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Alterations in Translatable Ribonucleic Acid After Heat Shock of *Saccharomyces cerevisiae*

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Changes in populations of translatable messenger ribonucleic acids (mRNA's) after heat shock of *Saccharomyces cerevisiae* were examined and found to correlate very closely with transient alterations in patterns of in vivo protein synthesis. Initial changes included an increase in translatable species coding for polypeptides synthesized during heat shock; this increase was found to be dependent on transcription but did not require ongoing protein synthesis. A decrease was observed in the level of translatable mRNA's coding for polypeptides whose synthesis was repressed after heat shock. This decrease was much more rapid than can be explained solely by termination of transcription. Requirements for this rapid loss of RNA from the translatable pool included both transcription and an active *rna1* gene product but not protein synthesis. After the initial changes in translatable RNA induced by heat shock, the patterns of both in vivo and in vitro translation products began to revert to the preshock levels. This recovery period, unlike the earlier changes, was dependent upon a requisite period of protein synthesis.

Responses to heat shock have now been described for a number of organisms, including *Escherichia coli* (11, 23), yeast (12, 14), *Drosophila* (reviewed in reference 1), and several eucaryotic cell lines (7). Although the experimental conditions and temperature ranges vary in these studies, the cellular responses involve similar coordinate changes in protein synthesis. After a shift to an elevated temperature, the synthetic rates of a small group of polypeptides increase significantly, whereas synthetic rates of some proteins made at high levels before the shift decrease. The nature of the molecular events which trigger these remarkable changes in translational patterns is not well understood, nor have functions been determined for the polypeptides induced to such high synthetic levels by heat shock. Considering the seemingly ubiquitous occurrence of the response, however, it seems likely that the heat shock-inducible proteins in different systems may be functionally homologous.

Wild-type strains of the yeast *Saccharomyces cerevisiae* exhibit the striking alterations in protein synthesis characteristic of a heat shock response when shifted from 23 to 36°C. This response is a transient one since within a single generation (2 to 3 h) at 36°C the yeast cells return to protein synthetic patterns resembling those of a 23°C culture (12). It is important to stress that both temperatures are considered to be within the normal growth range for yeast and as such have been used in a large number of studies as the permissive and nonpermissive temperatures for the isolation and examination of temperature-sensitive mutants (reviewed in reference 18).

We have begun an investigation of the molecular events responsible for the alteration of yeast protein synthesis after a shift in cultivation temperature by observing the effects of chemical and genetic inhibitors of specific macromolecular processes upon the heat shock response. Results presented here demonstrate that the initial changes in the synthesis of specific polypeptides in the heat shock response reflect corresponding alterations in the cellular levels of translatable mRNA's. Our results further suggest that the initial rapid depression in the synthesis of specific proteins is not due solely to a cessation of transcription of mRNA coding for the proteins, but involves a transcriptional event as well. We have also found that the initial changes in the cellular levels of translatable mRNA's after heat shock can occur in the absence of protein synthesis. The recovery of yeast cells from the heat shock-induced alterations, viz., a return to the preshift pattern of translatable mRNA's, does, however, require a prior period of protein synthesis.

MATERIALS AND METHODS

Strains and culture conditions. *S. cerevisiae* strain A364A (**a** *gal1 ade1 ade2 ura1 his7 lys2 tyr1*) and temperature-sensitive strains derived from A364A carrying the allele *rna1* (6) or *prt1* (4) were obtained

from the Yeast Genetic Stock Center, Berkeley, Calif. Cultures were grown to midlog phase (2×10^7 cells/ml) at 23°C in buffered low-sulfate medium supplemented to meet requirements of these strains(12). Temperature shifts were performed by pipetting cells into flasks preequilibrated in a 36 or 38°C water bath.

In vivo pulse-labeling and use of inhibitors. Pulse-labeling of 1-ml culture samples with [^{35}S]methionine or a ^{35}S-labeled yeast hydrolysate for periods of 5 to 10 min and preparation of sodium dodecyl sulfate (SDS)-soluble proteins were as previously described (12).

Lomofungin (kindly provided by G. Whitfield, The Upjohn Co., Kalamazoo, Mich.), prepared freshly at a concentration of 5 mg/ml in dimethyl sulfoxide, was added 5 min before heat shock of cultures to give a final concentration of 25 μg/ml. This concentration of inhibitor was chosen because it effectively and immediately inhibits pulse [^{3}H]uridine incorporation without affecting [^{35}S]methionine pulse incorporation for 20 to 30 min (data not shown). Higher concentrations resulted in immediate reduction of protein synthesis and were not used for this reason. Also, since addition of more than 1% dimethyl sulfoxide to our cultures resulted in an inhibition of protein synthesis, minimal volumes of inhibitor were used.

Cycloheximide from a sterile aqueous 5-mg/ml stock was used at a final concentration of 100 μg/ml.

