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A response of protein synthesis to temperature shift in the yeast *Saccharomyces cerevisiae*

(heat shock/transcription/translation/two-dimensional gel electrophoresis/*rna-1*)

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ABSTRACT **When *Saccharomyces cerevisiae* are subjected to a sudden increase in temperature (22°C to 37°C) they undergo extensive and, in some cases, extreme alterations in their rates of synthesizing individual polypeptides. These changes were monitored by pulse-labeling cells with [^{35}S]methionine and separating the total soluble proteins by two-dimensional gel electrophoresis. Incorporation of ^{35}S into individual proteins was measured by a computer-coupled autoradiogram-scanning method. The rates of synthesis of most proteins are transiently changed; 10-fold or greater induction or repression is common. This temperature response has also been studied in a mutant strain that is temperature sensitive for the nucleus-to-cytoplasm transport of RNA. In this mutant, not only the induction, but also a part of the repression in response to temperature upshift is largely inhibited. Conceivable mechanisms are discussed.**

Sudden temperature changes can generate greatly changed patterns of protein synthesis. Although particular attention has been focused on the heat shock response in *Drosophila* (1–5), similar effects have also been observed in other eukaryotes and in prokaryotes (6, 7). Induction of the heat shock proteins is often transient and involves the synthesis of new mRNA. On the other hand, the inhibition of *Drosophila* protein synthesis appears to be translational: the conjugate mRNA remains in the cytoplasm, quiescent but potentially functional (3).

We have used a two-dimensional analytical gel method to examine the proteins synthesized by the yeast, *Saccharomyces cerevisiae*, after a sudden increase in temperature. Gorenstein and Warner (8) have shown that the synthesis of *S. cerevisiae* ribosomal proteins is coordinately and transiently inhibited by temperature upshift. We report here that these cells undergo temporary and, in some cases, extreme alterations in the rates with which they synthesize many other proteins. When similar temperature shifts are applied to cells that are temperature sensitive for delivery of mRNA to the cytoplasm (9–11), both the induction *and* certain aspects of repression in the thermal response are eliminated.

MATERIALS AND METHODS

Cells and Media. The following strains (12, 13) were kindly provided by T. Cooper.

$$\text{M25: } \frac{\text{a,}}{\alpha,}\ \frac{his6,}{+}\ \frac{ur1,}{+}\ \frac{lys2,}{+}\ \frac{+}{ade6,}\ \frac{+}{leu1}$$

$$\text{M304: } \frac{\text{a,}}{\alpha}\ \frac{his7,}{+}\ \frac{lys2,}{+}\ \frac{rna\text{-}1,}{rna\text{-}1,}\ \frac{+}{ade1,}\ \frac{+}{tyr1}$$

The *rna-1* allele in M304 is derived from the mutant *ts-136* originally isolated by Hutchison *et al.* (9).

Cells were grown in the MV-A minimal medium described by Hartwell (14), supplemented with 20 μg of L-tyrosine per ml. We have found that addition of tyrosine to this minimal medium stimulates the incorporation of L-[^{35}S]methionine up to 40-fold.

Labeling Conditions. Cells in midlogarithmic phase of growth (less than 5×10^6 cells per ml) were rapidly filtered on membrane filters (47 mm, type HA; Millipore) and transferred to aerated medium at 37°C. At intervals, aliquots of cells were removed and labeled for 5 min with L-[^{35}S]methionine [New England Nuclear or made from hydrolysis of ^{35}S-labeled *Escherichia coli* (15)], followed by a chase with 1 mM unlabeled L-methionine for 1 min. Cells were then cooled for 5 sec in dry ice/acetone, centrifuged, washed once in 20 mM Tris·HCl/2 mM $CaCl_2$, pH 8.8, pelleted in 1.5-ml Eppendorf centrifuge tubes (Bio-Rad), frozen on dry ice, and kept at −70°C until they were to be processed.

Sample Preparation. Unless otherwise noted, all steps were carried out at 0–4°C. Frozen cells were resuspended in 0.2 ml of Tris/$CaCl_2$ and broken by shaking with glass beads in a Braun homogenizer, equipped with a multiple chamber sample holder (16). They were then treated for 5 min with 10 μl of micrococcal nuclease (1.0 mg/ml in Tris/$CaCl_2$). Twenty microliters of 2% sodium dodecyl sulfate/10% 2-mercaptoethanol was added next, followed by 20 μl of pancreatic DNase I (1.0 mg/ml), RNase A (2.0 mg/ml), 0.5 M Tris·HCl, pH 7.0/50 mM $MgCl_2$ (all enzymes from Worthington), and the incubation was continued for 5 min. They were then lyophilized and resuspended at room temperature in buffer A of O'Farrell (17) at a protein concentration of approximately 3 mg/ml.

