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Hierarchy of Elements Regulating Synthesis of Ribosomal Proteins in *Saccharomyces cerevisiae*

DONNA RUSSO KIEF[1] AND JONATHAN R. WARNER[1,2]*

Departments of Cell Biology[1] and Biochemistry,[2] Albert Einstein College of Medicine, Bronx, New York 10461

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Saccharomyces cerevisiae cells respond to a heat shock by temporarily slowing the synthesis of ribosomal proteins (C. Gorenstein and J. R. Warner, Proc. Natl. Acad. Sci. U.S.A. **73**:1547-1551, 1976). When cultures growing oxidatively on ethanol as the sole carbon source were shifted from 23 to 36°C, the synthesis of ribosomal proteins was coordinately inhibited twice as rapidly and 45% more severely than in comparable cultures growing fermentatively on glucose. Within 15 min, the relative rates of synthesis of at least 30 ribosomal proteins declined to less than one-sixth their initial values, whereas the overall rate of protein synthesis increased at least threefold. We suggest that this is due primarily to controls at the level of synthesis of messenger ribonucleic acid for ribosomal proteins but may also involve changes in messenger ribonucleic acid stability. In contrast, a nutritional shift-up causes a stimulation of the synthesis of ribosomal proteins. Experiments designed to determine the hierarchy of stimuli affecting the synthesis of these proteins demonstrated that temperature shock was dominant to glucose stimulation. When a culture growing on ethanol was shifted from 23 to 36°C and glucose was added shortly afterward, the decline in ribosomal protein synthesis continued unabated. However, in wild-type cells ribosomal protein synthesis began to recover within 15 min. In mutants temperature sensitive for ribosome synthesis, e.g., *rna2*, there was no recovery in the synthesis of most ribosomal proteins, suggesting that the product of *rna2* is essential for the production of these proteins under all vegetative conditions.

In the yeast *Saccharomyces cerevisiae* the synthesis of ribosomes is a sensitive indicator of the physiological state of the cell. Experiments described in the accompanying paper (8) demonstrate that cells growing on ethanol as a carbon source synthesize ribosomes at a substantially slower rate, and consequently have a lower ribosome concentration, than cells growing on glucose. On the addition of glucose to such a culture, the synthesis of ribosomes accelerates before that of other cell components. Conversely, when cells are deprived of an essential amino acid, there is preferential inhibition of the synthesis of ribosomal components (3, 20). A similar inhibition occurs when cells are deprived of a nitrogen source and begin the process of sporulation (14). In such experiments it has been possible to analyze both the transcription of ribosomal precursor ribonucleic acid (RNA) and the translation of 30 to 40 of the ribosomal proteins. In all cases the syntheses of ribosomal RNA and ribosomal proteins change in parallel, suggesting that the cell possesses mechanisms to coordinate the syntheses of the components of the ribosome.

However, other conditions (e.g., after a temperature upshift or in certain temperature-sensitive mutants [2, 4, 15, 19, 21]) lead to an inhibition of the synthesis of ribosomal proteins with relatively little effect on the transcription of ribosomal precursor RNA. In wild-type cells the inhibition is transient, but in cells containing recessive temperature-sensitive mutations in any of several genes (e.g., *rna2*, *rna4*, and *rna6*) the synthesis of ribosomal proteins is inhibited permanently (2, 19).

Our eventual aim is to ask (i) what are the molecular mechanisms responsible for the coordinate regulation of ribosomal RNA and ribosomal proteins, and (ii) is the same regulatory pathway involved in the response to different stimuli? As an approach to these questions we have attempted to determine the hierarchy of different stimuli. In particular, we have subjected a culture growing on ethanol to a temperature upshift either shortly before or shortly after the addition of glucose. An analogous experiment was performed using cells with a temperature-sensitive lesion in the gene *rna2*.

The results suggest that a temperature shift is

dominant to a nutritional shift, and that controls operating at the level of synthesis of functional messenger RNA (mRNA) are primarily responsible for the coordinate stimulation or repression of ribosomal protein synthesis in response to these stimuli.

MATERIALS AND METHODS

Strains and growth conditions. Experiments were performed on diploid *S. cerevisiae* strains obtained from N. Pearson. Two closely related diploids were used, one homozygous for *rna2* and temperature sensitive for growth and the other heterozygous for *rna2* and capable of growing at both 23 and 36°C. Their genotypes are as follows: NP1, *MATa/MATα rna2/rna2 tyr1/+ ura1 + +/ade1 +/ade2 +/lys2 +/his7 +/thr4*; NP2, *MATa/MATα rna2/+ ade1/+ ade2/+ ura1/+ his7/+ lys2/+ tyr1/+ +/thr4.*

By the criteria of growth rate at 36°C, of recovery of ribosomal protein synthesis after a temperature shift, and of ability to sporulate at 36°C, the *rna2* marker in strain NP2 is fully recessive (13; N. J. Pearson, Ph.D. thesis, Brandeis University, Waltham, Mass., 1980).