RNA extraction and cell-free translation. Autoclaved glassware and solutions were used in all manipulations involving RNA. Total cellular RNA was extracted from 10-ml samples of log-phase cultures by a slight modification of the method of Sripati and Warner (21). Pelleted cells were suspended in 0.5 ml of LETS buffer (0.1 M LiCl, 0.01 M EDTA, 0.01 M Tris, pH 7.4, 0.2% SDS) supplemented with SDS to 1% and broken by blending in a Vortex mixer for 1 min with sterile glass beads. After addition of 2.5 ml of LETS and 60 μl of diethyl pyrocarbonate (Sigma Chemical Co., St. Louis, Mo.), the lysate was extracted with 3 ml of LETS-saturated redistilled phenol-chloroform-isoamyl alcohol (25:24:1). The phenol extraction was repeated one or two times more until no interface of precipitated proteins was observed. RNA was precipitated overnight at −20°C from the final aqueous phase by addition of 0.3 ml of 5 M LiCl and 8 ml of ethanol. Before translation, the RNA samples were twice precipitated from 1.0 ml of LET buffer (0.1 M LiCl, 0.01 M EDTA, 0.01 M Tris, pH 7.4) by the addition of 0.67 ml of 5 M LiCl for 12 to 24 h at 4°C, and finally from LET buffer with 2.5 volumes of ethanol. Routine yields were 100 to 150 μg of RNA per 2×10^8 cells.

To allow examination of all translatable species, total cellular RNA was used for cell-free translation. The efficiency of translation of yeast RNA was compared in wheat germ extracts (19) and in the rabbit reticulocyte lysate system (16). Synthesis of high-molecular-weight translation products was more reproducible in the reticulocyte lysate assays, and all in vitro RNA translation was subsequently performed with this system by using kits obtained from New England Nuclear Corp., Boston, Mass. Optimum concentrations of Mg^{2+} and K^+ determined for each reticulocyte lysate kit ranged from 0.7 to 1.0 mM for Mg^{2+} and from 60 to 80 mM for K^+. Incorporation of [^{35}S]-methionine into trichloroacetic acid-precipitable counts per minute was linear for RNA concentrations from 0.25 to 1.5 μg per 12.5-μl assay, with the higher concentrations of RNA stimulating incorporation approximately 10-fold above blanks containing no added RNA. Incorporation was carried out for 60 min at 37°C, and the reactions were stopped by the addition of 200 μl of 7% trichloroacetic acid. The acid precipitate was resuspended in 100 μl of SDS sample buffer (12), and 25-μl portions were examined by gel electrophoresis as described below.

Gel electrophoresis. Pulse-labeled in vivo samples and cell-free translation products solubilized in SDS sample buffer were resolved by electrophoresis on 8% polyacrylamide-SDS slab gels and visualized by autoradiography as previously described (12). The autoradiographic patterns shown here represent those portions of the gels resolving polypeptides with apparent molecular weights greater than approximately 50,000. We have found that reproducible resolution of high-molecular-weight polypeptides by this one-dimensional gel system is very dependent on the source of SDS. We have encountered some lot-to-lot variation with SDS from the same supplier. The SDS used in these experiments was obtained from Gallard Schlesinger (BDH lot no. 2050410).

RESULTS

Alterations in translatable RNA. When wild-type strains of *S. cerevisiae* were shifted from 23 to 36°C, the patterns of pulse-labeled proteins were strikingly, but only transiently, altered. The full time course and quantitative analysis of synthetic changes during the yeast heat shock response have been previously reported (12). Synthetic patterns for strain A364A at representative points are presented in Fig. 1 (lanes A–E). Within 15 min after a shift to 36°C (lane B), the synthetic rates of a small set of proteins increased at least 10-fold above preshift levels (lane A). We have previously demonstrated by a comparison of one- and two-dimensional electrophoretic analyses that a number of bands on SDS-polyacrylamide gels corresponding to certain molecular weight polypeptides are unique species and we have also validated the use of such one-dimensional gels for analysis of these specific proteins (12). The major polypeptide species induced by heat shock which may be quantitated by one-dimensional gel electrophoresis correspond to bands labeled a, b, and c in Fig. 1. In contrast to the increased synthesis of this specific group of proteins, the synthetic rates of a number of "repressible" proteins (including bands 1 and 2, Fig. 1) dropped 5- to 10-fold after a shift to 36°C. Although other proteins showed coordinate induction or repression by two-dimensional gel electrophoretic analysis, they were not amenable to one-dimensional analysis (12, 14) and were not further considered

