

Heat Shock Response of *Saccharomyces cerevisiae* Mutants Altered in Cyclic AMP-Dependent Protein Phosphorylation

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When *Saccharomyces cerevisiae* cells grown at 23°C were transferred to 36°C, they initiated synthesis of heat shock proteins, acquired thermotolerance to a lethal heat treatment given after the temperature shift, and arrested their growth transiently at the G1 phase of the cell division cycle. The *bcy1* mutant which resulted in production of cyclic AMP (cAMP)-independent protein kinase did not synthesize the three heat shock proteins hsp72A, hsp72B, and hsp41 after the temperature shift. The *bcy1* cells failed to acquire thermotolerance to the lethal heat treatment and were not arrested at the G1 phase after the temperature shift. In contrast, the *cyr1-2* mutant, which produced a low level of cAMP, constitutively produced three heat shock proteins and four other proteins without the temperature shift and was resistant to the lethal heat treatment. The results suggest that a decrease in the level of cAMP-dependent protein phosphorylation results in the heat shock response, including elevated synthesis of three heat shock proteins, acquisition of thermotolerance, and transient arrest of the cell cycle.

The temperature shift-up referred to as heat shock seems to cause the alteration of a variety of cellular functions in many organisms. This set of changes is known as the heat shock response and includes the induction of synthesis of heat shock proteins (hsps) and the acquisition of thermotolerance to the lethal heat treatment (25). In *Saccharomyces cerevisiae* cells heat shock induces synthesis of hsps and simultaneously results in the acquisition of thermotolerance (15–18). It is known that stationary-phase *S. cerevisiae* cells are more thermally resistant than actively growing cells (7, 24), and when the temperature is shifted *S. cerevisiae* cells are transiently arrested at the G1 phase of the cell cycle (8). This shows that growth arrest at the G1 phase may be related in some manner to the acquisition of thermotolerance to the lethal heat treatment. A heat-resistant mutant isolated by Iida and Yahara (7), designated *hsr1*, constitutively synthesized some hsps and was altered in its growth control properties. Thus, the heat shock response seems to be related to the negative control of growth. Because cyclic AMP (cAMP)-requiring *S. cerevisiae* mutants such as *cyr1* are arrested at the G1 phase and suppressor mutants such as *bcy1*, which result in cAMP-independent protein phosphorylation, are not arrested at the G1 phase under conditions such as nitrogen starvation that arrest normal *S. cerevisiae* cells at the G1 phase (12, 14), it has been suggested that cAMP is involved in the positive control of growth. This led us to examine the heat shock response in mutants altered in cAMP-dependent protein phosphorylation. In this report we present evidence which suggests that the heat shock response is affected by cAMP-dependent protein phosphorylation.

MATERIALS AND METHODS

Yeast strains. *Saccharomyces cerevisiae* AM248-6D (α *his7*), AM248-1B (α *cyr1-2 his7*), and AM180-2B (α *bcy1 his7*) were from our stock. AM203-1A (α *bcy1 his7*), AM203-1B (α *bcy1 his7*), AM203-1C (α *his7*), and AM203-1D (α *his7*) were segregants from the same ascus obtained from the cross AM180-2B (α *bcy1 his7*) \times G211-6A (α *his7*). RA1-13D-1 (α *ura3 leu2 trp1 his3 bcy1::URA3*) was obtained by disrupting the *BCY1* gene of RA1-13D (α *ura3 leu2 trp1 his3*) by inserting the *URA3* gene. RA1-13D-1 (pSY2-2) was constructed by transforming RA1-13D-1 with plasmid pSY2-2, which carries the *BCY1* gene. These three strains were obtained from K. Tanaka, T. Oshima, and A. Toh-e.

Media. Complete medium (YPD) was composed of 10 g of yeast extract (Difco Laboratories, Detroit, Mich.)–20 g of peptone–20 g of glucose in 1 liter of distilled water. Nitrogen-free synthetic minimal medium was composed of 6.7 g of yeast nitrogen base without amino acids and ammonium sulfate (Difco) and 20 g of glucose in 1 liter of distilled water. A synthetic liquid medium with a low methionine content used for labeling proteins with L-[³⁵S]methionine was prepared based on a synthetic liquid medium described previously (4). The medium was composed of the following: 6.7 g of yeast nitrogen base without amino acids; 10 g of succinic acid; 6 g of NaOH; 2 g of glucose; 20 mg each of adenine and uracil; 40 mg each of L-histidine hydrochloride, DL-tryptophan, L-tyrosine, and L-lysine; 0.25 g of yeast extract; and 1 liter of distilled water. If necessary the medium was supplemented with substances required by auxotrophic mutants.

