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# Mild Temperature Shock Alters the Transcription of a Discrete Class of *Saccharomyces cerevisiae* Genes

CHUNG H. KIM† AND JONATHAN R. WARNER*

*Departments of Biochemistry and Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461*

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In *Saccharomyces cerevisiae* the synthesis of ribosomal proteins declines temporarily after a culture has been subjected to a mild temperature shock, i.e., a shift from 23 to 36°C, each of which support growth. Using cloned genes for several *S. cerevisiae* ribosomal proteins, we found that the changes in the synthesis of ribosomal proteins parallel the changes in the concentration of mRNA of each. The disappearance and reappearance of the mRNA is due to a brief but severe inhibition of the transcription of each of the ribosomal protein genes, although the total transcription of mRNA in the cells is relatively unaffected by the temperature shock. The precisely coordinated response of these genes, which are scattered throughout the genome, suggests that either they or the enzyme which transcribes them has unique properties. In certain *S. cerevisiae* mutants, the synthesis of ribosomal proteins never recovers from a temperature shift. Yet both the decline and the resumption of transcription of these genes during the 30 min after the temperature shift are indistinguishable from those in wild-type cells. The failure of the mutant cells to grow at the restrictive temperature appears to be due to their inability to process the RNA transcribed from genes which have introns (Rosbash et al., Cell 24:679–686, 1981), a large proportion of which appear to be ribosomal protein genes.

The assembly of the ribosome of *Saccharomyces cerevisiae* involves the coordinated synthesis of 4 RNA and 75 protein species. This coordination occurs not only in exponentially growing cells (10, 34) but also under conditions of substantial change in growth conditions, e.g., the change from ethanol to glucose as a carbon source (13), or the deprivation of an amino acid (35) or of a nitrogen source (23). Yet the genes for ribosomal proteins are scattered throughout the genetic map, as shown both by classical genetic techniques (11, 19, 27) and by gene cloning (3, 7–9, 36, 37). Of the 20 or more ribosomal protein genes that have been cloned, only 2 are near each other, and those are separated by several hundred base pairs (4, 7). Thus, it seems that each gene is under separate control, a situation in substantial contrast to that for *Escherichia coli*, in which clusters of several genes are under the regulation of a single promoter and a single translational repressor (5, 20, 38, 39).

To study the regulation of synthesis of *S. cerevisiae* ribosomal proteins, we have made use of cloned genes (7, 36) to prepare probes with which to measure such parameters as the concentrations of individual mRNAs and the transcription of their genes during the coordinated response to a physiological stress. A clear example of such a coordinated response occurs after a mild heat shock. When cells growing at 23°C are shifted to 36°C, a temperature which still permits growth, the synthesis of all ribosomal proteins begins an immediate decline, reaching 20 to 40% of its original level within 20 min. The synthesis of these proteins then begins to increase and reaches a normal value by 60 to 90 min (10). An analysis of the concentration of mRNA by cell-free translation suggested that the temporary inhibition of ribosomal protein synthesis reflects a temporary decline in the concentration of their translatable mRNAs (34). This suggestion has been confirmed by the direct analysis of the concentration of mRNA for three ribosomal proteins as a function of time after a temperature shift (25). A comparison of the rate of decline of the synthesis of ribosomal proteins with the half-life of mRNA suggested that the temperature shift leads to a brief halt in the transcription of the genes for all ribosomal proteins (10; C. H. Kim and J. R. Warner, J. Mol. Biol., in press).

Parallel experiments with a temperature-sensitive mutant, *ts*368, showed that after a shift to

† Present address: Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

36°C, the synthesis of ribosomal proteins declines with the same kinetics as in the wild type, but never recovers (10). Recently, Rosbash et al (25) have observed that these cells accumulate transcripts larger than the normal mRNA for two of the ribosomal proteins. These transcripts appear to be precursors which, at the restrictive temperature, the cell is unable to splice.

We now report that a temperature shift does, in fact, cause a severe but temporary inhibition of the transcription of five ribosomal protein genes, but not of two control genes. The mutation in strain *ts*368 had little effect on either the inhibition or the resumption of the transcription of the ribosomal protein genes.

(Portions of this work have been submitted by C.H.K. to the Sue Golding Division of the Albert Einstein College of Medicine in partial fulfillment of the requirements for the Ph.D. degree.)

## MATERIALS AND METHODS

**Strains, plasmids, media, and isotopic labeling of molecules.** *S. cerevisiae* strain A364A (*MATa gal1 ade1 ade2 ura1 his7 lys2 tyr1*) (ATCC 22244) and its derivative *ts*368 [*rna2* (Ts)] (13) were grown to $10^7$ cells per ml at 23°C in synthetic medium (33). For pulse-labeling experiments, spontaneous *ura*$^+$ revertants of the two strains were grown in synthetic medium lacking uracil. The culture was shifted to 36°C, and at various times samples were labeled with [5-$^3$H]uracil (Schwarz-/Mann, Orangeburg, N.Y.; 19 Ci/mmol) at a final concentration of 200 μCi/ml in the same medium for 7 min. The culture was poured onto crushed ice, and the cells were harvested and washed with 10 ml of ice-cold water. From these cells RNA was isolated as described previously (34). Approximately 25 μg of total cellular RNA was obtained per ml of culture.

The plasmids used and their origins are listed in Table 1. We are grateful to J. Woolford and M. Rosbash for supplying plasmid pY11-40 and to D. Botstein for supplying plasmid YIp5. Plasmid DNA from *E. coli* cultures was prepared according to the method of Clewell (6).