The cells were cultivated at 23°C with rotary shaking in minimal medium with either ethanol (EMM) or glucose (GMM) as a carbon source (8). Growth was monitored turbidimetrically in a Klett-Summerson colorimeter with a red filter.

Separation and quantitation of ribosomal proteins and ribosomal RNA. The methods used for separation and quantitation of ribosomal proteins and ribosomal RNA are described in the accompanying paper (8).

RESULTS

Transient inhibition of the synthesis of ribosomal proteins. Evidence has been presented in the accompanying paper showing that *S. cerevisiae* grown in EMM synthesize ribosomal proteins at a rate approximately one-seventh that of cells grown in GMM (8). To determine whether a further depression in ribosomal protein synthesis would occur when cells maintained in EMM were subjected to heat shock, we measured the relative rates of synthesis (A_i) of individual ribosomal proteins after a temperature shift from 23 to 36°C. The synthesis of total protein increased more than twofold immediately after the shift. Nevertheless, Fig. 1A clearly shows an immediate and dramatic decline in the relative rates of synthesis of four ribosomal proteins. Their synthesis remained repressed for at least 60 min, and even by 90 min only a small amount of derepression had occurred. Derepression was eventually complete since cells did recover and remain viable after heat shock. Table 1 shows that a similar situation existed for all of the ribosomal proteins analyzed. On the other hand, nonribosomal proteins on the average were unaffected by the temperature shift, although the synthesis of individual proteins may be inhibited, enhanced, or unchanged.

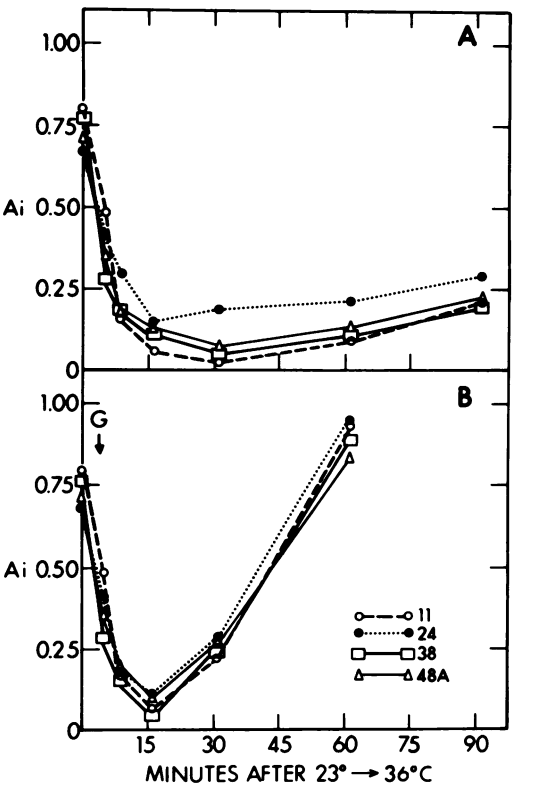

FIG. 1. *Strain NP2 grown in EMM: relative rates of synthesis of ribosomal proteins after a temperature upshift. (A) Effect of temperature upshift alone. (B) Effect of glucose addition 3 min after temperature upshift. An EMM culture uniformly labeled with [^{14}C]leucine was pulse-labeled for 5 min with [^{3}H]-leucine and chased for 1 min with cold leucine at 23°C or at various times after a shift to 36°C. Protein was extracted and analyzed as described in reference 8. A_i, a measure of the synthesis of an individual protein relative to total protein synthesis (8), was determined for a large number of ribosomal proteins, of which four are shown. The points are plotted at the midpoint of each pulse-chase.*

Comparison of the kinetics of the transient inhibition of ribosomal protein synthesis reveals several interesting differences between cultures grown in EMM and GMM (Fig. 2). First, the initial rate of decline appeared to be about twice as fast for cells grown in EMM. They exhibited a 50% inhibition in ribosomal protein synthesis within 5 to 6 min, whereas this level of inhibition was not reached until about 12 min when cells were grown in GMM. Second, within 15 min, a maximal inhibition of 85% was found for cells in EMM compared to a 60% inhibition for cells maintained in GMM. Finally, after 60 min at 36°C, cells grown in GMM had fully recovered

TABLE 1. *Strain NP2 (rna2/+) grown in EMM: relative rates of synthesis of ribosomal and nonribosomal proteins after shift in temperature from 23 to 36°C*[a]