Cell culture and heat shock treatment. *S. cerevisiae* cells were grown on solid or liquid medium at 23°C. The heat shock treatment in liquid medium was carried out by transferring exponentially growing cultures at 23°C to a water bath maintained at 36 or 52°C for the indicated times. For the heat shock treatment of plates, cells were inoculated on YPD plates and were then incubated at 23°C for 6 h to allow the

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cells to grow to the exponential phase. The plates were heated in a water bath at 38 or 57°C for the indicated times.

Determination of the proportion of unbudded cells. Small fractions of cell cultures were pipetted at the indicated times, briefly sonicated to dissociate the cell clumps, and examined under a microscope. To determine the proportion of unbudded cells, at least 600 cells were examined.

Determination of heat sensitivity. The cells were grown in liquid YPD medium at 23°C with shaking. Exponentially growing cultures were exposed to 52°C for the indicated times. In the case of the temperature shift experiments, the cultures were first challenged to 36°C for the indicated times and then exposed to 52°C for the indicated times. The heat treated cultures were then cooled by transferring them to an ice bath. The cells were briefly sonicated, spread onto plates containing YPD medium, and incubated at 23°C for 3 to 4 days. The viability of each culture was determined by comparing it with the culture that received no heat treatment.

Genetic analysis procedure. The methods used for genetic analyses were described by Nogi et al. (20).

Labeling and extraction of proteins. *S. cerevisiae* cells were grown with shaking at 23°C in liquid synthetic medium with a low methionine content. Exponentially growing cells grown to 10⁶ cells per ml or cells treated at 36°C for the indicated times were pulse labeled with 10 µCi of L-[³⁵S]methionine (1,200 Ci/mmol; (Amersham Corp., Arlington Heights, Ill.) per ml for 10 min, chased for an additional 3 min by the addition of nonradioactive L-methionine to 5 mg/ml, and cooled for 1 min in an ice bath. The radiolabeled cells were chilled, washed twice with 20 mM Tris hydrochloride (pH 8.8) containing 2 mM CaCl₂, and kept frozen until protein extraction. Protein extraction from whole cells to prepare the samples for two-dimensional gel electrophoresis was performed at 4°C. Frozen *S. cerevisiae* cells were vortexed four times for 30 s each time with 0.3 g of glass beads (0.5 mm in diameter) and 2.5 µl of 100 mM phenylmethylsulfonyl fluoride. Lysates were incubated for 5 min with 200 µl of 20 mM Tris hydrochloride (pH 8.8) containing 2 mM CaCl₂ and 10 µl of 1 mg of micrococcal nuclease (Sigma Chemical Co., St. Louis, Mo.) per ml. The samples were mixed with 20 µl of a 2% sodium dodecyl sulfate (SDS)-10% 2-mercaptoethanol solution, 20 µl of 1 mg of pancreatic DNase I (Sigma) per ml, 2 mg of RNase A (Sigma) per ml dissolved in a 0.5 M Tris hydrochloride (pH 7.0) solution containing 50 mM MgCl₂ and incubated for 5 min. The mixture was then lyophilized and dissolved at room temperature in the lysis buffer described by O'Farrell (21).

Two dimensional gel electrophoresis. The method of two-dimensional polyacrylamide gel electrophoresis was essentially the same as that described by O'Farrell et al. (21, 22). Twenty microliters of protein sample which was dissolved in lysis buffer was electrophoresed. Electrophoresis in the first dimension was performed for 4 h at 400 V as a total of 1,600 V in a glass tube (2.5 by 130 mm) containing 4% acrylamide-bisacrylamide, 2% Ampholine (LKB, Bromma, Sweden; pH range, 3.5 to 10), 2% Nonidet P-40 (Nakarai Chemicals, Ltd., Tokyo), and 9.2 M urea. SDS-slab gel electrophoresis for the second dimension (SDS-polyacrylamide gel electrophoresis) was carried out on a discontinuous SDS-polyacrylamide gel with 11% acrylamide-bisacrylamide in the separation gel and 4.75% in the stacking gel. Electrophoresed gels were stained for 1 h with a solution containing 0.025% Coomassie brilliant blue G-250 (Difco), 25% isopropanol, and 10% acetic acid. They were destained twice for 1 h each time and then overnight with a 7% acetic acid

solution. To obtain autoradiograms the gels were dried and exposed to Kodak X-Omat AR film (XAR-5). The following molecular weight markers (Pharmacia, Uppsala, Sweden) were coelectrophoresed: rabbit muscle phosphorylase *b* (94,000), bovine serum albumin (67,000), egg white ovalbumin (43,000), carbonic bovine erythrocyte anhydrase (30,000), soybean trypsin inhibitor (20,000), bovine milk α-lactalbumin (14,000).

Protein measurement. Protein was measured by the method of Lowry et al. (11), with bovine serum albumin used as a standard.

