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# Production of Heat Shock Protein Is Independent of Cell Cycle Blockage in the Yeast *Saccharomyces cerevisiae*

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**In response to certain environmental stresses, cells display a response characterized by the production of heat shock proteins. In this study we showed that blockage of cells of the yeast *Saccharomyces cerevisiae* at specific points in the mitotic cell cycle was not in itself a stress that induced the production of heat shock proteins. Nevertheless, cell cycle blockage did not preclude a normal heat shock response in arrested cells subjected to elevated temperatures.**

In response to a number of environmental stresses (10), cells display an increased synthesis of a subset of proteins referred to as heat shock proteins (for a review, see reference 11). For cells of the yeast *Saccharomyces cerevisiae*, this characteristic stress response, or heat shock response, is exhibited after an increase in temperature (13, 14), starvation for required nutrients (1, 6), exposure to high concentrations of ethanol (2, 16), or addition of certain amino acid analogs (3). Many of these same treatments (notably an increase in temperature [9] and starvation [6]) have been shown to bring about a specific blockage of the cell division cycle. Indeed, the imposition of any condition that leads to a differential block in progress through the cell division cycle (4) and thereby causes "unbalanced growth" as cellular growth activities continue in the absence of cell cycle progress (7) may be expected to promote a stress response. Many mutations and inhibitors are available to block specific aspects of the cell cycle of yeast cells, and we have used some of these to investigate whether cell cycle blockage itself is a stress that induces a stress response evidenced by increased heat shock protein production.

For these investigations into the effects of cell cycle blockage on the stress response, we used both inhibitors and conditional cell division cycle (*cdc*) mutations (4) to block the yeast cell cycle at specific positions. We found that cell cycle blockage itself did not induce a heat shock response. Furthermore, neither the imposition of cell-cycle-blocking conditions nor the consequent blockage of cells in the cell cycle prevented a cell from mounting a characteristic heat shock response when subsequently subjected to elevated temperatures.

## MATERIALS AND METHODS

**Strains and growth conditions.** Haploid *S. cerevisiae* GR2 (*MAT***a** *his6 ura1*) and DBY1252 (*MAT*α *cdc51-1 his4*) have been described elsewhere (8, 15). Strain MD4-2-6 is the product of multiple outcrosses of a cold-sensitive mutant isolated in our laboratory by M. A. Drebot; it carries a cold-sensitive mutation termed *cdc69-1*, which was shown by reciprocal-shift analysis (5) to arrest the cell cycle at "start" (see below) upon transfer of the mutant cells to 14°C (Fig. 1). Cells were grown in liquid YNB medium (7). Cell concentrations were determined by using a Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.), and cell morphologies were determined by microscopic examination. Hydroxyurea (HU), *o*-phenanthroline (OP), canavanine, and the yeast mating pheromone α-factor were all obtained from Sigma Chemical Co., St. Louis, Mo.

**Radioactive labeling and gel electrophoresis.** Cells were pulse-labeled with [$^{35}$S]methionine (New England Nuclear Corp., Boston, Mass.) for 10 min prior to protein extraction essentially as described previously (13). The contents of extract volumes containing equal amounts of acid-precipitable radioactivity were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described previously (13) and visualized by autoradiography.

## RESULTS

The criterion used here to assess the activation of the heat shock or stress response was the increased synthesis, as monitored by pulse-labeling and one-dimensional gel analysis, of yeast heat shock proteins of 100, 90, and 70 kilodaltons. This procedure (13) has been used to assess yeast heat shock protein production in response to many environmental stimuli (2) and was validated by analysis of the effect of the amino acid analog canavanine, previously shown to induce the heat shock response (3). Actively dividing cells treated with canavanine ceased cell proliferation uniformly in the cell cycle, with an increased proportion of cells without buds (Fig. 2A and B). Under this particular cell-cycle-blocking condition, we observed the expected production of heat shock proteins (Fig. 2C).

Other cell-cycle-blocking treatments were used to assess whether cell cycle blockage itself would induce heat shock protein production. Cell cycle inhibitors such as OP and the yeast mating pheromone α-factor were used to cause specific blockage of the cell cycle regulatory step referred to as start (4, 8), while HU was used to block cells in the S phase (18).