**Northern analysis.** Each sample of RNA was titrated with [$^3$H]polyuridylate to determine the amount of polyadenylated [poly(A)$^+$] RNA (mRNA) (28; C. H. Kim, Ph.D. thesis, Albert Einstein College of Medicine, Bronx, N.Y., 1982). Samples of total cellular RNA containing equal amounts of poly(A)$^+$ RNA (approximately 5 μg of total RNA) were mixed with 20 μl of sample buffer (50% formamide, 6% formaldehyde, 0.01 M $NaPO_4$ [pH 7]), heated at 50°C for 15 min, and cooled to room temperature, and then 3 μl of 50% glycerol–0.01 M $NaPO_4$–0.1% bromophenol blue was added. The samples were electrophoresed on horizontal 1.5% agarose gels in 0.01 M $NaPO_4$ (pH 7)–6% formaldehyde for 800 V-h with a constant circulation of the buffer (0.01 M $NaPO_4$ [pH 7], 6% formaldehyde). The RNA was then transferred to nitrocellulose by the technique of Thomas (30). The blots were probed with DNA restriction fragments which had been excised from agarose gels (32) and labeled by nick translation (7). In each case only a single RNA species was observed with the RNA grown at 23°C, indicating that each clone carries only the gene indicated in Table 1. Densitometry of the bands was carried out after several exposures to ensure linearity of the film response (Kim, Ph.D. thesis).

**Specific activity of the UTP pool.** The specific activity of UTP after the 7-min pulse was determined as described previously (Kim, Ph.D. thesis; Kim and Warner, in press). Briefly, an $HClO_4$ extract was subjected to thin-layer chromatography. The spot containing UTP was eluted, the content of UTP was determined by a luciferase assay, and its radioactivity was determined by scintillation counting.

**Measurement of poly(A)-containing RNA.** Poly(A)-containing RNA from cells labeled with [$^3$H]uracil was determined by chromatography on polydeoxythymidylate-cellulose (29). Approximately 3.0 μg of $^3$H-labeled total RNA and 44 μg of *S. cerevisiae* rRNA were dissolved in 500 μl of binding buffer (10 mM Tris-hydrochloride [pH 7.4], 10 mM EDTA, 0.5 M LiCl, 0.2% [wt/vol] sodium dodecyl sulfate), heated to 60°C for 5 min rapidly cooled on ice, and chromatographed in a Pasteur pipette column containing 1 g of oligodeoxythymidylate-cellulose (T-2; Collaborative Research, Inc., Waltham, Mass.) previously equilibrated in binding buffer, and the void volume was collected and reapplied. The column was washed four times with 1 ml of binding buffer and three times with 1 ml of buffer containing 0.2 M LiCl instead of 0.5 M LiCl. Poly(A)$^+$ RNA was eluted by washing with five 1-ml

TABLE 1. Plasmids used

| Plasmid | Origin | Vector | Size of insert (kilobases) | *S. cerevisiae* gene[a] |
|---|---|---|---|---|
| pRp1 | pTCM (3.2)[b] | pBR325 | 3.2 | Rp1 |
| pRp9 | λC6[a] | pBR322 | 0.9 | Rp9 |
| pRp29 | λA13[a] | pBR322 | 1.5 | Rp29 |
| pRp39 | pY11-40[c] | pMB9 | 1.8 | Rp39 |
| pRp73 | λA14[a] | pBR325 | 2.7 | Rp73 |
| pA83 | λA83[a] | pBR325 | 1.6 | Unidentified protein of $M_r$ ≈42,000 |
| pURA3 | YIp5[d] | pBR322 | 1.1 | Orotidine monophosphate decarboxylase |

[a] Fried et al. (7).
[b] Fried and Warner (8). See also reference 7 for identity of ribosomal proteins.
[c] Woolford et al. (36).
[d] Bach et al. (2).

aliquots of 10 mM Tris-hydrochloride (pH 7.4)–10 mM EDTA–0.2% (wt/vol) sodium dodecyl sulfate.

**Preparation of DNA filters and hybridization.** To prepare filters, 82 μg of plasmid DNA in water was diluted to 205 μg/ml in 0.1 N NaOH. The solution was heated at 100°C for 5 min to nick and denature the DNA and cooled quickly on ice. Twenty volumes of 1 M ammonium acetate (room temperature) was added, and the pH was monitored with pH paper to ensure neutrality. The solution was loaded, without vacuum, onto a 25-mm Millipore nitrocellulose filter which had been soaked in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and washed twice with 10 ml each of 6× SSC under full vacuum. The filter was baked in a vacuum oven for at least 2 h at 80°C and cut into three pieces with a no. 6 cork bore (10-mm diameter).

Hybridization of $^3$H-labeled *S. cerevisiae* RNA to plasmid DNA was carried out in a manner similar to that described by Zitomer et al. (40). A 10-mm-diameter filter containing pBR325 DNA and a 10-mm-diameter filter containing plasmid DNA were added to a 0.5-ml solution of 50% deionized formaide, 2× SSC, 0.2% sodium dodecyl sulfate, 0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.02% bovine serum albumin, and 35 μg of [$^3$H]RNA. This mixture was incubated at 40°C with gentle shaking for 69 h. The filters were then washed at 40°C twice by shaking with 50 ml of 2× SSC–40% formamide for 1 h, and at 40°C 10 times by shaking with 200 ml of 2× SSC–0.2% sodium dodecyl sulfate for 5 min each. The filters were then rinsed with 100 ml of 2× SSC and with 50 ml of 95% ethanol at room temperature. The filters were dried, and the radioactivity bound to each filter was determined in a toluene scintillation cocktail.