Two-Dimensional Gel Electrophoresis. Electrophoresis was carried out as described by O'Farrell (17) except that separate 17-cm-long isoelectric focusing gels with narrower pH ranges (pH 5–7 and pH 6–8) were used (18). Most of the overlapping segments of the two gels (i.e., 4 cm from the basic ends of pH 5–7 gels and 4 cm from the acidic ends of pH 6–8 gels) were cut off before the second dimension slab gels were run. Only pH 5–7 gels are shown here. Autoradiographs of dried gels were made on Kodak No-Screen film. The spot-density distributions in these films were analyzed and quantitated on a computer-coupled film scanner, the basic features of which are described elsewhere (19).

RESULTS

Heat-shock: Wild-type cells

When wild-type *S. cerevisiae* were shifted from 22°C to 37°C in minimal medium with tyrosine, their rate of incorporating radioactive methionine into acid-precipitable material immediately increased 1.5- to 3-fold. The rate of incorporation then decreased for 40–60 min and subsequently increased again to about twice the 22°C rate (data not shown).

Fig. 1 illustrates the synthesis of proteins in a portion of a pH 5–7 gel at 22°C (*A*) and at 20 min (*B*) and 100 min (*C*) after the

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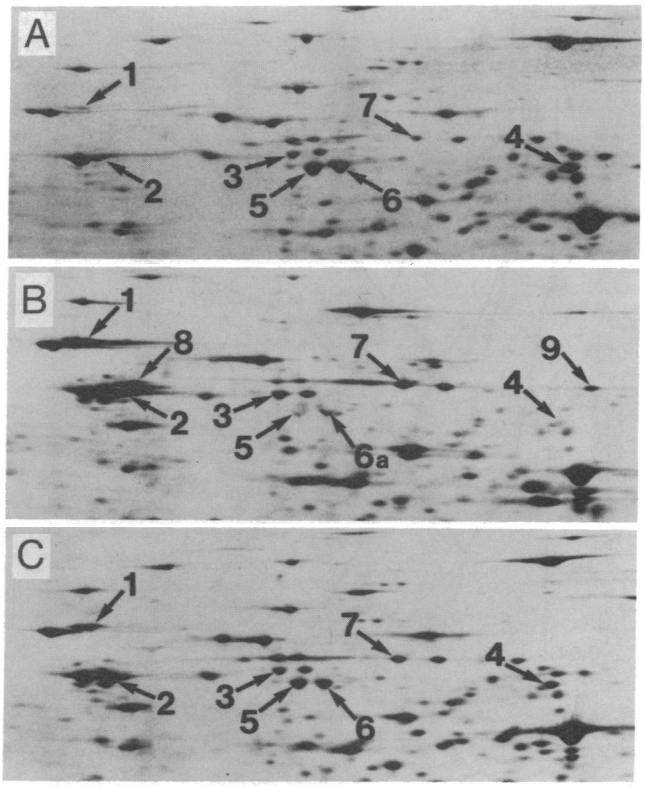

FIG. 1. Effect of a temperature shift on the synthesis of proteins in wild-type *S. cerevisiae* (M25). Cells were grown and labeled with L-[^{35}S]methionine (2 μCi/ml); 2.75×10^4 cpm of radioactive protein (27–48 μg) was layered on each first dimension gel. Corresponding portions of autoradiograms (12-day exposures) made from pH 5–7 gels are shown with the acidic end to the left. The molecular weight range is approximately 50,000 (bottom) to 100,000 (top). Cells were labeled: (*A*) at 22°C; (*B*) after 20 min at 37°C; (*C*) after 100 min at 37°C. The numbered spots are discussed in the text.

shift to 37°C. Shortly after the cells were shifted to 37°C, the relative rates of synthesis of many proteins were dramatically altered. To varying degrees, their synthesis appeared to be either stimulated (e.g., proteins 1, 2, 7, 8, and 9 in Fig. 1 *A* and *B*) or inhibited (e.g., proteins 4, 5, and 6). Some were little affected (e.g., protein 3). Some of the "heat-shock" proteins (8 and 9 in Fig. 1*B*) were made at such a low rate at 22°C that they could not be detected in this exposure of the autoradiogram.