To bring about a mutational block of the cell cycle, we could not use the members of the well-studied collection of temperature-sensitive *cdc* mutations (4). To elicit a cell cycle block, mutations of that type generally require the transfer of mutant cells to high temperatures (usually 36 to 37°C), a temperature shift that induces the heat shock response even in wild-type yeast cells (13, 14) and thereby obscures any effects of the cell cycle block on heat shock protein production. Other *cdc* mutations that are cold sensitive and produce cell cycle blockage at 14°C were more useful here. It was found that for wild-type cells the transfer from 29 to 14°C did

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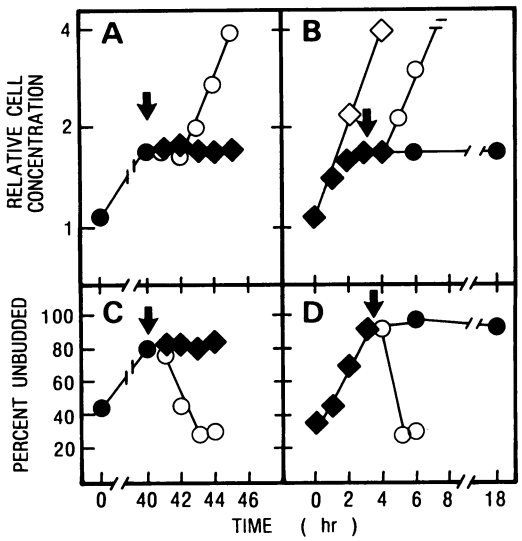


FIG. 1. Order-of-function analysis of the cold-sensitive *cdc69-1* block and the α-factor-sensitive step. (A and C) At time zero, exponentially dividing cells of the cold-sensitive strain MD4-2-6 (*MAT***a** *cdc69-1 leu2-3*) were transferred from 29 to 14°C and incubated for 40 h. The culture was then split, and cells were transferred (arrows) to the permissive temperature of 29°C and incubated with or without α-factor. Arrested cells transferred to medium containing α-factor failed to divide or bud; thus, the cold-sensitive *cdc69-1* mutation blocked cells at or before the α-factor-sensitive step start. (B and D) *cdc69-1* mutant cells dividing at the permissive temperature of 29°C were treated at time zero with α-factor. After the cells had become arrested, α-factor was removed by centrifugation (arrows), the culture was split, and incubation was continued in fresh medium at either the permissive temperature of 29°C or the restrictive temperature of 14°C. Cells at 14°C failed to divide or bud; thus, the cold-sensitive block arrested cells at or after the α-factor-sensitive step. Taken together, these reciprocal-shift experiments show that the cold-sensitive *cdc69-1* block is interdependent with the α-factor-sensitive step and that mutant cells are arrested at start. Symbols: ◇, untreated cells at 29°C; ○, treated cells transferred back to 29°C; ●, cells at 14°C; ◆, cells at 29°C in the presence of α-factor.

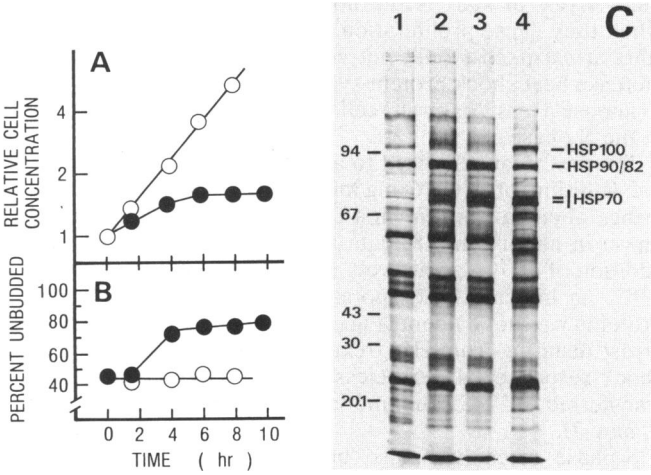


FIG. 2. Effect of canavanine on cell kinetics and heat shock protein production. At time zero, canavanine (10 μg/ml) was added to one portion of a culture of strain GR2 cells actively dividing at 23°C. At intervals cells were removed to assess cellular parameters. (A and B) Cells in the presence (●) or absence (○) of canavanine. (C) Autoradiograms of electrophoretic patterns of proteins radiolabeled for 10 min in the absence of canavanine (lane 1), 30 min (lane 2) and 60 min (lane 3) after the addition of canavanine, and 15 min after transfer to 37°C in the absence of canavanine (lane 4). The positions of molecular weight standards (in thousands) and heat shock protein (HSP) bands are indicated on the left and right, respectively. The heat shock protein band at the position corresponding to a molecular weight of 90,000 is designated HSP90/82 to indicate recent changes in nomenclature (19).

not induce the synthesis of heat shock proteins (Fig. 3, lanes 2 to 4); thus, the temperature shift needed to impose cell cycle blockage by cold-sensitive *cdc* mutations would not obscure any stress responses brought on by the cell cycle block. Therefore, we used the cold-sensitive S-phase mutation *cdc51-1* (15) and a newly isolated cold-sensitive start mutation (see Materials and Methods).