To establish that the hybridization was carried out in DNA excess, we hybridized increasing amounts of labeled RNA to a set of identical filters. The hybridization was linear to an input of 80 μg of RNA. Therefore, we chose to do the hybridizations with 35 μg of RNA, with which we estimate there is a 500- to 1,000-fold excess of DNA (Kim, Ph.D. thesis; Kim and Warner, in press).

## RESULTS

**Concentration of ribosomal protein mRNAs.** Rosbash et al. (25) have shown for ribosomal proteins Rp39, Rp51, and Rp52 that the temporary decline in their synthesis, which follows a temperature shift (10), is due to a decline in the concentration of their mRNA sequences, as determined by a Northern blot. We confirmed that result for four additional ribosomal proteins, Rp1, Rp9, Rp29, and Rp73 (Fig. 1 and Table 2). The concentration of mRNA for one nonribosomal protein remained relatively unchanged. In wild-type cells, the concentration of mRNA reached a minimum at approximately 20 min after the temperature shift and then increased to a normal value by about 60 min. These data are consistent with the kinetics of synthesis of ribosomal proteins after a temperature shift (10).

When cells of strain *ts*368, which contain a *ts* mutation in the gene *rna2*, were shifted from 23°C to the nonpermissive temperature of 36°C, the concentration of mRNA for most ribosomal proteins declined continuously to almost undetectable levels (Fig. 1, Table 2). Approximately 20 min after the temperature shift, a larger species of RNA appears, which has sequences in common with the mature RNA. These molecules are presumed, and in one case have been shown (25), to be unspliced precursors. They accumulate substantially less in the mutant than does mature mRNA in wild-type cells.

Such presumptive precursors are characteristic of most yeast ribosomal protein genes (4, 7, 25). The genes for two proteins, Rp1 and Rp39, do not, however, have introns (7, 25; J. Woolford, personal communication; L. Schultze, personal communication). In *ts*368 maintained at 36°C, the concentration of the mRNA for Rp39 returns almost to normal levels after 60 min, as expected if the mutation involves only splicing. However, the levels of mRNA for Rp1, while higher than those of spliced mRNAs, do not return to normal. The reason for this result is not clear. However, the mRNA for Rp1 turns over more rapidly when it is translated inefficiently,

TABLE 2. Change in mRNA concentration after shift from 23 to 36°C

| Strain | Time after temp shift (min) | Concn of individual mRNA molecules (% of concn at 23°C)[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ribosomal protein gene products | | | | | Nonribosomal protein gene product A83 |
| | | Rp1 | Rp9[b] | Rp29[b] | Rp39 | Rp73[b] | |
| A364A | 10 | 46 | 39 | 36 | 34 | 31 | 115 |
| | 20 | 44 | 34 | 27 | 20 | 12 | 120 |
| | 60 | 95 | 105 | 102 | 77 | 70 | 93 |
| *ts*368 | 10 | 51 | 35 | 37 | 31 | 43 | 102 |
| | 20 | 40 | 12 (12) | 9 (16) | 32 | 12 (17) | 118 |
| | 60 | 31 | 8 (11) | 3 (17) | 60 | 4 (21) | 103 |

[a] Autoradiograms such as those shown in Fig. 2 were scanned and quantitated by densitometry with a Joyce-Loebl recording densitometer. Values for each band were compared with values for mRNA from cells growing at 23°C.

[b] Numbers within parentheses are values for precursor mRNA.

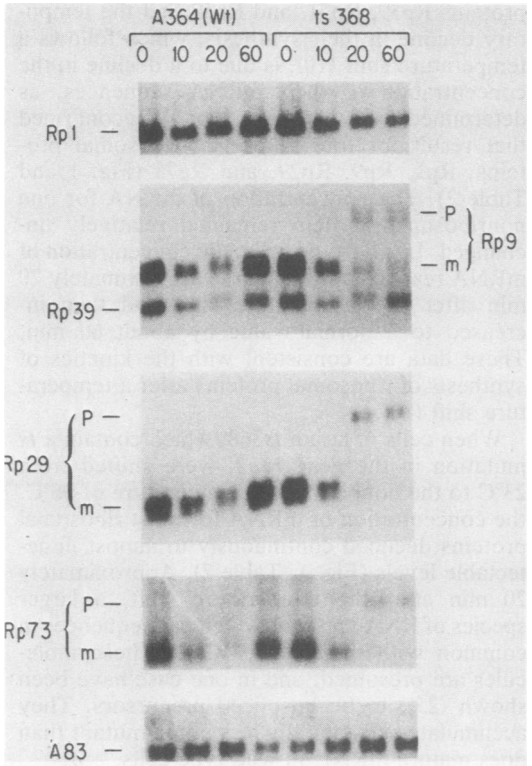


FIG. 1. Concentration of mRNAs for individual proteins as a function of time after temperature upshift in A364A wild-type cells and *ts*368 mutant cells. Cultures of strains A364A and *ts*368 were grown to $10^7$ cells per ml at 23°C in synthetic medium and shifted to 36°C, and at various times (indicated at top, in minutes) RNA was prepared by phenol-sodium dodecyl sulfate extraction. The concentration of mRNA was determined by hybridization to polyuridylate. Samples (~5 μg) of total RNA, containing equal amounts of mRNA, were treated with formaldehyde, fractionated on a 1.5% agarose gel, and transferred to nitrocellulose paper. The *S. cerevisiae* DNA inserts in the plasmids (Table 1) were labeled with $^{32}P$ by nick translation and hybridized to a filter containing RNA, which was then autoradiographed at −70°C with intensifying screens. Only that section of the filter with a signal is shown. The sample at time zero was isolated from cells growing at 23°C, m, mRNA; p, putative precursor.

due perhaps to excess Rp1 (22). The disruption in ribosome synthesis in *ts*368 may lead to some accumulation of Rp1 with a secondary effect on the level of its mRNA.