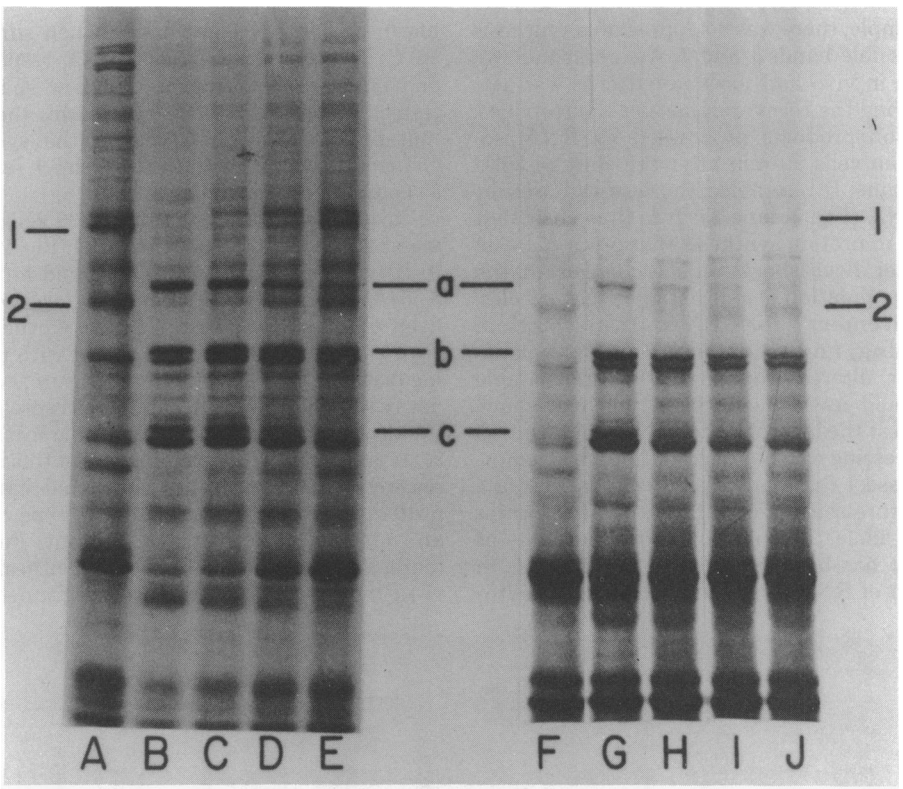

FIG. 1. *Alterations of in vivo and in vitro translation products in yeast cells after heat shock. A log-phase culture of strain A364A grown at 23°C was shifted to 36°C. Before and at times after the shift, 1-ml samples were pulse-labeled for 5 min with a ^{35}S-labeled yeast hydrolysate for analysis of in vivo translation products (lanes A–E), and 10-ml samples were taken for isolation of RNA for cell-free in vitro translation (lanes F–J). Shown are autoradiographic patterns of SDS-solubilized in vivo and in vitro translation products of molecular weights greater than 50,000 resolved by SDS-polyacrylamide gel electrophoresis (as described in Materials and Methods) from culture samples taken at 23°C (A, F) and at 15 (B, G), 30 (C, H), 60 (D, I), and 90 (E, J) min after a shift to 36°C. Apparent molecular weights of 100,000 for band a, 90,000 for band b, and 79,000 for band c were determined by comparison with protein standards of known molecular weights (not shown).*

in these studies.

Changes in synthetic rates for both the inducible and repressible proteins peaked 15 to 20 min after a shift to 36°C. After this time, the translational patterns began to return to the preshift 23°C pattern. That is, the synthetic rates of the heat shock-inducible proteins began to decline, whereas the rates of the repressible proteins increased (Fig. 1, lanes D and E). After 120 min at 36°C (not shown), the pulse-labeled protein pattern was almost indistinguishable from that observed before the shift. Thus, the period from 20 to 120 min after a shift appeared to be a recovery from the initial shock to translational patterns characteristic of undisturbed logarithmically growing cultures.

To examine the basis for preferential synthesis of specific proteins in response to heat shock of yeast, samples of total cellular RNA extracted from cells at various times after a 23 to 36°C shift were used as templates for cell-free translation, using a reticulocyte lysate system. We found that some of the proteins that could be uniquely examined by one-dimensional gel electrophoresis were translated at a lower efficiency in vitro as compared with in vivo translation. However, the relative in vitro synthesis of proteins within this limited molecular weight range was quite reproducible. Although comparisons are necessarily qualitative due to this reduced efficiency of cell-free synthesis of high-molecular-weight polypeptide species, the resulting in vitro synthetic patterns (Fig. 1, lanes F–J) were found to correspond very closely at each time point to in vivo pulse-labeled patterns. Maximum in vitro synthesis of inducible bands a, b, and c was obtained with RNA isolated from cells 15 min after the shift (Fig. 1, lane G). In the

same sample, there was no appreciable synthesis of repressible bands 1 and 2. A correspondence between in vivo and in vitro patterns was also seen during the recovery phase of the response: translation products programed by RNA isolated from cells 90 min after the shift to 36°C (Fig. 1, lane J) resembled those of the preshift 23°C RNA (Fig. 1, lane F). It is thus clear that changes in protein synthesis that occur in yeast cells after heat shock reflect changes in the cellular population of translatable RNA species.