Protein	Relative rate of synthesis at time (min) after shift to 36°C						
	0	3	7	15	30	60	90
Ribosomal protein							
2	0.91	0.45	0.38	0.24	0.15	0.24	ND[b]
6	0.85	0.45	0.31	0.22	0.08	0.15	ND
9	0.70	0.35	0.12	0.05	0.03	0.07	ND
11	0.80	0.49	0.16	0.06	0.03	0.09	0.22
12	0.73	0.48	0.18	0.11	0.07	0.15	ND
13[c]	0.66	0.38	0.18	0.18	0.08	0.14	0.17
14	0.89	0.56	0.34	0.24	0.24	0.48	0.48
16	0.49	0.45	0.14	0.04	0.03	0.07	0.14
18	0.52	0.36	0.17	0.11	0.07	0.08	ND
24	0.68	0.41	0.30	0.15	0.19	0.22	0.29
26	0.71	0.32	0.17	0.07	0.07	0.10	ND
27	0.60	0.31	0.13	ND	0.09	0.14	ND
30	0.53	0.35	0.17	0.08	0.03	0.10	0.29
31	0.71	0.27	0.13	0.06	0.03	0.10	0.17
38	0.78	0.28	0.19	0.11	0.06	0.11	0.20
39	0.87	0.33	0.23	0.10	0.11	0.24	0.34
41	ND	0.41	0.22	0.23	0.11	ND	ND
42	1.11	0.47	0.35	0.21	0.19	0.40	0.48
48A	0.71	0.36	0.18	0.13	0.08	0.14	0.23
50	0.70	0.39	0.19	0.08	0.04	0.10	0.17
52	0.67	0.41	0.20	0.07	0.04	0.11	0.17
55	0.86	0.48	0.25	0.18	0.20	0.30	0.38
59	0.83	0.65	0.39	0.32	0.30	0.33	0.57
60	0.74	0.45	0.28	0.16	0.17	0.24	0.32
61	0.67	0.44	0.18	0.08	0.07	0.11	0.26
Nonribosomal protein							
A	1.26	1.25	1.27	1.22	1.74	ND	ND
B	1.06	1.83	0.55	0.47	0.57	ND	ND
C	0.98	1.24	0.91	1.01	1.93	ND	ND
D	1.08	0.91	0.93	0.61	0.94	ND	ND

[a] Experiments identical to those described in the legend to Fig. 1A were carried out, and the value of A_i was determined for a large number of ribosomal proteins and some nonribosomal proteins (A through D) whose identity is unknown.

[b] ND, Not determined.

[c] Boldface type indicates a ribosomal protein showing noncoordinate behavior either in the experiment presented or under other cellular conditions.

from heat shock, whereas a culture in EMM had barely begun to recover.

We conclude that transient repression and derepression of ribosomal protein synthesis occurs coordinately in cells under both growth conditions, but that cells growing on ethanol show a substantially greater sensitivity to heat shock than those growing on glucose.

Effect of glucose on transient inhibition. The accompanying paper (8) demonstrates that the addition of glucose to cells growing on ethanol causes an immediate and selective stimulation of the synthesis of ribosomal proteins. We now ask whether addition of glucose can suppress the inhibition of ribosomal protein synthesis after a temperature shift.

From Fig. 1A and B it is clear that there was relatively little change in the rate or extent of the selective inhibition of the synthesis of the four ribosomal proteins shown when glucose was added 3 min after heat shock. However, the duration of the inhibition was markedly shortened. The situation after glucose addition appeared remarkably similar to that found when cells were maintained continuously with glucose, i.e., derepression began after 15 min and was complete after 60 min. Table 2 shows that under these physiological conditions the synthesis of most ribosomal proteins remained coordinate. Interestingly, several of the previously identified "exceptional proteins," which are printed in boldface type, showed a somewhat less steep repression-derepression curve.

These data indicate that initially heat shock is dominant to glucose addition. After the adaptation to elevated temperature has occurred, i.e., about 15 min, glucose exerts its effect: ribosomal protein synthesis increases approximately four-

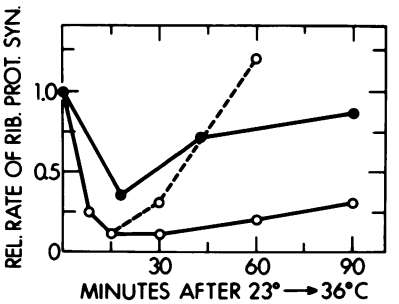

FIG. 2. *Comparison of the kinetics of the transient inhibition of ribosomal protein synthesis between cells grown on glucose or ethanol. Symbols: ●——●, cells grown on glucose; ○——○, cells grown on ethanol; ○- - -○, cells grown on ethanol and glucose added 3 min after temperature upshift. Data for glucose-grown A364A wild-type cells are from Gorenstein (C. Gorenstein, Ph.D. thesis, Albert Einstein College of Medicine, Bronx, N.Y., 1977). Very similar data for glucose-grown cells of strain NP2 were obtained by Pearson (N. J. Pearson, Ph.D. thesis).*

fold between 15 and 60 min.