**Transcription of ribosomal protein genes.** The alteration in the concentration of mRNAs which follows a temperature upshift could arise from either a decrease in the rate of transcription or a decrease in the lifetime of the mRNAs. To distinguish between these alternatives, we measured the rate of transcription of a number of genes after cells were shifted from 23 to 36°C. As a compromise between incorporating sufficient radioactivity and avoiding complications due to turnover of the transcripts, a labeling period of 7 min was chosen. Cultures were labeled either before or at intervals after shifting from 23 to 36°C. Samples were taken to measure the specific activity of the UTP pool, and the remainder was used to prepare RNA. One portion of the RNA was used to determine the proportion of poly(A)$^+$ RNA in the labeled transcripts. A temperature shift, even to another temperature at which the doubling time is about the same, had broad effects on RNA metabolism (Table 3). There was a substantial but temporary inhibition of the labeling of the UTP pool which was consistently, but inexplicably, more apparent in strain A364A than in *ts*368. There was a temporary inhibition of synthesis of stable [non-poly(A)$^+$] RNA (Table 3 and Fig. 2A) (31). The total synthesis of poly(A)$^+$ RNA, however, was relatively constant (Table 3 and Fig. 2A).

Each of these RNA samples was hybridized to the DNA from seven plasmids carrying an *S. cerevisiae* gene and one with no *S. cerevisiae* gene (see above). The raw data are presented in Table 4, along with corrections for hybridization to the DNA with no *S. cerevisiae* genes. Table 5 presents a summary of the data for two experiments, corrected for the specific activity of the UTP pools and arranged to show the change in transcription of individual genes as a function of time after a temperature shift. Some of the data are also plotted in Fig. 3A.

Several features are clear. After a temperature shift, the transcription of different genes gyrates rather wildly, e.g., the transcription of the unidentified gene on clone A83 increased 2.5-fold or more in the first few minutes and then declined to less than the original value. On the other hand, the rates of transcription of the genes for ribosomal proteins proceed in lockstep. Within the first 10 min they fell by 70 to 90%, by 30 min they had recovered to nearly normal levels, and by 55 min they were nearly twofold greater than the rates at 23°C. We draw two conclusions: (i) a temperature shift temporarily interrupts the transcription of ribosomal protein genes, and (ii) the response of ribosomal protein genes to a temperature shift is highly coordinated.

**Transcription in *ts*368.** Similar measurements of the transcription of ribosomal and nonribosomal protein genes were carried out with strain *ts*368 to determine whether the product of gene *rna2* is involved in transcription as well as in the processing of mRNA for ribosomal proteins. The overall synthesis of mRNA after a shift to 36°C was relatively unaffected by the temperature-sensitive allele of *rna2*, although the syn-

TABLE 3. Summary of the effect of temperature upshift on RNA synthesis[a]

| Strain | Time after temp shift (min) | Sp act of total RNA cpm/μg ($\times 10^{-4}$) | % of value at 23°C | Sp act of UTP pool (% of value at 23°C) | Poly(A)-containing RNA (% of total [$^3$H]RNA) | Total RNA synthesis (U) | Total mRNA synthesis (U) | Total stable RNA synthesis (U) |
|---|---|---|---|---|---|---|---|---|
| A364A | 0 | 8.4 | 100 | 100 | 21 | 100 | 21 | 79 |
| | 10 | 4.07 | 49 | 72 | 38 | 68 | 26 | 42 |
| | 30 | 1.97 | 23 | 39 | 39 | 60 | 23 | 37 |
| | 55 | 6.94 | 83 | 67 | 26 | 124 | 32 | 92 |
| *ts*368 | 0 | 8.09 | 100 | 100 | 25 | 100 | 25 | 75 |
| | 10 | 5.41 | 67 | 80 | 35 | 83 | 29 | 54 |
| | 30 | 3.73 | 46 | 80 | 47 | 57 | 27 | 30 |
| | 55 | 3.43 | 42 | 90 | 45 | 47 | 21 | 26 |

[a] Cultures were labeled with [$^3$H]uracil either before or after a shift from 23 to 36°C. The sample at time zero was labeled at 23°C. The other times indicate the midpoint of a 7-min pulse. From one portion, the specific activity of the UTP was determined as described in the text. From the rest of the sample, RNA was prepared, and its specific activity was determined and compared with the value at 23°C. A portion of the RNA was subjected to oligodeoxythymidylate-cellulose chromatography (see text) to determine the proportion of poly(A)-containing RNA. The total RNA and mRNA syntheses care expressed in arbitrary units and were determined by the following equations: $D = (A/B) \times 100$, and $E = (C \times D) - 100$, where $A$ is the percent specific activity of total RNA, $B$ is the specific activity of the UTP pool, $C$ is the proportion of poly(A)-containing RNA, and $D$ and $E$ are the total RNA and total mRNA syntheses, respectively. The total stable RNA synthesis is the difference between the total RNA and total mRNA syntheses.

thesis of stable RNA declined (Fig. 2B). Some of that apparent decline may represent turnover of unprocessed rRNA transcripts (26, 31). Table 5 and Fig. 3B show the hybridization of these RNA samples to the various individual genes. Just as in wild-type cells, the temperature shift caused a brief interruption of the transcription of ribosomal protein genes. Within 30 min, transcription returned almost to normal. Only on prolonged exposure to the restrictive temperature did *ts*368 differ appreciably from the wild type.