Requirement for RNA transcription, processing, and transport. Possible explanations for altered populations of translatable RNA could involve control of the heat shock response at the level of either RNA transcription and processing or RNA degradation. In attempting to dissect these processes, we made use of a temperature-sensitive strain (ts136) that carries an *rna1* allele. This mutant grows normally at 23°C but has been reported to be defective in transport of RNA from the nucleus to the cytoplasm and in RNA processing when shifted to 36°C (5, 6, 8). If the heat shock response is dependent on the transport of new species of translatable RNA to the cytoplasm, then this mutant should be defective in the synthetic changes associated with the heat shock response as compared with wild-type cells.

Pulse-labeled proteins from ts136 cells growing at 23°C or after a shift to 36°C (Fig. 2, lanes A-E) were similar to those observed for strain A364A, the wild-type parent, at 23°C. As has been observed by others, protein synthesis decayed exponentially in this mutant with time of incubation at the elevated temperature, and this decay time has been interpreted as representing the decay of preexisting cytoplasmic mRNA (2, 9). It is clear from an examination of this in vivo response to a temperature shift that inducible proteins a, b, and c seen in a wild-type culture are not synthesized by this mutant. Furthermore, an examination of proteins synthesized in vitro by a reticulocyte lysate programed with

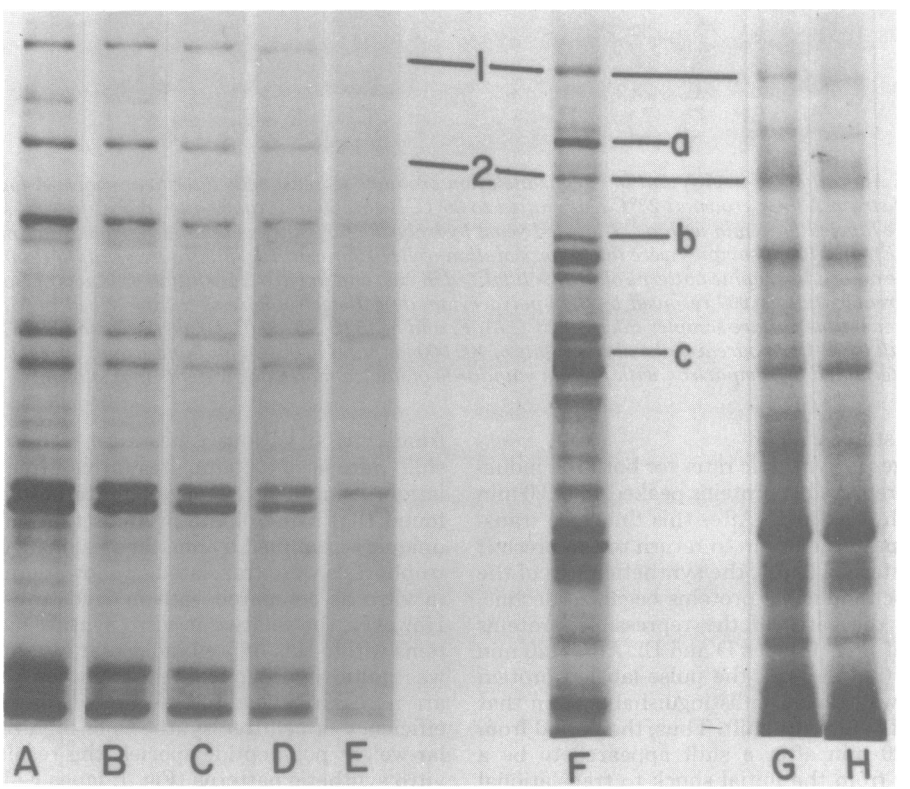

FIG. 2. *Effect of rna1 mutation on the yeast heat shock response. Samples of an exponentially growing culture of ts136 were pulse-labeled for 10 min with [^{35}S]methionine at 23°C (A) and at pulse times beginning 10 (B), 20 (C), 30 (D), and 60 (E) min after a shift to 36°C. A portion of the culture shifted to 36°C for 30 min was returned to 23°C, and a sample was labeled 30 min later (F). In vitro translation products were analyzed from reticulocyte lysate cell-free translation assays using RNA isolated from ts136 cultured at 23°C (G) and 20 min after a shift to 36°C (H). SDS-solubilized proteins were analyzed as in Fig. 1.*

whole-cell RNA isolated from ts136 either before or after a temperature shift showed no significant increases in the levels of translatable RNA coding for the inducible proteins (Fig. 2, lanes G and H). Thus, the appearance of the heat shock-inducible proteins as well as translatable mRNA's coding for these polypeptide species requires a functional *RNA1* allele.