**Effect of start arrest on heat shock protein production.** Start arrest was brought about by use of the conditional mutation *cdc69-1* (see Materials and Methods). Cells bearing the cold-sensitive *cdc69-1* mutation were grown at 29°C, a temperature that did not induce the heat shock response (13) (Fig. 3, lanes 1 and 6); to impose start arrest, we transferred the cells to the restrictive temperature of 14°C, a treatment that itself also did not induce the heat shock response (Fig. 3, lanes 2 to 4). After 24 h at 14°C, mutant cells had become uniformly blocked at start (data not shown). In those blocked cells there was no evidence of the heat shock response, as assessed by our gel analysis (Fig. 3, lane 10). These start-blocked cells were then transferred from 14 to 37°C to test the inducibility of heat shock proteins. Heat shock proteins were induced within 15 min after transfer of these arrested cells to 37°C (Fig. 3, lane 11). Thus, *cdc* mutant cells blocked at start were still able to mount a heat shock response.

Similar results were found after start arrest by treatment with OP (Fig. 4), other start-arresting agents (17), or the yeast mating pheromone α factor.

**Effect of S-phase blockage on heat shock protein produc-**

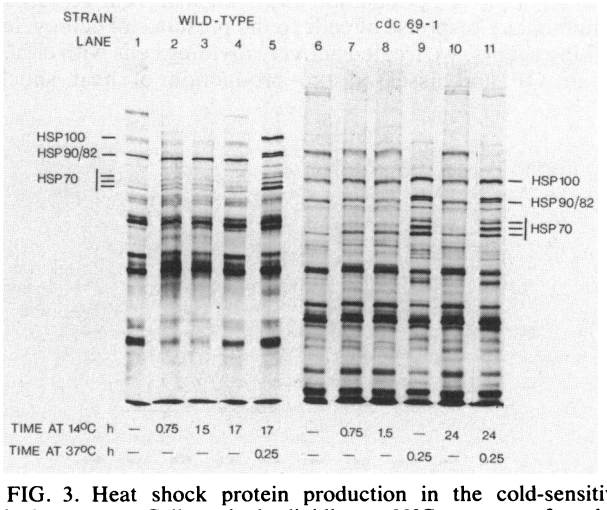


FIG. 3. Heat shock protein production in the cold-sensitive *cdc69-1* mutant. Cells actively dividing at 29°C were transferred to 14°C and incubated as indicated, followed in some cases by transfer to 37°C. The production of heat shock proteins was assessed after 10 min of incubation in the presence of radioactive methionine, followed by extraction, electrophoretic separation, and autoradiography of labeled proteins. Heat shock protein bands are indicated as in Fig. 2.

**tion.** Arrest at start is the normal response of yeast cells when they cease proliferation, so it might be expected that start arrest itself would not constitute a stress that would increase heat shock protein synthesis. Therefore, we sought to impose a less "natural" cell cycle response, such as arrest in the S phase.

The first treatment used to arrest cells in the S phase was the addition of HU (6 mg/ml); at this concentration HU brings about a uniform first-cycle arrest of cells in the S phase in about 5 h (18; data not shown). At 6 h after the addition of HU to an actively dividing population of cells at 23°C, an increased incorporation of label into heat shock proteins was not evident (Fig. 5, lane 6). Therefore, S-phase arrest resulting from HU treatment did not induce the heat shock response. Nevertheless, such S-phase-arrested cells transferred to 37°C could mount a heat shock response (Fig. 5, lane 7).

S-phase arrest was also brought about by use of the cold-sensitive S-phase mutation *cdc51-1* (15). After transfer of actively dividing mutant cells from the permissive temperature of 25°C to the restrictive temperature of 14°C and incubation for 17 h, cells were uniformly arrested in the S phase, as judged by bud morphology (18). In these arrested cells there was no evidence of heat shock protein production. When these arrested cells were transferred to 37°C, they also could induce the characteristic heat shock response (data not shown).

These experiments show that arrest of the cell division cycle at start or in the S phase is not a stress that induces the usual heat shock response; furthermore, blockage of the cell division cycle does not prevent cells from synthesizing heat shock proteins when subsequently subjected to heat shock.