Thus, the gene *rna2* seems to have little immediate effect on the transcription of ribosomal protein genes. By 20 min after a temperature shift, there is a substantial accumulation of precursor molecules derived from the genes for Rp9, Rp29, and Rp73 (Fig. 1 and Table 2). In fact, at 20 min the total concentration of molecules, taking mature and precursor together, was roughly the same in wild-type and mutant cells. However, by 55 to 60 min the amount of mRNA in the mutant was less than 25% of that of the wild type. Since the rate of transcription in the mutant is at least one-half of that in the wild type, the lifetime of the product of transcription, i.e., the precursor, must be substantially less than that of the mature mRNA.

Summarizing the data of Fig. 1 and 3, it seems not unlikely that the low concentration of transcripts in *ts*368 at the restrictive temperature is due solely to the rapid turnover of unprocessed molecules and that the rate of transcription of ribosomal protein genes is totally unaffected by the mutation in *rna2*.

## DISCUSSION

The results presented above demonstrate that a mild temperature shock causes an immediate inhibition of the transcription of five ribosomal protein genes. The concentration of mRNA for ribosomal proteins falls as preexisting molecules decay (Table 3). These kinetics parallel rather precisely the rate of synthesis of ribosomal proteins after a temperature shift (10), suggesting that the cells do not alter the efficiency of translation of the mRNA in response to a declining supply.

An extrapolation from previous studies show-

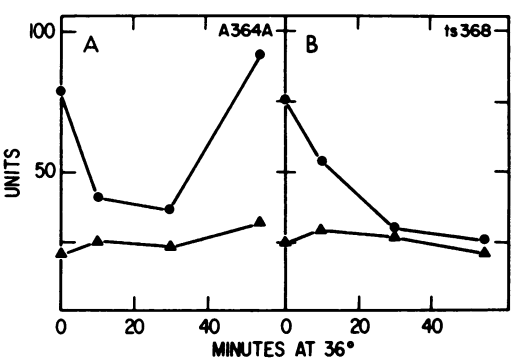


FIG. 2. Kinetics of total stable RNA synthesis and total mRNA synthesis after temperature upshift in A364A wild-type cells and *ts*368 mutant cells. The rate of synthesis of total stable RNA (●) and total mRNA (▲) in A364A cells (A) and *ts*368 cells (B) is plotted as a function of time after temperature upshift (data are from Table 3).

TABLE 4. Hybridization of individual mRNAs pulse-labeled after temperature upshift in A364A wild-type cells and *ts*368 mutant cells[a]

| Strain | Time after temp shift (min) | Input cpm ($\times 10^{-6}$) | Filter | cpm hybridized on filter | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Ribosomal protein gene products | | | | | Nonribosomal protein gene products | | |
| | | | | Rp1 | Rp9 | Rp29 | Rp39 | Rp73 | A83 | URA3 | pBR325 |
| A364A | 1 | 2.94 | 1 | 1,619 | 687 | 610 | 765 | 509 | 301 | 199 | 76 |
| | | | 2 | 1,700 | 620 | 677 | 757 | 506 | 242 | 210 | 62 |
| | | | Net avg[b] | 1,591 | 585 | 575 | 692 | 439 | 203 | 136 | 0 |
| | 10 | 1.43 | 1 | 320 | 121 | 131 | 154 | 114 | 454 | 193 | 16 |
| | | | 2 | 310 | 133 | 136 | 174 | | 441 | 238 | 25 |
| | | | Net avg | 294 | 111 | 113 | 143 | 93 | 427 | 195 | 0 |
| | 30 | 0.69 | 1 | 576 | 266 | 245 | 307 | 194 | 179 | 140 | 37 |
| | | | 2 | 555 | 239 | 262 | 305 | 185 | 163 | 146 | 20 |
| | | | Net avg | 537 | 224 | 224 | 277 | 161 | 142 | 114 | 0 |
| | 55 | 2.43 | 1 | 2,078 | 883 | 792 | 923 | 651 | 154 | 153 | 74 |
| | | | 2 | 2,285 | | | 939 | 612 | 145 | 208 | 47 |
| | | | Net avg | 2,121 | 822 | 731 | 870 | 570 | 89 | 120 | 0 |
| *ts*368 | 0 | 2.83 | 1 | 2,009 | 894 | 875 | 926 | 647 | 369 | 244 | 98 |
| | | | 2 | 1,700 | 921 | 835 | 933 | 565 | 353 | 219 | 136 |
| | | | Net avg | 1,738 | 791 | 738 | 813 | 489 | 244 | 115 | 0 |
| | 10 | 1.89 | 1 | 403 | 235 | 241 | 312 | 175 | 587 | 174 | 70 |
| | | | 2 | 431 | 263 | 213 | 272 | 148 | 563 | 170 | 35 |
| | | | Net avg | 364 | 196 | 174 | 239 | 109 | 522 | 119 | 0 |
| | 30 | 1.3 | 1 | 1,216 | 571 | 528 | 813 | 371 | 446 | 201 | 62 |
| | | | 2 | 1,170 | 597 | 493 | 810 | 357 | 456 | 217 | 56 |
| | | | Net avg | 1,134 | 525 | 452 | 753 | 305 | 392 | 150 | 0 |
| | 52 | 1.2 | 1 | 1,394 | 653 | 608 | 866 | 412 | 310 | 224 | 95 |
| | | | 2 | 1,491 | 623 | 631 | 903 | 434 | 302 | 234 | 40 |
| | | | Net avg | 1,375 | 570 | 551 | 817 | 355 | 238 | 161 | 0 |

[a] $^3$H-labeled total RNA (35 μg) from the samples described in Table 3 were hybridized to excess filter-bound DNA of each of the subcloned sequences (see text). Two filters were hybridized to separate portions of RNA.
[b] The net average is the average of values from filters 1 and 2, less that from the blank.

ing that the temperature shift inhibits the synthesis of at least 40 ribosomal proteins (10) suggests that the transcription of all ribosomal protein genes is affected by a temperature shift. However, the overall synthesis of mRNA continues normally after the temperature shift (Fig. 2). It will be interesting to determine whether other genes related to protein synthesis, such as those for tRNA synthetases, are also responsive to a temperature shift, as appears to be the case in *E. coli* (14).