RESULTS

Comparison of protein profiles between the wild-type and *bcy1* mutant strains. The profiles of proteins synthesized under normal and heat shock conditions in the wild-type (AM203-1C) and *bcy1* (AM203-1B) strains were examined. Cells of each strain were incubated in a liquid synthetic medium with a low methionine content at 23°C with shaking. Exponentially growing cultures at 23°C or cultures incubated further at 36°C for 30 min were pulse-labeled with L-[³⁵S]methionine for 10 min and chased for 3 min in the presence of an excess amount of unlabeled L-methionine. The protein sample was prepared for each culture and was analyzed by two-dimensional gel electrophoresis. Synthesis of at least 15 proteins was stimulated after the wild-type cells were shifted to 36°C (Fig. 1A and B). Among these hsp synthesis of six proteins was not stimulated after the *bcy1* mutant cells were shifted to 36°C (Fig. 1C and D). The molecular weights of these six proteins were 84,000 (hsp84), 72,000 (hsp72), 49,000 (hsp49), 45,000 (hsp45), and 41,000 (hsp41); two proteins with molecular weights of 72,000 were identified and referred as hsp72A and hsp72B according to their mobility on the first dimension (A, acidic form; B, basic form).

To confirm that synthesis of these hsp was repressed by the *bcy1* mutation, a tetrad, AM203-1A (*bcy1*), -1B (*bcy1*), -1C (*BCY1*), -1D (*BCY1*), derived from heterozygous diploids carrying the *bcy1* mutation was analyzed for the pattern of protein synthesis after the cells were shifted to 36°C. The repression of synthesis of hsp72A, hsp72B, and hsp41 after the shift to 36°C cosegregated with the *bcy1* mutation; but that of hsp49 did not cosegregate, and that of hsp84 and hsp45 was not reproducible (Fig. 2). The result means that the ability to synthesize hsp84, hsp49, and hsp45 after the shift to 36°C is independent of the *bcy1* mutation. To confirm further that synthesis of hsp72A, hsp72B, and hsp41 is repressed by the *bcy1* mutation, the pattern of protein synthesis in the mutant strain RA1-13D-1 (*bcy1::URA3*), in which the *BCY1* gene was disrupted by the insertion of *URA3* gene, was compared with that in the original strain RA1-13D (*BCY1*) and in a transformant RA1-13D-1(pSY2-2) carrying the *BCY1* gene. Synthesis of hsp72A, hsp72B, and hsp41 was not stimulated after the *bcy1::URA3* mutant cells were shifted to 36°C, but it was observed in cells of the original strain and the transformant carrying the *BCY1* gene. From these results it is concluded that the ability to synthesize hsp72A, hsp72B, and hsp41 after the shift to 36°C is repressed by the *bcy1* mutation.

Comparison of protein profiles between the wild-type and *cyr1-2* mutant strains. If synthesis of some hsp is repressed by higher levels of intracellular cAMP or cAMP-dependent protein phosphorylation, it is expected that the *cyr1-2* mutant that produces a low level of cAMP at 23°C may synthesize hsp without the shift to 36°C. To examine this