Mutants temperature sensitive for ribosome synthesis. When a culture is shifted from 23 to 36°C, mutants with a temperature-sensitive allele of the gene *rna2* undergo a permanent depression in the synthesis of ribosomal proteins. We asked (i) for cells growing on ethanol how do the kinetics of inhibition of ribosomal protein synthesis in the mutant compare to that of the wild type and (ii) can the addition of glucose suppress, even partially, the mutant phenotype?

Figure 3A shows the relative rates of synthesis of four ribosomal proteins in mutant cells maintained in EMM after a temperature shift. Initially, the results were almost identical to those obtained in wild-type cells (Fig. 1A). As expected, however, the mutant showed no resumption in the synthesis of ribosomal proteins. Table 3 shows similar results for the majority of the 25 ribosomal proteins analyzed, with the exception of those indicated in boldface type, whose syntheses were repressed less or for a shorter period of time. An example, no. 24, is illustrated in Fig. 3A.

Experiments were then performed with the addition of glucose before and after the temperature shift. The addition of glucose 2 min after a temperature shift had no apparent effect on the rates of synthesis of three of the four ribosomal proteins shown (Fig. 3B). At 36°C their rates of synthesis were substantially reduced, whereas those of nonribosomal proteins remained normal. The synthesis of protein no. 24, however, was repressed to a lesser degree and recovered by 45 min. Composite data for 25 ribosomal proteins are presented in Table 4. The rates of synthesis for most of the proteins were coordinated. Several, those indicated in boldface type, were exceptional.

When glucose was added to cells growing on ethanol, the relative rate of ribosomal protein synthesis increased about 25% within 5 min (Fig. 4) (8). When the temperature was subsequently raised, the familiar inhibition of ribosomal protein synthesis was observed (Fig. 4). However, there was a reproducible delay before the inhibition occurred (compare Fig. 3A and 4). This finding suggests that the addition of glucose to cells at 23°C sets in motion events that cannot be immediately reversed by a temperature shift. In the long run, however, the effects of a temperature shift, both for wild-type and *rna2(Ts)* cells, were dominant to the effects of glucose for the majority of the ribosomal proteins. The exceptional proteins were again refractory (com-

TABLE 2. *Strain NP2 (rna2/+) grown in EMM: relative rates of synthesis of ribosomal proteins when glucose is added 3 min after temperature upshift*[a]

Ribosomal protein	Relative rate of synthesis					
	At 23°C	At time (min) after glucose addition at 36°C				
		0	4	12	27	57
2	0.91	0.45	0.17	ND[b]	0.23	0.74
6	0.85	0.45	0.17	ND	0.24	0.90
9	0.70	0.35	0.13	ND	0.20	1.16
11	0.80	0.49	0.17	0.07	0.23	0.94
12	0.73	0.48	0.16	0.14	0.19	0.72
13[c]	0.66	0.38	0.16	0.09	0.17	0.66
14	0.89	0.56	0.27	0.35	0.30	0.85
16	0.49	0.45	0.13	0.05	0.19	0.86
18	0.52	0.36	0.13	ND	0.17	0.77
24	0.68	0.41	0.19	0.16	0.28	0.94
26	0.71	0.32	0.16	ND	0.18	0.80
27	0.60	0.31	0.13	ND	0.21	0.83
30	0.53	0.35	0.17	0.09	0.20	0.98
31	0.71	0.27	0.15	0.04	0.17	0.74
38	0.78	0.28	0.16	0.05	0.24	0.89
39	0.87	0.33	0.20	0.11	0.28	0.89
41	ND	0.41	0.18	ND	0.21	0.88
42	1.11	0.47	0.27	0.25	0.31	0.86
48A	0.71	0.36	0.19	0.10	0.28	0.84
50	0.70	0.39	0.15	0.05	0.18	0.90
52	0.67	0.41	0.18	0.06	0.20	0.91
55	0.86	0.48	0.24	0.14	0.28	0.88
59	0.83	0.65	0.34	0.31	0.29	0.70
60	0.74	0.45	0.25	0.14	0.23	0.69
61	0.67	0.44	0.21	0.08	0.19	0.92

[a] Experiments identical to those described in the legend to Fig. 1B were carried out, and the value of A_i was determined for a large number of ribosomal proteins.

[b] ND, Not determined.

[c] Boldface type indicates a ribosomal protein showing noncoordinate behavior either in the experiment presented or under other cellular conditions.