One could argue that the measurements shown in Table 5 do not distinguish between an inhibition of transcription and a destabilization of the transcripts. The latter seems unlikely for several reasons. The stability of unspliced transcripts, although less than that of mature mRNA, is great enough to permit substantial accumulation of RNA (25) (Fig. 1). Furthermore, the inhibition is observed for both intron-free and intron-containing genes. Finally, if transcription were continuing at a normal rate, yet accumulating in 7 min only the radioactivity shown in Table 5, one can calculate that the lifetime of the transcript would be comparable to the transcription time, thus reducing the argument to one of semantics. At this stage it is not, of course, possible to exclude the possibility that the regulation of transcription occurs at the level of premature termination (21), rather than initiation.

The data shown in Table 5 are not sufficiently sensitive to determine whether the shutoff of transcription occurs immediately after the temperature shift and whether transcription falls briefly to zero. On the other hand, we consistently observed an overshoot; i.e., the rate of transcription of ribosomal protein genes after 1 h

TABLE 5. Kinetics of transcription of the ribosomal protein genes after temperature upshift in A364A wild-type cells and *ts*368 mutant cells

| Strain | Time after temp shift (min) | Expt | % of original rate of transcription[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Ribosomal protein gene products | | | | | Nonribosomal protein gene products | |
| | | | Rp1 | Rp9 | Rp29 | Rp39 | Rp73 | A83 | URA3 |
| A364A | 10 | 1 | 26 | 27 | 28 | 39 | 30 | 293 | 200 |
| | | 2 | 14 | | 14 | 19 | 9 | 218 | 89 |
| | 30 | 1 | 87 | 98 | 100 | 103 | 94 | 179 | 214 |
| | | 2 | 83 | | 85 | 76 | 82 | 188 | 94 |
| | 55 | 1 | 199 | 210 | 205 | 188 | 194 | 65 | 131 |
| | | 2 | 155 | | 171 | 180 | 207 | 77 | 128 |
| *ts*368 | 10 | 1 | 26 | 31 | 30 | 37 | 30 | 267 | 130 |
| | 30 | 1 | 82 | 83 | 77 | 116 | 78 | 201 | 105 |
| | 55 | 1 | 88 | 80 | 83 | 112 | 81 | 108 | 156 |

[a] The net average values from Table 4 were corrected for UTP pool specific activity (SA; Table 3) and compared with the transcription at 23°C. For example; for gene Rp1 in A364A cells labeled after 10 min at 36°C, experiment 1: $\{(\text{net average})_t \div [(SA)_t/(SA)_0] \div (\text{value at } 23°C)\} \times 100 = (294 \div [72/100] \div 1{,}591) \times 100 = 26\%$.

at 36°C was nearly twice the rate at 23°C. Such an overshoot would clearly hasten the return of the concentration of mRNA to normal levels.

Whereas most of the experiments have been carried out after a shift from 23 to 36°C, one can observe an inhibition of synthesis of ribosomal proteins even after a shift from 23 to 30°C, which is the optimal temperature for growth. The magnitude of the inhibition is roughly proportional to the magnitude of the shift (J. R. Warner, *in* J. Strathern et al., ed., *The Molecular Biology of the Yeast Saccharomyces*, in press). Since ribosomes are used largely for growth, the phenomenon may represent the unwillingness of the cell to invest in new production facilities in the face of a changing, uncertain environment. It remains to be seen whether the effect of a mild temperature shock on ribosome synthesis is a sensitive manifestation of the more general effect of a severe temperature shock, in which synthesis of most of the cell proteins ceases, to be replaced by the vigorous synthesis of a new class, the heat shock proteins, e.g., in *Drosophila* (1) or in yeasts (15). However, after even a mild temperature shock, there is a substantial alteration in the spectrum of proteins synthesized by yeast cells (16–18, 24). Many are elevated, many are depressed. It is not surprising, therefore, that we have found substantial fluctuation in the transcription of our control genes. It is noteworthy that the synthesis of actin, the one other yeast protein whose gene is known to contain an intron, is relatively unaffected by a mild heat shock (24).