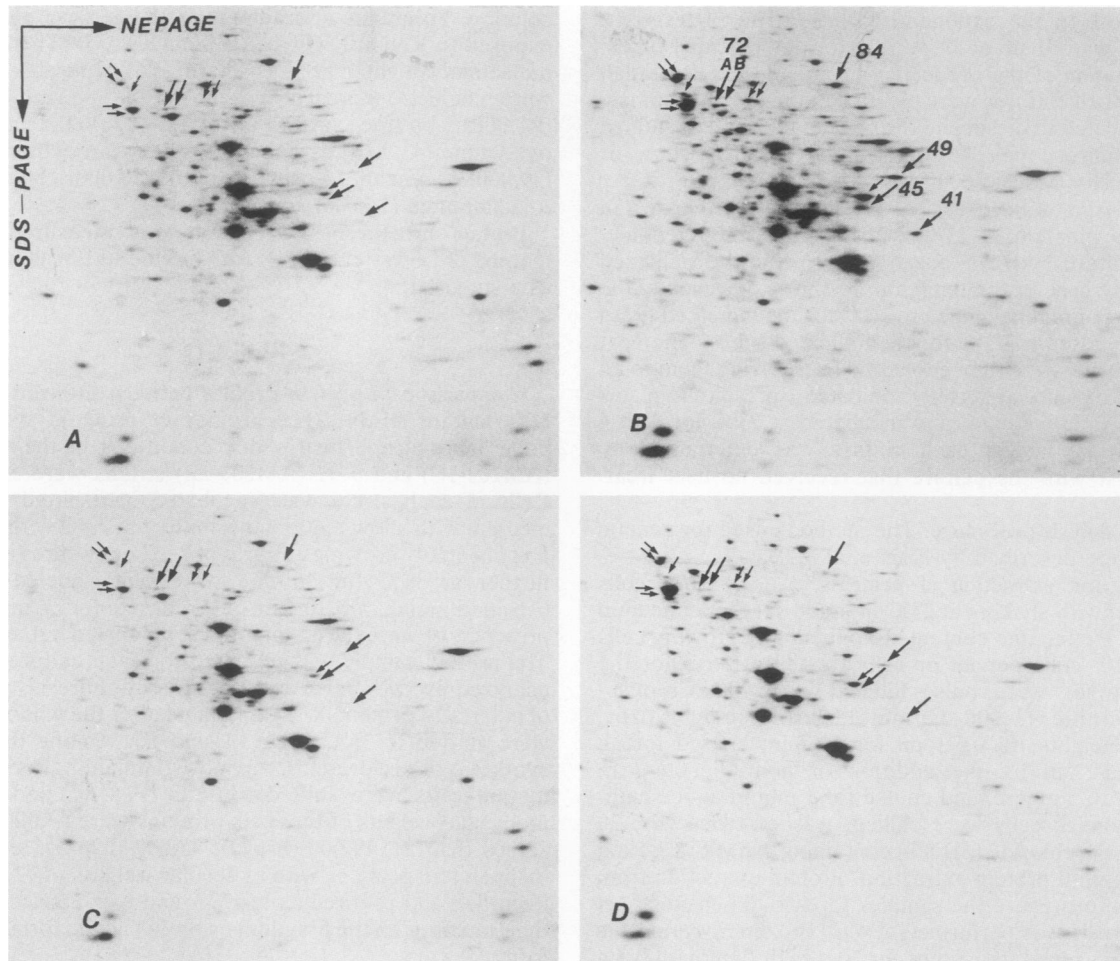


FIG. 1. Two-dimensional gel electrophoretic analysis of [^{35}S]methionine-labeled proteins synthesized in wild-type and *bcy1* strains. The wild-type and mutant cells were grown on synthetic medium with a low methionine content at 23°C. Exponentially growing cultures at 23°C and cultures heat shocked at 36°C for 30 min were pulse-labeled with 10 μCi (1,200 Ci/mmol) of [^{35}S]methionine per ml for 10 min and chased for 3 min with nonradioactive L-methionine (0.5 mg/ml). Total proteins extracted from labeled cells (20 μg) were analyzed by two-dimensional gel electrophoresis. (A and B) AM203-1C (*BCY1*); (C and D) AM203-1B (*bcy1*). (A and C) Cells were grown and labeled at 23°C; (B and D) cells were heat shocked at 36°C for 30 min. Arrows indicate hsps identified on this gel. The synthesis of hsps indicated by the larger arrows was not observed in the *bcy1* mutant. Numbers next to the arrows indicate molecular weights of the proteins (in thousands). NEPAGE, non-equilibrium pH gradient electrophoresis; SDS-PAGE, SDS-polyacrylamide gel electrophoresis.

possibility, proteins synthesized before and after the shift to 36°C in the wild-type (AM248-6D) and *cyr1-2* (AM248-1B) strains were analyzed by two-dimensional gel electrophoresis. Significant amounts of proteins with molecular weights of 73,000 (p73), 72,000 (hsp72A and hsp72B), 56,000 (p56), 48,000 (p48), 43,000 (p43), and 41,000 (hsp41) were synthesized in *cyr1-2* cells that were exponentially growing at 23°C when compared with those synthesized in wild-type cells grown at 23°C (Fig. 3). Among these proteins, hsp72A and hsp72B were synthesized in the *cyr1-2* cells grown at 23°C to levels almost identical to those found in *cyr1-2* and wild-type cells incubated at 36°C. The level of hsp41 synthesis in the *cyr1-2* cells grown at 23°C was significantly higher than that in the wild-type cells grown at 23°C but lower than those observed in the *cyr1-2* and wild-type cells incubated at 36°C. Synthesis of four proteins (p73, p56, p48, and p43) was observed in the *cyr1-2* cells before and after the shift to 36°C, but not in the wild-type or *bcy1* cells incubated under the same conditions. The results indicate that synthesis of hsp72A, hsp72B, hsp41, and several other proteins may be

negatively controlled by cAMP or cAMP-dependent protein phosphorylation.

Heat-sensitivity of cAMP-mutants. The wild-type (AM203-1C), *bcy1* (AM203-1B), and *cyr1-2* (AM248-1B) cells grown in liquid YPD medium to the exponential phase at 23°C were exposed to 52°C for 1 to 5 min. The heat-treated cultures were then cooled in an ice bath, diluted appropriately, and spread onto plates containing YPD medium. The plates were incubated at 23°C for 3 to 4 days, and the viability of each culture was determined. The *bcy1* mutant strain was more sensitive to the elevated temperature than was the wild-type strain, but the *cyr1-2* mutant strain was more resistant than was the wild-type strain (Fig. 4). The presence of cAMP in the growth medium was effective in reducing cell survival of *cyr1-2* cells (Fig. 4).

