escherichia coli K-12. Proc. Natl. Acad. Sci. USA. 79:860-864.