The genes for ribosomal proteins are scattered over the genome, yet under normal conditions their transcription is coordinated to yield equimolar amounts of mRNA (Kim and Warner, in press). After a mild temperature shock, their transcription falls and recovers in a precisely coordinate fashion (Fig. 3). During the early stages of sporulation, the mRNA for ribosomal proteins also declines (23). How does such coordination of dozens of unlinked genes come about? One is first attracted by the possible role of intervening sequences, since most ribosomal

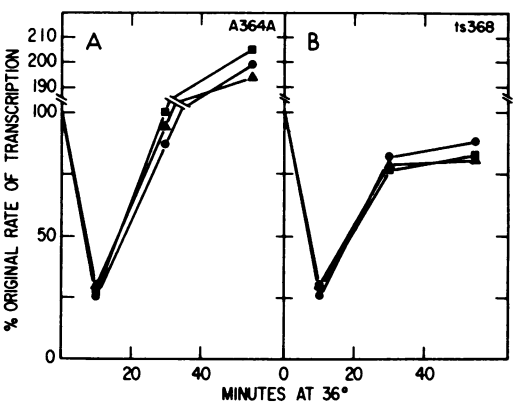


FIG. 3. Transcription of ribosomal protein genes after temperature upshift in A364A wild-type cells and *ts*368 mutant cells. The relative transcription of ribosomal protein genes in A364A cells (A) and *ts*368 cells (B) at various times after a shift from 23 to 36°C is plotted from the data in Table 5. Symbols: ●, Rp1; ■, Rp29; ▲, Rp73.

protein genes have them, and they are rare elsewhere in the genome. However, two genes without intervening sequences, those for Rp1 and Rp39, seem to coordinate their transcription with that of the rest of the genes. Alternatively, each gene may have a promoter-like element which is individually responsive to the signal generated by the temperature shift. Sequence analyses in this laboratory and others should soon reveal common sequence elements if they exist. Nevertheless, the problem of mechanism would remain. What are the signals of coordination and what are the receptors? Perhaps one of the many subunits of RNA polymerase II is a sigma-type molecule specific for ribosomal protein genes. Since transcription of these genes makes up 15% or more of polymerase II transcription, the suggestion is not totally unreasonable. The alteration of such a subunit, e.g., by phosphorylation, could lead to a common effect on the transcription of all ribosomal protein genes. Alternatively, some small effector molecule might be a necessary cofactor for transcription of these genes, a molecule whose concentration would be subject to numerous controls (Warner, in press). In any case, the ribosomal protein genes should prove to be valuable in efforts to understand the basis of the transcription of eucaryotic genes and of its regulation.

An original impetus for carrying out this investigation was to determine whether the inhibition of synthesis of ribosomal proteins in *ts*368 at the restrictive temperature is related to the temporary decline in synthesis of ribosomal proteins after a mild temperature shock. It is clear now that the two phenomena are independent. Rosbash et al. (25) have shown that the *ts* mutation leads to the accumulation of an unspliced pre-mRNA for ribosomal protein genes (Fig. 1) (7). Indeed, Rp1 and Rp39, whose genes do not have intervening sequences (25; J. Woolford, personal communication; L. Schultze, personal communication), are synthesized at substantial rates in *ts*368 at 36°C (11). On the other hand, for at least 30 min after a temperature shift, the transcription of ribosomal protein genes in the mutant is indistinguishable from that in wild-type cells (Fig. 3). Nevertheless, it remains to be seen whether the gene *RNA2* and the related genes *RNA3* through *RNA11*, whose mutants show a similar phenotype (12), are simply structural components of a complex splicing apparatus or are themselves part of a regulatory process.

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LITERATURE CITED

1. **Ashburner, M., and J. J. Bonner.** 1979. The induction of gene activity in *Drosophila* by heat shock. Cell **17**:241–254.
2. **Bach, M. L., F. Lacroute, and D. Botstein.** 1979. Evidence for transcriptional regulation of orotidine-5′-phosphate decarboxylase in yeast by hybridization of mRNA to the yeast structural gene cloned in *E. coli*. Proc. Natl. Acad. Sci. U.S.A. **76**:386–390.
3. **Bollen, G. H. P. M., L. H. Cohen, W. H. Mager, A. W. Klaassen, and R. J. Planta.** 1981. Isolation of cloned ribosomal protein genes from the yeast *Saccharomyces carlsbergensis*. Gene **14**:279–287.
4. **Bollen, G. H. P. M., C. M. T. Molenaar, L. H. Cohen, M. M. C. van Raansdonk-Duin, W. H. Mager, and R. J. Planta.** 1982. Ribosomal protein genes of yeast contain intevening sequences. Gene **18**:29–38.
5. **Brot, N., P. Caldwell, and H. Weissbach.** 1980. Autogenous control of *Escherichia coli* ribosomal protein L10 synthesis *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **77**:2592–2595.
6. **Clewell, D. B.** 1972. Nature of Col $E_1$ plasmid replication in *Escherichia coli* in the presence of chloramphenicol. J. Bacteriol. **110**:667–676.
7. **Fried, H. M., N. J. Pearson, C. H. Kim, and J. R. Warner.** 1981. The genes for fifteen ribosomal proteins of *Saccharomyces cerevisiae*. J. Biol. Chem. **256**:10176–10183.
8. **Fried, H. M., and J. R. Warner.** 1981. Cloning of yeast gene for trichodermin resistance and ribosomal protein L3. Proc. Natl. Acad. Sci. U.S.A. **78**:238–242.
9. **Fried, H. M., and J. R. Warner.** 1982. Molecular cloning and analysis of yeast gene for cycloheximide resistance and ribosomal protein L29. Nucleic Acids Res. **10**:3133–3148.
10. **Gorenstein, C., and J. R. Warner.** 1976. Coordinate regulation of the synthesis of eukaryotic ribosomal proteins. Proc. Natl. Acad. Sci. U.S.A. **73**:1547–1551.
11. **Grant, P. G., D. Schinder, and J. E. Davies.** 1976. Mapping of trichodermin resistance in *Saccharomyces cerevisiae*: a genetic locus for a component of the 60S ribosomal subunit. Genetics **83**:667–673.
12. **Hartwell, L. H., C. S. McLaughlin, and J. R. Warner.** 1970. Identification of ten genes that control ribosome formation in yeast. Mol. Gen. Genet. **109**:42–56.
13. **Kief, D. R., and J. R. Warner.** 1981. Coordinate control of syntheses of ribosomal ribonucleic acid and ribosomal proteins during nutritional shift-up in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **1**:1007–1015.
14. **Lemaux, P. G., S. L. Herendeen, P. L. Block, and F. C. Neidhardt.** 1978. Transient rates of synthesis of individual polypeptides in *E. coli* following temperature shifts. Cell **13**:427–434.
15. **Lindquist, L.** 1981. Regulation of protein synthesis during heat shock. Nature (London) **293**:311–314.
16. **McAlister, L. and D. B. Finkelstein.** 1980. Alterations in translatable ribonucleic acid after heat shock of *Saccharomyces cerevisiae*. J. Bacteriol. **143**:603–612.
17. **McAlister, L., S. Strausberg, A. Kulaga, and D. B. Finkelstein.** 1979. Altered patterns of protein synthesis induced by heat shock of yeast. Curr. Genet. **1**:63–74.
18. **Miller, M. J., N.-H. Xuong, and E. P. Geiduschek.** 1979. A response of protein synthesis to temperature shift in the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **76**:5222–5225.
19. **Mortimer, R., and D. Hawthorne.** 1966. Genetic mapping in *Saccharomyces*. Genetics **53**:165–173.
20. **Nomura, M., E. A. Morgan, and S. R. Jaskunas.** 1977. Genetics of bacterial ribosomes. Annu. Rev. Genet. **11**:297–347.
21. **Oxender, D. L., G. Zurawski, and C. Yanofsky.** 1979.