To confirm the direct relationship between the mutation and heat sensitivity, the tetrads obtained from heterozygous diploids carrying *bcy1* and *cyr1-2* were analyzed for the mutant phenotype and heat sensitivity. The tetrads were inoculated onto plates containing YPD medium and incu-

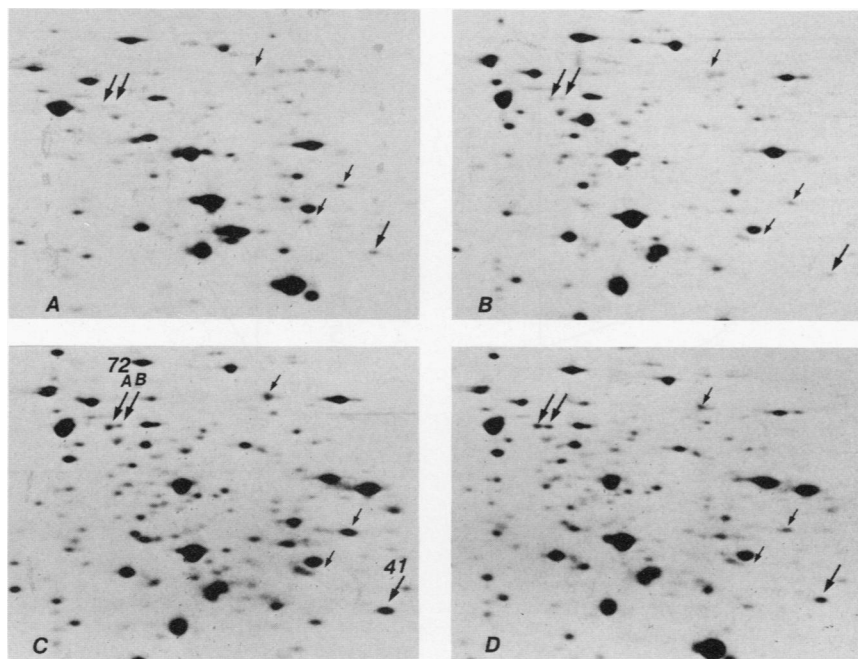


FIG. 2. Cosegregation of the *bcy1* mutation and the induction of hsp synthesis in the tetrad obtained from a diploid heterozygous for the *bcy1* mutation. Exponentially growing cultures of each strain were heat shocked at 36°C for 30 min and pulse-labeled with [³⁵S]methionine, as described in the legend to Fig. 1. (A) AM203-1A (*bcy1*); (B) AM203-1B (*bcy1*); (C) AM203-1C (*BCY1*); (D) AM203-1D (*BCY1*). Arrows indicate hsps that were not synthesized in the *bcy1* mutant, as shown in Fig. 1. The synthesis of hsps indicated by large arrows was cosegregated with the *bcy1* mutant. In panel C, the numbers next to the arrows indicate molecular weights of proteins (in thousands).