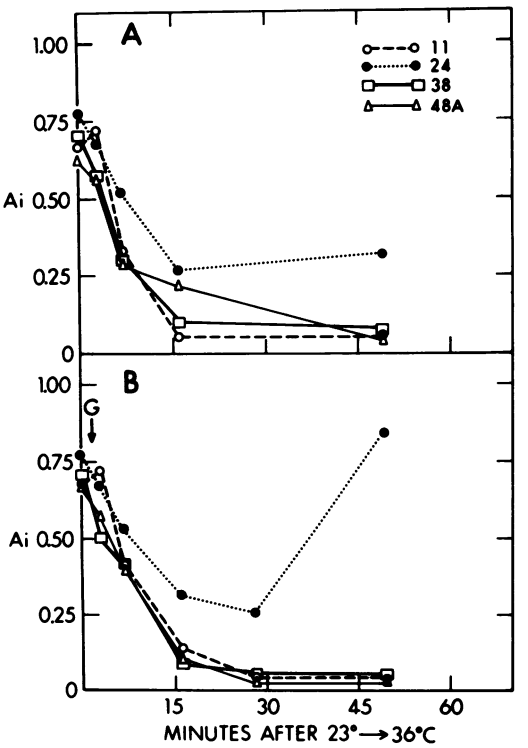

FIG. 3. *Strain NP1 (rna2/2) grown in EMM: relative rates of synthesis of ribosomal proteins after a temperature upshift. (A) Effect of temperature shift alone. (B) Effect of glucose addition 2 min after temperature upshift. These experiments are identical to their counterparts in Fig. 1, except that a diploid rna2(Ts) strain was used.*

pare Fig. 3B and 4); only a transient inhibition in their synthesis rate was observed.

Exceptional ribosomal proteins. The syntheses of a number of proteins, e.g., no. 14, 24, 39, and 42, are not always coordinated with those of the other ribosomal proteins (2, 14, 20). Whereas their syntheses are inhibited by a temperature shift (19) and stimulated by glucose (8), they are refractory to the effects of *rna2(Ts)*. The present investigation has extended the analysis of these exceptional proteins. The most striking example occurred when mutant cells were given glucose after a temperature shift (Fig. 3B). Initially, synthesis of these proteins declined coordinately with that of the others. However, the inhibition of their synthesis was far less severe and was transitory. The synthesis of these proteins escaped the effects of a lesion in *rna2* and responded to stimulation by glucose as in the wild type. These "noncoordinated" proteins appeared to respond in a similar fashion in the mutant after heat shock alone, but a longer time was necessary for derepression to occur.

Other ribosomal proteins, e.g., 2, 6, 12, 55, and 59, appeared to have intermediate responses. The syntheses of these proteins were characterized by two properties: they were coordinated or noncoordinated with that of other ribosomal proteins depending on the stimulus involved, and when noncoordinated their synthesis was usually at an intermediate level.

These findings raise the possibility that the ribosomal proteins are a less homogeneous group than has been previously thought. Furthermore, these noncoordinated proteins appear to define a qualitative difference between the transient inhibition characteristic of wild-type cells and the permanent inhibition characteristic of mutant cells and may provide an opportunity to distinguish between the two experimentally.

TABLE 3. *Strain NP1 (rna2/2) grown in EMM: relative rates of synthesis of ribosomal proteins and nonribosomal proteins after shift in temperature from 23 to 36°C*[a]

Protein	Relative rate of synthesis at time (min) after shift to 36°C				
	0	2	6	15	48
Ribosomal protein					
2	0.52	0.49	0.33	0.06	0.18
6	0.60	0.58	0.30	0.03	0.05
9	0.73	0.68	0.22	0.02	0.03
11	0.67	0.72	0.33	0.06	0.06
12	0.85	0.63	0.36	0.04	0.13
13[b]	0.69	0.59	0.40	0.10	0.20
14	0.95	0.58	0.57	0.28	0.61
15	0.54	0.47	0.27	0.07	0.03
16	0.79	0.61	0.48	0.04	0.07
18	0.60	0.41	0.26	0.03	0.02
24	0.78	0.68	0.53	0.26	0.32
26	0.65	0.52	0.30	0.04	0.05
27	0.69	0.50	0.25	0.04	0.03
30	0.74	0.58	0.35	0.08	0.09
31	0.72	0.48	0.24	0.04	0.05
38	0.71	0.57	0.30	0.10	0.07
39	0.66	0.57	0.40	0.06	0.20
42	0.70	0.75	0.68	0.09	0.33
48A	0.67	0.58	0.29	0.22	0.03
50	0.84	0.59	0.30	0.04	0.09
52	0.76	0.66	0.34	0.08	0.12
55	0.76	0.62	0.40	0.09	0.16
59	0.76	0.74	0.50	0.18	0.19
61	ND[c]	0.56	0.34	0.27	0.08
Nonribosomal protein					
A	1.08	ND	ND	ND	ND
B	0.88	ND	ND	ND	ND
C	0.99	ND	ND	ND	ND
D	0.90	ND	ND	ND	ND

[a] Experiments identical to those described in the legend to Fig. 3A were carried out, and the value of A_i was determined for a large number of ribosomal proteins and some nonribosomal proteins (at steady state only).

[b] Boldface type indicates ribosomal proteins showing exceptional behavior in this experiment.

[c] ND, Not determined.