What is even more intriguing about the response of ts136 to a temperature shift is that the decay of synthesis of the heat shock-repressible proteins as well as the decay in the level of translatable mRNA encoding these proteins was considerably slower in this mutant than in the wild-type strain. Even after the mutant was held at 36°C for 30 min, bands 1 and 2 were easily visualized by in vivo pulse-label (Fig. 2, lane D) or by in vitro synthesis (Fig. 2, lane H), whereas little or no isotope was incorporated into these bands in wild-type cells 15 min after a shift to 36°C (Fig. 1, lanes B and G). Thus, the half-lives of the mRNA's coding for the heat shock-repressible proteins appear by these indirect measurements to be much shorter after heat shock of the wild type as compared with the half-life estimated in the ts136 mutant. This implies that a functional *rna1* gene product is required for the rapid loss of these translatable mRNA's in response to heat shock.

It is interesting to note that if a ts136 culture held at 36°C for 30 min was subsequently shifted back to 23°C, the heat shock response was then observed in vivo (Fig. 2, lane F) and in vitro (data not shown). Inducible proteins a, b, and c reached high synthetic levels within 20 to 30 min of the temperature lowering. With regard to the repression of bands 1 and 2, it is more difficult to evaluate the response since many proteins were showing a synthetic recovery at the permissive temperature.

The absence of in vivo or in vitro changes in protein synthesis in ts136 cultures shifted to 36°C implies that the function of the *rna1* gene product (presumably transport of RNA from the nucleus to the cytoplasm or processing of RNA) is a prerequisite for these changes. To examine whether processing or transport of preexisting nuclear RNA, or both, is involved in the initiation of the heat shock response, we made use of the zinc-chelating antibiotic lomofungin, which has been used to effectively inhibit transcription in yeast (10). In testing the effect of this antibiotic on the heat shock response, we found that experimental conditions must be carefully chosen to eliminate pleiotropic effects (see Materials and Methods). Also, complete inhibition of transcription required a 23 to 38°C temperature shift. As shown in Fig. 3 (lane B), the addition of 25 μg of lomofungin per ml 5 min before shifting a culture of A364A from 23 to 38°C resulted in inhibition of the in vivo changes in protein synthesis associated with heat shock relative to an untreated sample of cells similarly shifted and pulse-labeled (lane C). Cell-free translation confirmed that the in vivo protein pattern in lomofungin-treated cells accurately reflected translatable RNA populations (not shown). It thus appears that transcriptional processes are necessary for all the early changes in the levels of translatable RNA which result in the altered patterns of protein synthesis associated with the heat shock response.

Initial heat shock changes do not require protein synthesis. To evaluate whether transcriptional changes alone are sufficient to alter the patterns of translatable RNA, we examined the effect of blocking protein synthesis, using strain ts187. This strain carries the mutant locus

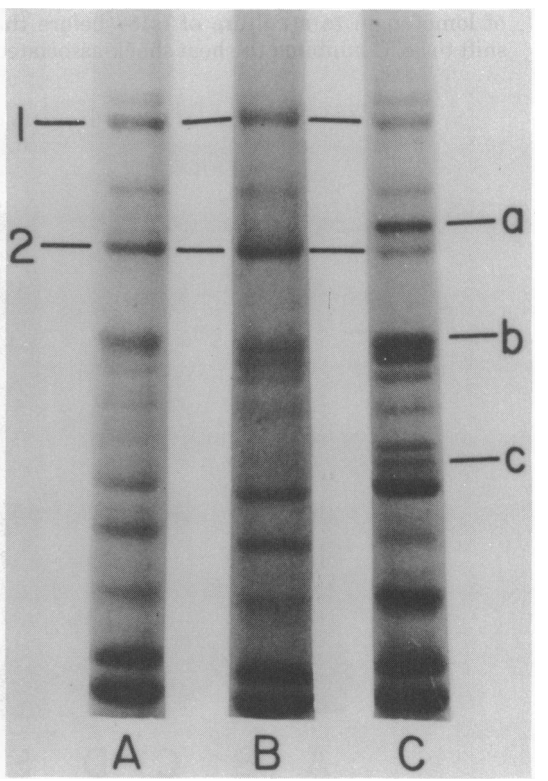

FIG. 3. *Effect of lomofungin on the yeast heat shock response. A sample of a culture of A364A was pulse-labeled with [^{35}S]yeast hydrolysate for 5 min at 23°C (A) and 20 min after a shift to 38°C in the presence (B) or absence (C) of lomofungin as outlined in Materials and Methods. Shown are the autoradiographic patterns of SDS-solubilized proteins resolved by electrophoresis on 8% polyacrylamide gels.*

prt1, which causes a rapid breakdown of polysomes at 36°C due to a temperature-sensitive defect in the initiation of protein synthesis (4). At 23°C, pulse-labeled proteins (Fig. 4, lane A) and in vitro translation products (Fig. 4, lane F) in the mutant resembled those of the parental strain A364A. When ts187 was shifted to 36°C, pulse-label incorporation into proteins ceased (Fig. 4, lane B). However, the translation products of RNA isolated from ts187 cells 20 min after a shift to 36°C clearly reflected the transcriptional changes associated with heat-shocked cells (Fig. 4, lane H). Similar results were obtained if protein synthesis was inhibited by addition of cycloheximide 5 min before heat shock of a wild-type culture (data not shown). Thus, ongoing protein synthesis was not required for the changes in translatable RNA associated with the induction of bands a, b, and c and with the repression of bands 1 and 2. As was the case for the wild-type response, the addition of lomofungin to a culture of ts187 before the shift to 38°C inhibited the heat shock-associated transcriptional changes when the RNA was monitored by in vitro translation (Fig. 4, lane G).