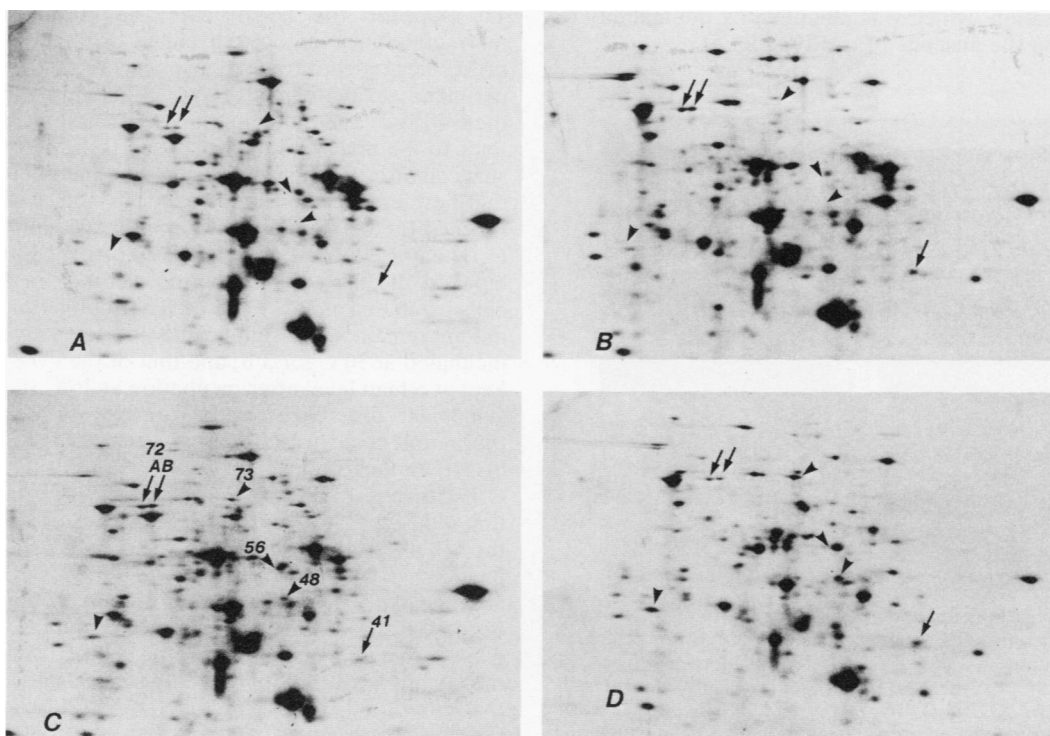


FIG. 3. Two-dimensional gel electrophoretic analysis of [³⁵S]methionine-labeled proteins synthesized in wild-type and *cyr1-2* strains. Two-dimensional gel electrophoretic analysis of [³⁵S]methionine-labeled proteins was performed as described in the legend to Fig. 1. (A and B) AM248-6D (*CYR1*); (C and D) AM248-1B (*cyr1-2*). (A and C) Cells were grown and labeled at 23°C; (B and D) cells were heat shocked at 36°C for 30 min. Arrows and arrowheads indicate hsps and other proteins specifically synthesized in the *cyr1-2* mutant, respectively. In panel C, the numbers next to the arrows indicate molecular weights of proteins (in thousands).

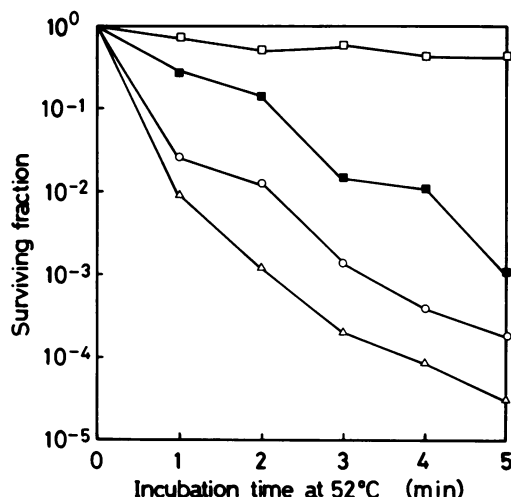


FIG. 4. Response of wild-type, *bcyl*, and *cyr1-2* cells to elevated temperatures. Exponentially growing AM203-1C (○), AM203-1A (△), and AM248-1B (□, ■) cells were exposed to 52°C with (closed symbols) or without (open symbols) cAMP (2 mM) for various lengths of time. The viability was then determined.

bated at 23°C for 6 h, and the plates were exposed to 57°C for 8 or 16 min. The plates were incubated at 23°C for 2 to 3 days, and the heat sensitivity was observed. The heat-sensitive phenotype cosegregated with the *bcyl* mutation, which was identified by its sensitivity to nitrogen starvation (12), while the heat-resistant phenotype cosegregated with the *cyr1-2* mutation, which was identified by the inability to grow at 37°C in the absence of cAMP (Fig. 5).

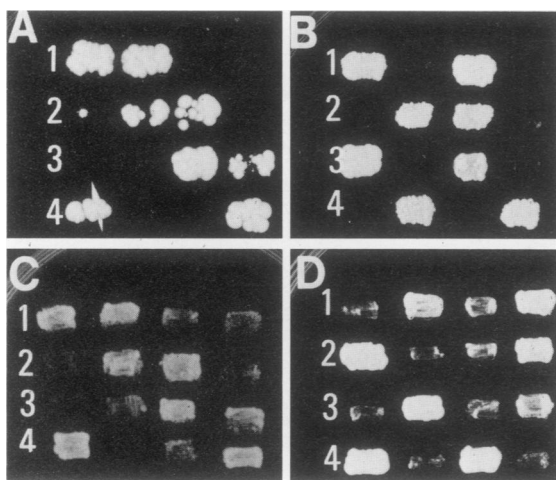


FIG. 5. Segregation of phenotypes with regard to heat sensitivity in tetrads obtained from the heterozygous diploids carrying the *bcyl* or *cyr1-2* mutation. (A and C) Four tetrads obtained from the AM203-1C (*BCY1*) × AM203-1B (*bcyl*) cross; (B and D) four tetrads obtained from the AM248-6D (*CYR1*) × AM248-1B (*cyr1-2*) cross. The tetrads were inoculated on plates containing YPD medium and incubated at 23°C for 6 h to allow the cells to grow to the exponential phase. The plates were incubated at 38°C for 90 min and then heat treated in a 57°C water bath for 8 min (A) or 16 min (B). The tetrads shown in panel A were inoculated on nitrogen-free synthetic minimal medium and incubated at 23°C for 9 days. The colonies were replica-plated onto plates containing YPD medium and incubated at 23°C for 1 day (C). The tetrads shown in panel B were incubated on YPD medium at 37°C for 2 days (D).