TABLE 4. *Strain NP1 (rna2/2) grown in EMM: relative rates of synthesis of ribosomal proteins when glucose is added 2 min after temperature upshift*[a]

Ribosomal protein	Relative rate of synthesis					
	At 23°C	At time (min) after glucose addition at 36°C				
		0	4	13	25	46
2[b]	0.52	0.49	0.36	0.12	0.21	0.34
6	0.60	0.58	0.33	0.07	0.17	0.49
9	0.73	0.68	0.36	0.05	0.02	0.06
11	0.67	0.72	0.41	0.14	0.04	0.04
12	0.85	0.63	0.51	0.06	0.16	0.54
13	0.69	0.59	0.47	0.20	0.19	0.76
14	0.95	0.58	0.61	0.76	0.33	0.83
15	0.54	0.47	0.46	ND[c]	0.05	0.15
16	0.79	0.61	0.46	0.11	0.09	ND
18	0.60	0.41	0.32	0.05	0.01	0.11
24	0.78	0.68	0.54	0.31	0.26	0.84
26	0.65	0.52	0.36	0.06	0.02	0.02
27	0.69	0.50	0.40	0.07	0.02	0.03
30	0.74	0.58	0.47	0.11	0.10	0.08
31	0.72	0.48	0.29	0.05	0.03	0.04
38	0.71	0.57	0.43	0.09	0.05	0.06
39	0.66	0.57	0.50	0.16	0.27	1.11
42	0.70	0.75	0.59	0.20	0.27	0.61
48A	0.67	0.58	0.41	0.10	0.02	0.03
50	0.84	0.59	0.40	0.07	0.06	0.05
52	0.76	0.66	0.46	0.12	0.11	0.15
55	0.76	0.62	0.47	0.12	0.12	0.29
59	0.76	0.74	0.55	0.21	0.15	0.22
61	ND	0.56	0.42	0.07	0.07	0.10

[a] Experiments identical to those described in the legend to Fig. 3B were carried out, and the value of A_i was determined for a large number of ribosomal proteins.

[b] Boldface type indicates ribosomal proteins showing exceptional behavior in this experiment.

[c] ND, Not determined.

Effect of glucose on the synthesis of ribosomal RNA. The accompanying paper demonstrates that wild-type cultures growing in EMM respond to the addition of glucose by rapidly increasing the balanced synthesis of ribosomal precursor RNA and ribosomal proteins (8). Conversely, temperature upshifts in both wild-type and *rna2(Ts)* cells uncouple the synthesis of ribosomal precursor RNA from the synthesis of ribosomal proteins (19, 21). To determine which of the two patterns dominates in a double shift, glucose was added to a culture in EMM 3 min after a temperature upshift.

The methods used for determination of the rate of ribosomal RNA transcription and maturation are described in Table 5. In brief, wild-type cells were grown for several generations with [^{14}C]uracil at 27°C. They were then shifted to 37°C and pulse-labeled with [*methyl*-^{3}H]methionine at intervals before and after the addition of glucose. The RNA was extracted and analyzed on polyacrylamide gels, and the radioactivity in each species was determined. The data presented in Table 5 show that cells grown on ethanol responded to temperature shock as did cells grown on glucose. The rate of synthesis of ribosomal precursor RNA was relatively unaffected by the temperature upshift, although the rate of ribosomal precursor RNA processing was markedly decreased, presumably due to the absence of ribosomal proteins (2, 5). When glucose was added 3 min after the temperature shift, ribosomal precursor RNA synthesis was stimulated approximately twofold even in the face of declining ribosomal protein synthesis. Strikingly, within only 20 min of the addition of glucose the rate of ribosomal precursor RNA maturation reached normal levels, suggesting that the ribosomal proteins necessary for ribosomal precursor RNA processing were present at normal concentrations by this time.

Thus, these results confirm that the patterns of temperature shock dominate those of glucose enrichment. The normal regulatory mechanisms coordinating the synthesis of ribosomal RNA and ribosomal proteins, which are overridden by temperature shock, cannot be restored, at least initially, by glucose addition.

DISCUSSION

A physiological response to moderate or severe heat shock has now been described for a number of organisms, including *Escherichia coli* (9, 22), *Naegleria* (18), *Tetrahymena* (6), *Saccharomyces* (10–12), *Drosophila* (reviewed in reference 1), other insects (17), and several eucaryotic cell lines (7). Severe temperature shock can shut off the synthesis of nearly all cell proteins and induce the synthesis of a new set, the "heat-shock" proteins, e.g., in *Drosophila* (1, 16) or *Naegleria* (18). A moderate temperature shock, when cells are shifted from a lower to a higher temperature that will still support growth, leads to a transient stimulation or inhibition of the synthesis of many proteins. In yeasts (5, 19), *E. coli* (9), and *Tetrahymena* (6) the syntheses of proteins or RNA involved in the protein synthetic apparatus, in particular the ribosomal proteins, are especially sensitive to such a temperature shift. Although magic spot, guanosine 5′-diphosphate-3′-diphosphate, has been observed in yeast cells after a temperature shift (13), it does not appear to be involved in the repression of ribosomal protein synthesis because the repression is observed normally in cells in which no guanosine 5′-diphosphate-3′-diphosphate is synthesized, e.g., ρ^0 strains (unpublished observations). In summary, little is known about the mechanisms of the coordinate