The transcriptional changes at 36°C which were obvious in the in vitro translation products of the untreated culture (Fig. 4, lane H) were immediately expressed in vivo when the ts187 culture was returned to the permissive temperature (Fig. 4, lane C). Within 60 min at 23°C, the preshift pattern of protein synthesis was regained (Fig. 4, lane E). Thus, in the initial heat shock response, transcription alone was sufficient for alterations in translatable RNA, with translational processes being necessary only for in vivo expression of these transcriptional changes.

Recovery from heat shock requires protein synthesis. After undergoing the early changes in translatable RNA populations, wild-type cells began to recover from the heat shock, and after 120 min (Fig. 5, lane C) the in vitro translation pattern resembled that observed before the shift (Fig. 5, lane A). This recovery,

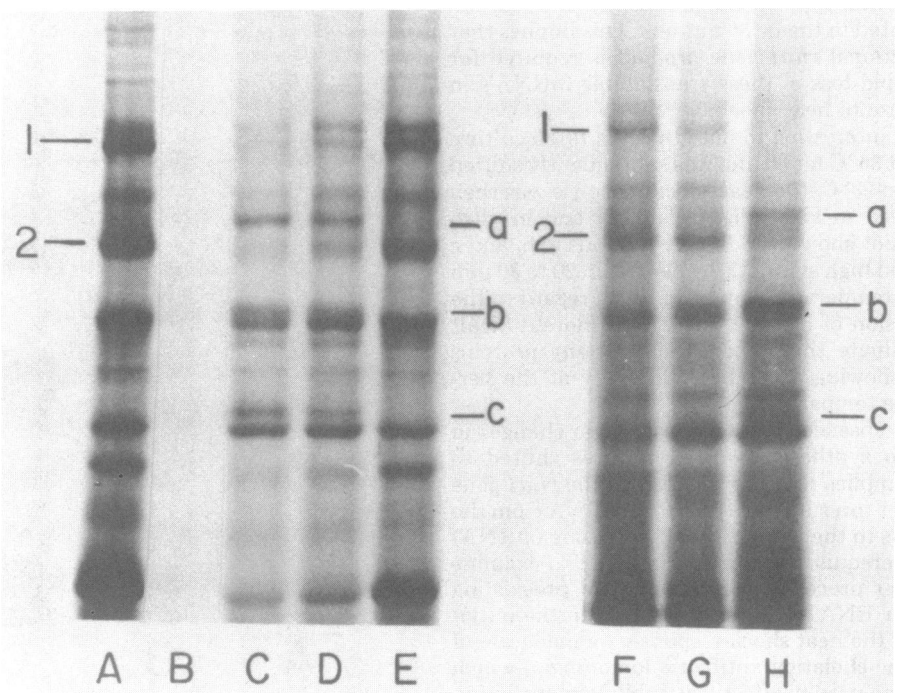

FIG. 4. *Effect of inhibition of translation on the yeast heat shock response. Samples of an exponentially growing culture of ts187 were pulse-labeled for 5 min with ^{35}S-labeled yeast hydrolysate at 23°C (A) and 10 min after a shift of the culture to 36°C (B). A portion of the culture was returned to 23°C after a 30-min exposure to 36°C, and samples were labeled 10 (C), 30 (D), and 60 (E) min after return of the culture to 23°C. In vitro translation products were analyzed by using RNA isolated from a culture of ts187 grown at 23°C (F) and from cultures of ts187 shifted for 20 min to 38°C in the presence (G) or absence (H) of lomofungin. Shown are the autoradiographic patterns of SDS-solubilized translation products resolved by polyacrylamide gel electrophoresis.*

unlike the initial heat shock changes, did not occur in the absence of protein synthesis. Initial changes in translatable RNA populations 15 min after a shift to 36°C were similar in cultures of A364A (Fig. 5, lane B) and ts187 (lane D) and in a culture of A364A treated with cycloheximide before the shift (lane F). After 120 min, a time when the wild type had regained many of the characteristics of the preshift pattern of translation products (lane C), the heat shock pattern was still observed in the ts187 culture (lane E) and in the cycloheximide-treated wild-type culture (lane G). In the latter samples, proteins a and b were still the major high-molecular-weight translation products and no recovery in the synthesis of bands 1 and 2 was observed. The ts187 cells were still viable after 120 min at 36°C and, when shifted back to the permissive temperature, these cells synthesized high relative levels of the heat shock-inducible proteins as protein synthesis recovered (not shown).