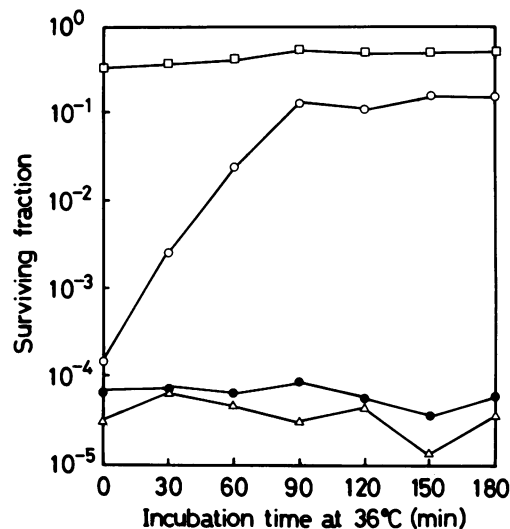


FIG. 6. Development of thermotolerance in wild-type, *bcyl*, and *cyr1-2* cells. Exponentially growing AM203-1C (○, ●), AM203-1B (△), and AM248-1B (□) cells were first incubated at 36°C for various times with (closed symbols) or without (open symbols) cAMP (2 mM) and were then exposed to 52°C for 4 min. The viability was then determined.

Development of thermotolerance in cAMP mutants. McAlister and Finkelstein (15) indicated that the rapid shift of actively growing yeast cultures from 23 to 36°C results in the protection of the culture from death due to exposure at 52°C. To examine the hypothesis that the acquisition of thermotolerance may be correlated with the cellular level of cAMP-dependent protein phosphorylation, the following experiment was performed. Exponentially growing cultures of the wild-type and mutant strains were first incubated at 36°C for 1 to 3 h and were then exposed to 52°C for 4 min; their survival after the heat treatment was then determined. The thermotolerance that developed in the wild-type strain reached the maximum level 2 h after the shift to 36°C (Fig. 6). The presence of cAMP in the culture medium completely blocked the increase of thermotolerance in the wild-type cells incubated at 36°C (Fig. 6). Thermotolerance of the *bcyl* mutant remained at a significantly low level, even after it was incubated at 36°C for 3 h; and that of the *cyr1-2* mutant was kept at a high level after incubation at 36°C (Fig. 6). Thus, a significant difference in the kinetics of development of thermotolerance was observed between the wild-type and cAMP mutant strains.

Transient G1 phase arrest by heat shock. It has been found that wild-type *S. cerevisiae* cells are transiently arrested at the G1 phase by a shift from 23 to 36°C (8). On the other hand, *cyr1* mutants are arrested at the G1 phase in the absence of cAMP (14), and *bcyl* mutants suppress the arrest of the G1 phase caused by the *cyr1* mutation (12). If cAMP plays a role as a negative effector for the transient arrest of the G1 phase caused by the temperature shift, it is expected that growth of the *bcyl* mutants may not be arrested at the G1 phase by the shift to 36°C, which is in contrast to the case in wild-type cells, which may be arrested at the G1 phase under the same conditions. To examine this possibility, the wild-type and *bcyl* cells grown at 23°C were shifted to 36°C. Small fractions of each culture were taken out at 20- or 30-min intervals and examined for the proportion of unbudded cells. The proportion of unbudded cells in wild-type cultures increased to 70% 80 min after the shift to 36°C

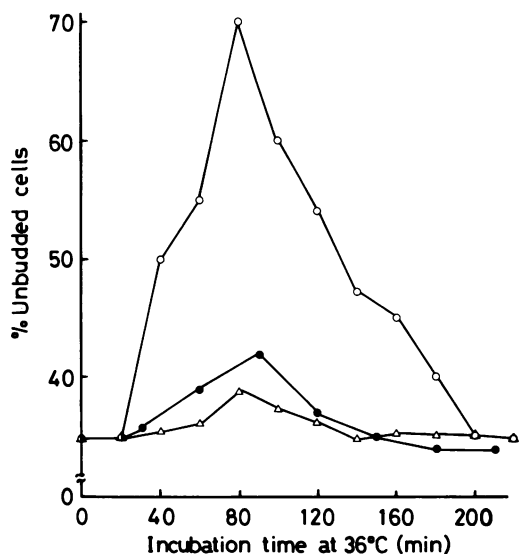


FIG. 7. Accumulation of unbudded cells in wild-type and *bcy1* cells incubated at 36°C. Exponentially growing cultures of AM203-1C (○, ●) and AM203-1B (△) were incubated at 36°C for various times with (closed symbols) or without (open symbols) cAMP (2 mM); the proportion of unbudded cells was then determined.

and then decreased to the level obtained in cultures incubated at 23°C. The proportion in the *bcy1* mutant cultures did not increase during incubation at 36°C (Fig. 7). The presence of cAMP in the growth medium blocked the transient arrest of the G1 phase observed in wild-type cells (Fig. 7). The results suggest that the decrease of the levels of cAMP or cAMP-dependent protein phosphorylation is responsible for the transient arrest of the G1 phase after the shift from 23 to 36°C.