In the immediate vicinity of spot 6 and, to a much lesser extent, spot 5, the changing pattern of protein synthesis created an interesting effect, which is worth explaining in more detail. The transient repression of protein 6 at 37°C permitted a closely apposed, less abundant protein, 6a, to be detected in Fig. 1*B*. The small, half-moon-shaped spot made by the labeled 6a protein was due to its displacement ahead of the much more abundant, but more slowly migrating, protein 6, which locally overloaded the gel and displaced protein 6a ahead of it. It is difficult to decide whether the synthesis of protein 6a was induced by the heat shock because it cannot be sufficiently well resolved from the heavily labeled protein 6 at 22°C (Fig. 1*A*).

The observed changes in labeling pattern were not due to protein breakdown or modification. When cells were prelabeled at 22°C and then shifted to 37°C in nonradioactive medium, the pattern remained essentially as in Fig. 1*A*. Spots 1, 2, 7, 8, and 9 were not enhanced; spots 4, 5, and 6 did not disappear.

Within 1 hr after the shift-up, the rate of total protein synthesis recovered and the relative rate of synthesis of most of the individual protein species returned to roughly their 22°C levels (e.g., proteins, 5, 6, 8, and 9 in Fig. 1*C*). However, the synthesis of several proteins was temperature dependent at the steady state (e.g., proteins 1, 2, and 7 in Fig. 1*C*, direct data not shown, but the pattern of synthesis seen after 100 min at 37°C is similar to that seen in cells grown for several days at this temperature). Although the proteins that are singled out here also showed a very large transient increase shortly after the temperature shift (Fig. 1*B*), not all proteins with large stimulatory transients were preferentially made at 37°C (e.g., protein 9 in Fig. 1*B*). The heat-shock response is reproducible, with visual inspection of the autoradiograms readily showing the same proteins being stimulated or repressed in numerous successive experiments (data not shown).

The most striking aspect of the temperature response is that it affects so large a fraction of the proteins. Autoradiograms of the entire pH 5–7 gels were analyzed by a computer-coupled scanning method to quantitate the distribution of incorporated L-[^{35}S]methionine among resolved protein species (19). The sensitivity was set to analyze spots containing at least approximately 0.8 cpm (7 dpm). Of 433 spots analyzed in the 22°C gel (from which Fig. 1*A* was made) and 491 spots analyzed after 20 min at 37°C (Fig. 1*B*), only 233 could be matched between the two autoradiograms. When we compare the same protein sample run on two different gels, we routinely match 90–95% of the analyzed spots. (The unmatched remainder is largely made up of spots that are just above threshold in one gel and below it in the other and of spots that are located at the edges of the scanned areas.) Fig. 2 illustrates the range of induction ratios when the synthesis of matchable proteins is compared after 20 min at 37°C and at 22°C. Induction ratios (which are defined in the legend of Fig. 2) exceeded 10 for 17 of the 233 matched proteins and were less than 0.1 (repression) for 10 proteins. In addition, 32 "strong" spots (defined as $\geq$10 cpm) in the pattern made after 20 min at 37°C (Fig. 1*B*) were below threshold at 22°C (Fig. 1*A*) whereas 17 "strong" spots in the 22°C gel were not detected after 20 min at 37°C. These 49 cases represent induction ratios *outside* the range of 0.1–10. Limitations of sensitivity prevent us from classifying the 409 re-

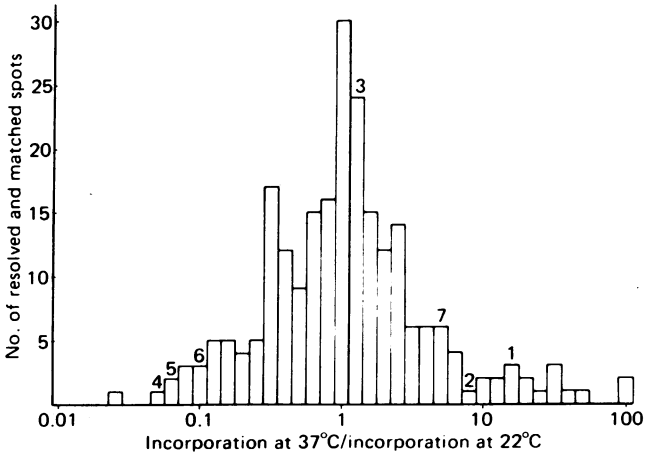

FIG. 2. Histogram of the induction ratios (incorporation of L-[^{35}S]methionine after 20 min at 37°C divided by the incorporation at 22°C) of individual proteins. Autoradiograms, parts of which are shown in Fig. 1 *A* and *B*, were analyzed essentially according to ref. 19 but with certain modifications of the computer programs. Note that the abscissa is plotted on a logarithmic scale. Numbers placed above the histogram refer to spots identified in Fig. 1 and show the quantitated induction ratios for these proteins. When the calculation is made on a single sample run on two gels, essentially the entire histogram lies in a symmetrical distribution between the limits of 0.5–2.

maining unmatched spots with respect to this "extreme" category. Induction ratios among matched spots outside the range of 0.5–2 are quite common (Fig. 2).