In an attempt to define the dependence of the recovery period on protein synthesis, we found that recovery of preshift in vitro patterns in A364A did not occur when cycloheximide was added as late as 10 min after a shift to 36°C (not shown). Recovery did occur, however, if the addition of cycloheximide was delayed to 20 or 30 min. Since the inducible heat shock proteins were synthesized at high levels during the time from 10 to 20 min after a shift, these proteins may play some role in the recovery of mRNA to preshift levels after the imposition of a heat shock.

DISCUSSION

The heat shock response in *S. cerevisiae* may be described as a programed series of events. After a shift of wild-type cells from 23 to 36°C, the synthetic rates of a large number of cellular proteins rapidly change to either higher or lower levels (3, 12, 14). Although the magnitudes of rate changes vary among different proteins (14), all changes peak within 15 to 20 min and are followed by a recovery period during which synthetic patterns become progressively similar to the preshift pattern. The yeast heat shock response is thus an appropriate model for investigating molecular mechanisms involving not only the coordinate induction and repression of

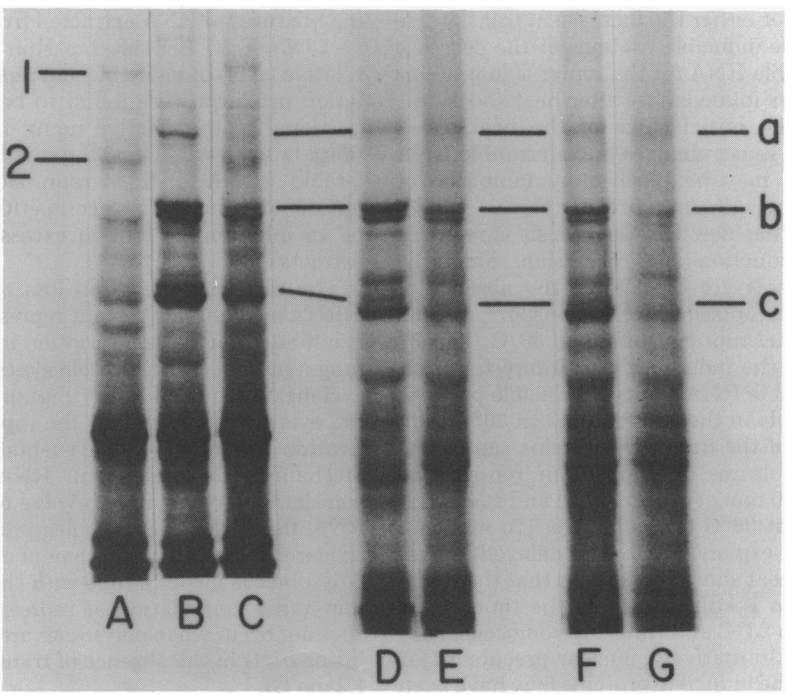

FIG. 5. *Effect of inhibition of protein synthesis on recovery from heat shock. Log-phase cultures of A364A and ts187 were grown at 23°C. The A364A culture was divided into two parts, and cycloheximide was added to one part 5 min before the three cultures were shifted to 36°C. Samples were taken from the untreated A364A culture at 23°C (A) and at 20 and 120 min, respectively, after the shift to 36°C from the untreated A364A culture (B, C), from the ts187 culture (D, E), and from the cycloheximide-treated A364A culture (F, G). RNA isolated from each sample was used for cell-free translation. Shown are autoradiographic patterns of SDS-solubilized in vitro translation products resolved by polyacrylamide gel electrophoresis.*

groups of polypeptides, but also the reversal of these processes.

The coordinate changes which occur throughout the time course of the heat shock response in yeast reflect changes in populations of translatable RNA. This conclusion is based on the similarity of in vivo and in vitro translation products at each time point (Fig. 1). Thus, preferential synthesis of a group of polypeptides at a particular stage in the response is due to the relative abundance of translatable RNA for those polypeptides. In contrast, some of the preexisting mRNA's of the *Drosophila* heat shock response are relatively stable and can be isolated from shocked cells and translated in vitro (15, 20). The increased synthesis of proteins induced by heat shock in *Drosophila* has been attributed by some investigators both to an increase in synthesis of corresponding mRNA's and to a preferential reformation of polysomes on these newly synthesized mRNA species (13). Such dramatic changes in polysome profiles are not observed in yeast after heat shock (17; D. Finkelstein and L. McAlister, unpublished observations). Also, as presented here (Fig. 4 and 5), blocking protein synthesis before heat shock does not alter either the increase in translatable RNA for the inducible proteins or the decrease in translatable RNA for the repressible proteins which occurs immediately after heat shock.