DISCUSSION

In this report we have shown that a decrease of cAMP levels and repression of cAMP-dependent protein phosphorylation are required for the heat shock responses in *S. cerevisiae*, including the synthesis of three hsp's, hsp72A, hsp72B, and hsp41; the acquisition of thermotolerance; and transient arrest of the G1 phase of the cell cycle. The induction of hsp synthesis in response to the temperature shift involves the transcriptional and translational activation of their genes or transcripts (25). Recently, it was indicated that the transcriptional activation of heat shock genes involves a transcriptional factor specific for these genes. This factor is designated HSTF (heat shock transcription factor) in *Drosophila melanogaster* (23) and sigma-32 in *Escherichia coli* (3). In this study the *bcy1* mutant, which produces cAMP-independent protein kinase (14), failed to induce the synthesis of three hsp's; and the *cyr1-2* mutant, which produces a low level of cAMP (13), constitutively synthesized these proteins even without the temperature shift. The synthesis of hsp's other than these three, however, was not affected by the *bcy1* and *cyr1* mutations. From these results it is expected that dephosphorylation of a substrate(s) for the cAMP-dependent protein kinase may be involved in the selective synthesis of three hsp's. Such substance(s) may well be transcriptional or translational factors specific for the selective synthesis of hsp's.

The wild-type strain developed thermotolerance after the shift to 36°C, but the increase of thermotolerance was

blocked by the addition of cAMP. On the other hand, the *cyr1-2* mutant strain exhibited thermotolerance without a shift to 36°C, and the addition of cAMP was effective in reducing the thermotolerance observed in *cyr1-2* cells. These experimental data imply that the intracellular level of cAMP in wild-type cells may be depressed by the shift to 36°C. Boutelet et al. (1) and Camonis et al. (2), however, reported that in wild-type cells the cAMP level increases after the shift to 36°C. Because the *bcy1* mutant cells, which are able to synthesize cAMP but produce cAMP-independent protein kinase, did not show thermotolerance, it is suggested that the temperature shift affects a later step of the cAMP cascade system, even though the cellular cAMP level does not decrease after the temperature shift.

A positive correlation between synthesis of hsp's and acquisition of thermotolerance has been reported in *S. cerevisiae* (15), *Dictyostelium discoideum* (10), *D. melanogaster* (19), and mammalian cells (9, 26). These data suggest that hsp's may function in the acquisition of thermotolerance to the lethal heat treatment. In this study the *bcy1* mutant, which did not induce the synthesis of three hsp's, failed to acquire thermotolerance, while the *cyr1-2* mutant, which synthesized three hsp's without a temperature shift, showed thermotolerance to the same degree as the heat-treated wild-type cells. Thus, it can be assumed that the synthesis of these three hsp's is involved in the acquisition of thermotolerance to the lethal heat treatment.

Another function of hsp's may be related to growth control. Johnston and Singer (8) reported that the temperature shift from 23 to 36°C caused transient arrest of the G1 phase in the *S. cerevisiae* cell cycle. We showed that *cyr1* cells are arrested at the G1 phase of the cell cycle in the absence of cAMP and that the *bcy1* mutation suppressed the arrest of the G1 phase caused by the *cyr1* mutation (12, 14). Because *bcy1* cells could not synthesize three hsp's even after heat shock treatment, these hsp's, if not all, may be directly or indirectly involved in the transient arrest of the G1 phase.

Constitutive synthesis of seven proteins was observed in *cyr1-2* cells without the temperature shift (Fig. 3). Among these proteins, three proteins (p73, p56, and p48) were identical to the proteins induced in the G0 phase identified by Iida and Yahara (5, 6) with respect to electrophoretic mobility. The heat shocked wild-type cells which were transiently arrested at the G1 phase did not produce these proteins, which were found in *cyr1-2* cells. The reason for this is unknown, but a continuously low level of cAMP in *cyr1-2* mutant cells might be responsible for this effect. Iida and Yahara (7) indicated that the *hsr1* mutant, which is heat shock resistant, constitutively synthesized those three proteins induced in the G0 phase found in the *cyr1-2* mutant cells. Thus, the *cyr1-2* and *hsr1* mutants had common characteristics in the heat shock response and production of proteins induced in the G0 phase. It is possible that the *HSR1* gene product may be related to the cAMP cascade system.

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