Effects of defective RNA metabolism

In attempting to distinguish transcriptional, translational, and intermediate components of the heat-shock response, we have examined proteins in M304, a mutant strain of *S. cerevisiae* whose *rna-1* lesion makes it defective in the transport of RNA from the nucleus to the cytoplasm and in the excision of certain intervening RNA sequences (9–11, 20). When M304 cells were shifted from 22°C to 37°C, the pattern of protein synthesis also changed (Fig. 3). Most of the changes, however, were quite different from those observed in the wild type. Comparing Figs. 1*B* and 3*B*, it is evident that the dramatic induction of wild-type heat-response proteins (e.g., proteins 1, 2, 8, and 9) is not seen in M304, implying that their induction requires the supply of new RNA to the cytoplasm. More surprisingly, the dramatic inhibition of synthesis of certain proteins (e.g., proteins 4, 5, and 6) in wild-type cells after shift to 37°C was also not seen in M304. Fig. 4 illustrates the kinetics of synthesis of proteins 4 and 5 in both strains. During the first 20 min after the shift-up in wild-type cells, the rate of incorporation into these proteins decreased by a factor of at least 8 and 26, respectively. (The incorporation into proteins 4 and 5 after 20 min at 37°C was probably overestimated. For reasons that have already been discussed, we judge that the shapes of these spots at 37°C may signify the presence of unresolved minor proteins.) In M304, the rate of incorporation into proteins 4 and 5 did not change greatly for 10–20 min and then decayed much more slowly, with a half-life of 11 min. A similar response (unpublished data) was seen in another mutant strain, M421, obtained from T. Cooper. Strain M421 is a diploid strain, constructed by J. Bossinger, which is homozygous for a temperature-sensitive mutation in RNA synthesis (*ts4472*; ref. 21).

We take 11 min to be the functional half-life of the residual mRNA for these two proteins at 37°C under conditions of blocked mRNA production (refs. 22 and 23 and unpublished data). The much more rapid thermal repression of proteins 4 and 5 in the wild type implies the intervention of some mechanism beyond mere cessation of mRNA production, although the latter is likely also to play a role here, just as it does for the ribosomal proteins (24).

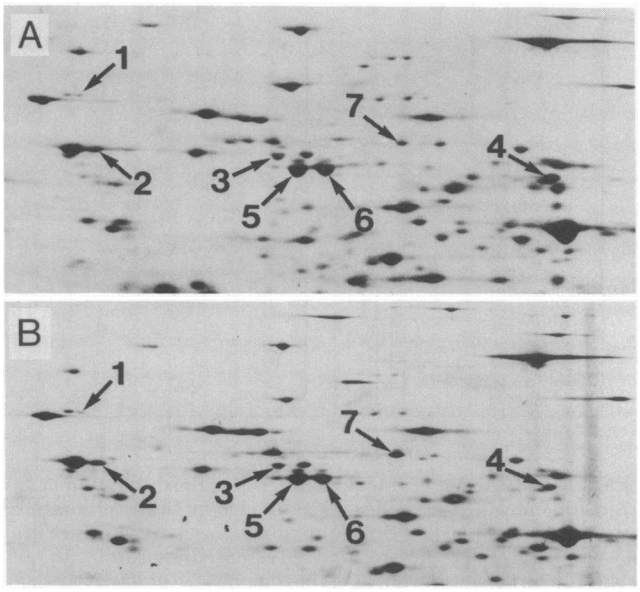

FIG. 3. Effect of a temperature shift on the synthesis of proteins in the mutant M304. The experiment was performed exactly as described in the legend to Fig. 1 except for L-[^{35}S]methionine content, as noted below. Cells were labeled: (*A*) at 22°C (2 μCi/ml); (*B*) after 20 min at 37°C (5.6 μCi/ml). Spots are numbered as in Fig. 1.