TABLE 5. *Strain NP2 (rna2/+): effect of glucose addition on the rate of ribosomal RNA synthesis and maturation after a temperature shift*[a]

Culture	Time (min) at 37°C	Total *methyl*-^{3}H (cpm)	Total [^{14}C]uracil (cpm)	^{3}H/^{14}C	(25S + 27S)/ 35S
Without glucose	0	4,796	1,822	2.63	0.71
	20	3,489	1,592	2.19	0.27
With glucose	20	7,537	2,193	3.44	0.85
	100	11,209	2,866	3.91	0.98

[a] An EMM culture uniformly labeled with [^{14}C]uracil was pulse-labeled for 3 min with [*methyl*-^{3}H]methionine at 27°C and at various times after a temperature upshift with and without glucose addition. Total RNA was extracted and analyzed as described in reference 8. From the gel analysis data, the "index of synthesis," the ratio of *methyl*-^{3}H/[^{14}C]uracil radioactivity, and the "index of maturation," the ratio of ^{3}H radioactivity in 27S + 25S RNA to the ^{3}H radioactivity in 35S RNA, were determined for each sample.

inhibition of ribosomal protein synthesis. We have attempted to dissect it by comparing the effects of a temperature shift on cells growing rapidly on glucose or slowly on ethanol and by determining whether the inhibition due to a temperature shift is dominant to the stimulation caused by the addition of glucose.

We found that the coordinate repression of ribosomal protein synthesis on temperature upshift was faster and more severe in cells grown on ethanol than in those grown on glucose. Furthermore, derepression occurred very slowly in ethanol. Thus, slowly growing oxidative cells were strikingly more sensitive to this environmental change than were rapidly growing fermentative cells.

Cell-free translation of RNA isolated from wild-type cells grown on glucose suggests that the transient inhibition of ribosomal protein synthesis that occurs after temperature shift is due largely to a limitation in the availability of functional mRNA (5, 19). The kinetics of decline of the synthesis of these proteins suggested that the synthesis of mRNA for ribosomal proteins ceases almost immediately after the upshift, and the available mRNA decays with its characteristic half-life (2). Assuming a similar mechanism in cells growing on ethanol, the data in Fig. 1 suggest that the half-life of mRNA is shorter than in cells growing on glucose. It would be interesting to determine whether this is characteristic of all mRNA's in cells growing in ethanol. If not, perhaps the cell modulates its concentration of ribosomes by coupling the stability of mRNA for ribosomal proteins with the growth rate. This notion predicts that a nutritional shift-up would lead, perhaps immediately, to an increased stability of mRNA for ribosomal proteins. Alternatively, the extreme response to heat shock of cells growing in ethanol may involve regulation at the translational level. One could argue that in cells grown on ethanol some component of the temperature upshift response must generate differential effects on the translational efficiency or the functional stability of different mRNA's.

A further objective of our study was to attempt to distinguish transcriptional and translational components of the nutritional shift-up response. The stimulation of ribosomal protein synthesis upon the addition of glucose to cells growing on ethanol is so rapid that it seems unlikely to be due solely to increased transcription (8). We attempted to distinguish the components of this response by observing the effects of adding glucose to a culture either shortly before or shortly after a temperature shift. If heat shock blocks the synthesis of mRNA for ribosomal proteins, then subsequent addition of glucose should have no immediate effect on the synthesis of ribosomal proteins if glucose acts only at the level of mRNA synthesis. The data in Fig. 1B suggest that this is true. On the other hand, Fig. 4 shows that when glucose was added before the temperature shock, there appeared to be a brief competition between the two effects before the characteristic decline of ribosomal protein synthesis commenced. This could indicate that glucose or its metabolites set in motion a process, e.g., a cohort of polymerases transcribing protein genes, that must run its course in spite of a temperature shift. In summary, the data suggest that the effects of glucose are likely to be at the level of synthesis of mRNA for ribosomal proteins, but may involve alterations in stability as well.