**Effect of cell-cycle-blocking conditions on the heat shock response in dividing cells.** The stress-induced synthesis of heat shock proteins can be a transient phenomenon, as exemplified by the response to heat shock itself (13, 14). Therefore, we considered the possibility that upon treatment with the cell-cycle-blocking agents used here, there may have been a heat shock response that was only transient and that would have subsided by the time complete cell cycle blockage of the cell population had been achieved. To assess the immediate response of cells to the presence of cell-cycle-blocking agents, we treated actively dividing cells with either HU or OP and assessed the production of heat shock proteins at intervals. During much of this time the majority of cells in the presence of cell-cycle-blocking agents were still actively progressing through the cell cycle, since most cells at the time of imposition of cell-cycle-blocking conditions were at a cell cycle stage different from that blocked by the particular treatment. At no time did we detect an increased synthesis of heat shock proteins during treatment with these inhibitors (Fig. 5, lane 5; Fig. 4, lanes 2 to 6).

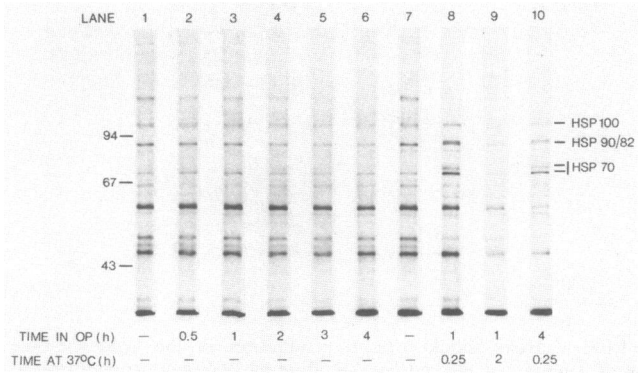


FIG. 4. Effect of OP on the heat shock response. A culture of wild-type cells actively dividing at 23°C was treated with OP (20 μg/ml). At intervals cells were transferred to 37°C, and the production of heat shock proteins was assessed as the experiments shown in Fig. 2 and 3. Molecular weight standards and heat shock protein bands are indicated as in Fig. 2.

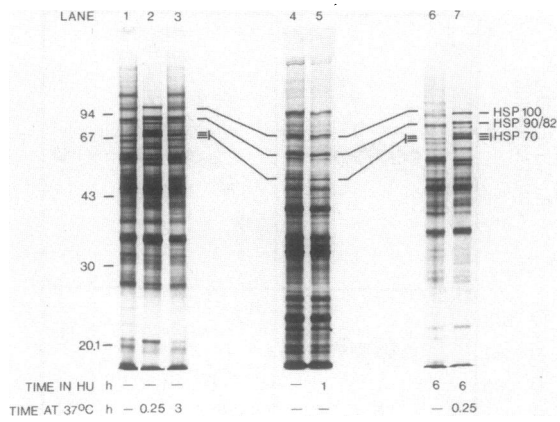


FIG. 5. Effect of HU on the heat shock response. A culture of wild-type cells actively dividing at 23°C was split, and one portion was treated with HU (6 mg/ml). At intervals cells were transferred to 37°C, and the production of heat shock proteins was assessed as in the experiments shown in Fig. 2 and 3. Molecular weight standards and heat shock protein bands are indicated as in Fig. 2.

We also assessed the ability of cells to mount a normal heat shock response while still dividing in the presence of cell-cycle-blocking agents by transferring the cells to 37°C. Here, too, cells in the presence of cell-cycle-blocking agents still underwent cell division in the initial stages of accumulation at a particular cell cycle stage. In the presence of OP or HU, these cells showed after 15 min at 37°C a heat shock response indistinguishable from that of untreated control cultures similarly transferred from 23 to 37°C (Fig. 4, lane 10; Fig. 5, lane 7).

## DISCUSSION

The experiments presented here suggest that intervention in the cell division cycle to block yeast cells in the S phase or at the cell cycle regulatory step start is not in itself a signal for activation of the stress response. This conclusion was substantiated by blocking cells in the S phase with mutations and inhibitors and by blocking start with mutations, α-factor, and a variety of inhibitors. We also found that cells blocked in the cell cycle were not prevented from synthesizing heat shock proteins if subjected to heat shock. These observations extend the work of Ludwig et al. (12), who showed that heat shock protein synthesis could be seen at all stages of the cell cycle in actively dividing yeast cells.

Although start arrest does not of necessity lead to the induction of heat shock protein synthesis, start arrest mediated by some temperature-sensitive *cdc* mutations or by sulfur starvation has been found to engender heat shock protein production in those start-arrested cells (6). This finding signifies that the synthesis of heat shock proteins in those situations is a result of the particular conditions used to arrest the cells and not of the actual arrest of cell proliferation. It was suggested that the arrest conditions that

induced the synthesis of heat shock proteins also caused arrested cells to attain a "$G_0$" state (6). On that basis, our results would indicate that even upon extended incubation of arrested cells, none of the start-arresting treatments studied here would cause cells to attain a similar $G_0$ status.

## ACKNOWLEDGMENT

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