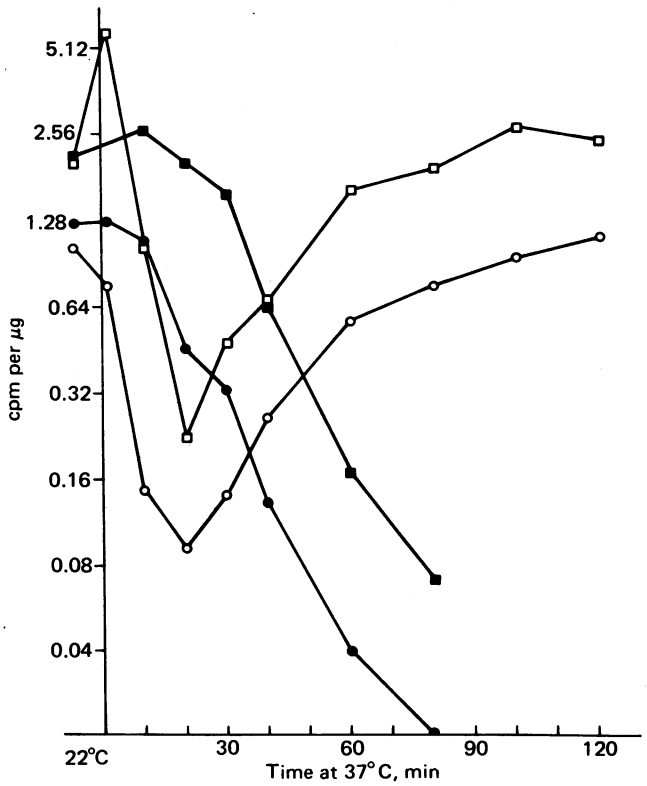

FIG. 4. Transcriptional and post-transcriptional components of the heat response. Kinetics of synthesis of proteins 4 and 5 (Fig. 1) after cells were shifted to 37°C. After the shift-up, aliquots of cells were taken at the indicated times, labeled with 5 μCi of L-[^{35}S]methionine per ml for 5 min, and separated by two-dimensional gel electrophoresis. The incorporation into proteins was measured essentially as described (19). It is expressed here (ordinate) as cpm of radioactivity in a particular spot per μg of total protein subjected to electrophoresis. ○ and □, Proteins 4 and 5, respectively, in M25; ● and ■, proteins 4 and 5, respectively, in M304.

DISCUSSION

We have shown that *S. cerevisiae* growing in glucose minimal medium produce certain characteristic proteins when subjected to a sudden temperature up-shift.‡ At the same time, the synthesis of many other proteins, including the ribosomal proteins (8), is inhibited. By and large, the synthetic rates return to their pre-shift levels within 2 hr. However, the synthesis of some proteins is elevated or depressed during steady growth at the higher temperature. Qualitative aspects of the temperature response have been analyzed with the help of a newly developed analytical method (19).

As a first step in understanding the mechanisms of the pleiotropic temperature response in *S. cerevisiae*, we have tried to distinguish components that act at the levels of transcription, translation, or intermediate steps of gene expression. To this end, we have looked at the effects of temperature-sensitive *rna-1* and RNA synthesis mutations. Both kinds of mutations eliminate the induction of heat-response proteins. One might, therefore, conclude that the induction requires new tran-

‡ Experiments on this subject were also presented at a just-concluded meeting (L. McAllister and D. B. Finkelstein, Abstracts, XIth International Congress of Biochemistry, Toronto, 1979).

scription at the elevated temperature. Although we believe the conclusion to be most probably correct, the reasoning is faulty. The same mutations also affect the kinetics of repression, substituting a delayed and progressive decay of capacity to synthesize certain proteins in the mutants for a much more rapid and extreme, but transient, loss of that capacity in the wild type. *In vitro* analysis of mRNA and nuclear RNA may be required in order to settle the issue of the transcriptional component of the transient heat response conclusively.

The rate of decay of the capacity to synthesize proteins at 37°C in *rna-1* mutant cells most probably reflects the stability of their conjugate messages (refs. 13, 22, and 23 and unpublished data). Any shut-off of protein synthesis that is even more rapid must therefore involve something that affects translation directly. Consequently, we would argue that there must be some component of the transient heat response in the wild type that generates differential effects on the translational efficiency or on the functional stability of different preformed messages. *In vitro* translation experiments should allow one to distinguish effects on translational efficiency from effects on functional stability.

Why should the *rna-1* lesion affect the above cytoplasmic translational components? We would argue that there must be a further nuclear involvement with these cytoplasmic components of the heat response. More specifically, we postulate that the transient change in translational efficiency or mRNA stability is due to one or more effectors that either come directly from the nucleus or are generated outside the nucleus (cf. ref. 25) in response to nuclear signals. Further studies with mutants and metabolic inhibitors may provide insight into the nature of the effector(s).

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