The experiments described in Fig. 3 and 4 and Tables 3 and 4 demonstrate that the *rna2* gene product is essential for the syntheses of ribosomal proteins in cells growing in ethanol as well as in cells growing in glucose (2). This is an important point because Pearson and Haber (14) found that the *rna2* gene product was no longer essential for ribosomal protein synthesis during or shortly after nitrogen deprivation where cell growth has stopped. Perhaps such dispensability is due to the cells entering a sporulation mode, but this is not clear, especially in light of recent

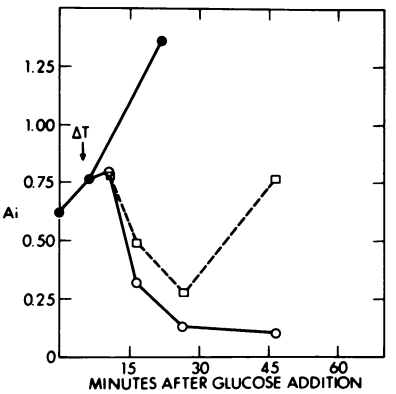

FIG. 4. *Strain NP1 (rna2/2) grown in EMM: relative rates of synthesis of ribosomal proteins when glucose is added 5 min before temperature upshift (ΔT). Symbols: ○, average A_i values for 17 coordinated ribosomal proteins; □, average A_i values for exceptional ribosomal proteins 2, 6, 12, 13, 14, 24, 39, 42; ●, average A_i values for 25 ribosomal proteins from cells maintained at 23°C.*

evidence suggesting that *rna2* is involved in a splicing reaction necessary for the maturation of mRNA for ribosomal proteins (14a).

Previous work showed that temperature shock uncouples the synthesis of ribosomal RNA from the synthesis of ribosomal protein in both wild-type and *rna2(Ts)* cells growing on glucose (2, 15, 21). Table 5 shows that this was true as well in cells growing on ethanol. The addition of glucose shortly after a temperature shock stimulated the transcription of ribosomal precursor RNA, even in the face of declining synthesis of ribosomal protein. This is a further indication that a temperature shock overrides the normal regulatory processes which maintain a balance between ribosomal RNA and ribosomal proteins.

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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. **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.
3. **Gross, K. J., and A. O. Pogo.** 1974. Control mechanism of ribonucleic acid synthesis in eukaryotes. J. Biol. Chem. **249:**568-576.
4. **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.
5. **Hereford, L., and M. Rosbash.** 1977. Regulation of a set of abundant messenger RNA sequences. Cell **10:**463-470.
6. **Hermolin, J., and A. M. Zimmerman.** 1976. RNA synthesis in division-synchronized *Tetrahymena* macronuclear and cytoplasmic RNA. J. Protozool. **23:**594-600.
7. **Kelley, P. M., and M. J. Schlesinger.** 1978. The effect of amino acid analogues and heat shock on gene expression in chick embryo fibroblasts. Cell **15:**1277-1286.
8. **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.
9. **Lemaux, P. G., S. L. Herendeen, P. L. Block, and F. C. Neidhart.** 1978. Transient rates of synthesis of individual polypeptides in *E. coli* following temperature shifts. Cell **13:**427-434.
10. **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.
11. **McAlister, L., and D. B. Finklestein.** 1980. Alterations in translatable ribonucleic acid after heat shock of *Saccharomyces cerevisiae*. J. Bacteriol. **143:**603-612.
12. **Miller, M. J., N. 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. **72:**1117-1121.
13. **Pao, C. C., J. Paietta, and J. A. Gallant.** 1977. Synthesis of guanosine tetraphosphate in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. **74:**314-321.
14. **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.

14a. **Rosbash, M., P. K. W. Harris, J. L. Woolford, Jr., 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.

15. **Shulman, R. W., and J. R. Warner.** 1978. Ribosomal RNA transcription in a mutant of *Saccharomyces cerevisiae* defective in ribosomal protein synthesis. Mol. Gen. Genet. **161:**221-223.
16. **Storti, R. V., M. P. Scott, A. Rich, and M. L. Pardue.** 1980. Translational control of protein synthesis in response to heat shock in *D. melanogaster* cells. Cell **22:** 825-834.
17. **Vincent, M., and R. M. Tanguaery.** 1979. Heat-shock induced proteins in the cell nucleus of *Chironomus tetans* salivary gland. Nature (London) **281:**501-503.
18. **Walsh, C.** 1980. Appearance of heat shock proteins during the induction of multiple flagellae in *Naegleria gruberi*. J. Biol. Chem. **255:**2629-2632.
19. **Warner, J. R., and C. Gorenstein.** 1977. The synthesis of eukaryotic ribosomal protein in vitro. Cell **11:**201-212.
20. **Warner, J. R., and C. Gorenstein.** 1978. Yeast has a true stringent response. Nature (London) **275:**338-339.
21. **Warner, J. R., and S. A. Udem.** 1972. Temperature sensitive mutations affection ribosome synthesis in *Saccharomyces cerevisiae*. J. Mol. Biol. **65:**243-257.
22. **Yamamori, T., K. Ito, Y. Nakamura, and T. Yura.** 1978. Transient regulation of protein synthesis in *Escherichia coli* upon shift-up of growth temperature. J. Bacteriol. **134:**1133-1140.