Attenuation in the *Escherichia coli* tryptophan operon: role of RNA secondary structure involving the tryptophan codon region. Proc. Natl. Acad. Sci. U.S.A. **76**:5524–5528.

22. **Pearson, N. J., H. M. Fried, and J. R. Warner.** 1982. Yeast use translational control to compensate for extra copies of a ribosomal protein gene. Cell **29**:347–355.
23. **Pearson, N. J., and J. E. Haber.** 1980. Changes in regulation of ribosomal protein synthesis during vegetative growth and sporulation of *Saccharomyces cerevisiae*. J. Bacteriol. **143**:1411–1419.
24. **Plesset, J., J. J. Foy, L. L. Chia, and C. S. McLaughlin.** 1982. Heat shock in *Saccharomyces cerevisiae*: quantitation of transcriptional and translational effect, p. 495–514. *In* B. Safer and M. Grunberg-Manago (ed.), Interaction of translational and transcriptional control in the regulation of gene expression. Elsevier Science Publishing Co., New York.
25. **Rosbash, M., P. K. W. Harris, J. Woolford, and J. L. Teem.** 1981. The effect of temperature sensitive RNA mutants on the transcription products from cloned ribosomal protein genes of yeast. Cell **24**:679–686.
26. **Shulman, R. W., C. E. Sripati, and J. R. Warner.** 1977. Non-coordinated transcription in the absence of protein synthesis in yeast. J. Biol. Chem. **252**:1344–1349.
27. **Skogerson, L., C. McLaughlin, and E. Wakatama.** 1973. Modification of ribosomes in cryptopleurine-resistant mutants of yeast. J. Bacteriol. **116**:818–822.
28. **Sogin, S. J., and C. A. Saunders.** 1980. Fluctuation in polyadenylate size and content in exponential- and stationary-phase cells of *Saccharomyces cerevisiae*. J. Bacteriol. **144**:74–81.
29. **Sripati, C., and J. R. Warner.** 1978. Isolation, characterization and translation of mRNA from yeast. Methods Cell Biol. **20**:61–81.
30. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. U.S.A. **77**:5201–5205.
31. **Udem, S. A., and J. R. Warner.** 1972. Synthesis and processing of ribosomal RNA in *Saccharomyces cerevisiae*. J. Mol. Biol. **65**:227–242.
32. **Vogelstein, B., and D. Gillespie.** 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. U.S.A. **76**:615–619.
33. **Warner, J. R.** 1971. The assembly of ribosomes in yeast. J. Biol. Chem. **246**:447–454.
34. **Warner, J. R., and C. Gorenstein.** 1977. The synthesis of eukaryotic ribosomal proteins *in vitro*. Cell **11**:201–212.
35. **Warner, J. R., and C. Gorenstein.** 1978. Yeast has a true stringent response. Nature (London) **275**:338–339.
36. **Woolford, J. L., Jr., L. M. Hereford, and M. Rosbash.** 1979. Isolation of cloned DNA sequences containing ribosomal protein genes from *Saccharomyces cerevisiae*. Cell **18**:1247–1259.
37. **Woolford, J. L., and M. Rosbash.** 1981. Ribosomal protein genes rp39 (10-78), rp39 (11-40), rp51, and rp52 are not contiguous to other ribosomal protein genes in the *Saccharomyces cerevisiae* genome. Nucleic Acids Res. **9**:5021–5036.
38. **Yates, J. L.,and M. Nomura.** 1980. *E. coli* ribosomal protein L4 is a feedback regulatory protein. Cell **21**:517–522.
39. **Zengel, J. M., D. Mueckl, and L. Lindahl.** 1980. Protein L4 of the *E. coli* ribosome regulates an eleven gene rprotein operon. Cell **21**:523–535.
40. **Zitomer, R., D. Montgomery, D. Nichols, and B. Hall.** 1979. Transcriptional regulation of the yeast cytochrome c gene. Proc. Natl. Acad. Sci. U.S.A. **76**:3627–3631.