During the initial stage of the heat shock response in yeast, changes in translatable RNA populations may be blocked by inhibition of transcription with lomofungin (Fig. 3 and 4), suggesting that new RNA synthesis is required for both induction and repression. Similarly, both processes are blocked in the absence of nuclear to cytoplasmic RNA transport, as seen with the *rna1* mutant shifted to 36°C (Fig. 2). In terms of the induction, it is interesting that no translatable RNAs for the inducible proteins are detectable in the *rna1* mutant at 36°C. Yet, expression of the inducible proteins can be observed in cultures of the mutant returned to 23°C after 30 min at 36°C (Fig. 2) and even after being held at 36°C for as long as 120 min (not shown). One explanation for this delayed expression of the heat shock response is that the signal for induction is still present at the time of the shift back to 23°C and transcription occurs subsequently. Alternatively, nuclear precursors for mRNA for the inducible proteins may have been synthesized in an untranslatable form at the time of the initial shift to 36°C and attain a translatable form only when the cells are returned to 23°C. The latter possibility is attractive since precursors for tRNA species have been identified in *rna1* cells shifted to 36°C (5, 8). Attempts to distinguish between these alternatives by inhibiting transcription by addition of lomofungin to the mutant at 36°C before shifting back to 23°C have been unsuccessful due to pleiotropic effects of this drug. We are currently pursuing this question by isolating appropriate sequence probes.

Transcription may also be required for the rapid decrease in translatable RNA for certain repressible proteins since repression is inhibited by lomofungin (Fig. 3) and in the *rna1* mutant shifted to 36°C (Fig. 2). Miller et al. (14) reported that decay in the rate of synthesis of specific heat shock-repressible proteins was more rapid in wild-type yeast cells than in an *rna1* mutant. We have confirmed this result and extended it by demonstrating that this differential loss of repressible protein synthesis is apparently not due to translational control but instead appears to be due to changes in the levels of translatable RNAs as seen by cell-free protein synthesis programed by whole-cell RNA from the wild type and mutant. We have no evidence from in vitro protein synthesis using heterologous systems for differential efficiency of translation of mRNA's encoding inducible and repressible proteins. Using mixtures of RNA extracted from cells grown at 23°C or 15 min after a shift to 36°C, the relative levels of inducible and repressible translation products were found to be directly proportional to the relative input of either RNA (data not shown). Thus, the rapid loss of translatable mRNA for these repressible proteins is not due to a masking or competition effect, nor to an inhibitor present in excess in the RNA extracts.

The fact that the rapid loss of translatable mRNA's coding for certain repressible proteins is inhibited by the transcription inhibitor lomofungin but not by the protein synthesis inhibitor cycloheximide or in a *prt1* mutant implies that the events responsible for the rapid RNA inactivation are transcriptional events. Although the mechanism for making an RNA species untranslatable may simply involve degradation of RNA, the notion that such degradation could be triggered by simple displacement of mRNA from polysomes is incompatible with the observation that rapid degradation of repressible mRNA's does not occur when polysomes are broken down (using *prt1*) in the absence of transcription (Fig. 4, lane G).

The fact that in an *rna1* mutant the rapid inhibition of certain repressible mRNA's does not occur implies that transcription alone is not sufficient to cause the repression, but presumably a further transport or processing event is also required. As stated above, we have no evi-

dence from in vitro experiments using heterologous systems for the inactivation of preexisting mRNA's by heat shock RNA. This would tend to imply that some preexisting yeast protein(s) may function in this regard as an RNA-inactivating enzyme with a heat shock transcript perhaps providing some essential cofactor. It is interesting to note that Warner and Gorenstein (3, 22) have reported that the decay of ribosomal protein synthesis is no different after heat shock of wild-type or *rna1* cells. Therefore, not all proteins synthetically repressed by heat shock require an active transcriptional process. Further investigation of the intriguing phenomenon of specific repression dependent upon ongoing transcription will require the isolation of appropriate sequence-specific probes in order to determine the mechanisms involved in this RNA modification or degradation.

During the recovery phase of the yeast heat shock response, synthesis of the repressible proteins recovers while synthesis of the inducible proteins declines. It is possible that, here too, transcription is required for the corresponding changes in translatable RNA populations. Again, attempts to selectively block transcription with lomofungin during the recovery phase of the heat shock response have been unsuccessful. The responsiveness of yeast cells to this antibiotic appears to be quite variable, and it has been an effective selective inhibitor of transcription in our hands only when present at the time of a 23 to 38°C shift.

Once the heat shock-induced changes in translatable mRNA's have occurred, protein synthesis is required for the reestablishment of the preshift mRNA pattern. Changes in translatable RNA populations during the recovery phase of the heat shock do not occur in yeast cells blocked in protein synthesis at the time of (or 10 min after) the 23 to 36°C shift (Fig. 5). Furthermore, shifting a heat-shocked culture of the *prt1* mutant back to 23°C in the presence of cycloheximide greatly retards the recovery process (data not shown). This suggests that some protein(s) made during the initial stages of the heat shock response is required for the reestablishment of the preshift translational patterns.

While some aspects of the response to heat shock are similar for different organisms, certain molecular features of the response in yeast are apparently unique and merit further investigation. Studies currently in progress which are aimed at evaluating the physiological significance of the heat shock response may provide selectable phenotypes for the isolation of mutants and eventual dissection of the regulation of this response.

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