JOURNAL OF BACTERIOLOGY, Nov. 1968, p. 1664-1671
Vol. 96, No. 5

Temperature-sensitive Mutants of Yeast Exhibiting a Rapid Inhibition of Protein Synthesis

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Received for publication 9 September 1968

Certain temperature-sensitive (ts^-) mutants of yeast which cannot be corrected by nutritional supplementation exhibited a rapid cessation of protein synthesis after a shift to the restrictive temperature. Genetic and biochemical tests permitted a division of these mutants into four classes. This division was based upon genetic complementation patterns among the mutants and an investigation of glucose incorporation into macromolecules and polyribosome content in the mutants after a shift to the restrictive temperature. A study of these parameters in the parent strain (ts^+) in the presence of certain well-characterized inhibitors allowed a tentative identification of the biochemical defects in each of the four classes. The properties of the mutants in class IA were consistent with the hypothesis that they result from a defect in the initiation of polypeptide chains or in ribonucleic acid synthesis; mutants in class IB from a defect in the elongation of polypeptide chains; mutants in class IIA from a defect in energy metabolism; and mutants in class IIB from a lesion affecting membrane function.

In a previous publication, macromolecule synthesis was examined in 400 independently derived temperature-sensitive mutants of yeast (3). These mutants do not grow at 36 C on highly enriched media, and we assume, therefore, that they are not temperature-sensitive auxotrophs but are defective in some indispensable gene function. In this study, we concentrated upon 16 of these mutants which exhibit a rapid cessation of protein and ribonucleic acid (RNA) synthesis after a shift to the restrictive temperature. One can imagine several types of defects which would result in this phenotype: defects in protein synthesis, RNA synthesis, energy metabolism, and membrane permeability. We attempted to devise a screening procedure employing simple physiological tests that would allow us to group mutants exhibiting a rapid inhibition of protein synthesis according to the biochemical nature of their defect.

After studying a number of biochemical parameters, we decided that two, glucose incorporation into macromolecules and polyribosome content, are particularly indicative of the site of inhibition in a mutant. The mutants were, accordingly, divided into four classes on the basis of an investigation of glucose incorporation and an analysis of polyribosome content at the restrictive temperature; complementation studies on these mutants support this classification scheme. To correlate the properties of the mutants in each of the four classes with a particular biochemical lesion, we investigated these two parameters in the parent strain in the presence of a number of well-characterized inhibitors. The latter study indicated that the properties of the mutants in these four classes are consistent with the hypothesis that they result from the following types of biochemical defects: polypeptide chain initiation or RNA synthesis (class IA); polypeptide chain elongation (class IB); energy metabolism (class IIA); and membrane function (class IIB).

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MATERIALS AND METHODS

Cell strains and media. The parent strain (A364A), the origin of the mutants from it by nitrosoguanidine mutagenesis, and the composition of the media (YM-1, YM-5, and YEPD-TAU plates) were described previously (3). Mutant 296* is a segregant of a cross between our original ts^- 296 mutant and a ts^+ strain; the segregant was used in most experiments, since the original mutant was a double ts^- mutant. Cells were grown overnight in YM-1 medium, harvested by centrifugation while in the log phase of growth, and suspended in YM-5 medium for labeling experiments involving whole cells or the cells that were converted to spheroplasts (5). Spheroplasts were incubated for 3 hr in YM-5 medium containing 0.4 M $MgSO_4$ at 23 C before the start of an experiment. In a few experiments, a defined medium of the following composition was used: yeast nitrogen base minus amino acids (Difco), 6.7 g; alanine, 40 mg;

arginine, 40 mg; aspartic acid, 100 mg; cysteine, 40 mg; glutamic acid, 200 mg; glycine, 40 mg; histidine, 40 mg; isoleucine 40 mg; leucine 40 mg; lysine, 40 mg; methionine, 40 mg; phenylalanine, 40 mg; proline, 40 mg; serine, 40 mg; threonine, 40 mg; tryptophan, 40 mg; tyrosine, 40 mg; valine, 40 mg; adenine, 10 mg; uracil, 10 mg; succinic acid, 10 g; sodium hydroxide, 6 g; glucose, 20 g; distilled water, 1 liter (final *p*H 5.8). For experiments with spheroplasts, the medium was supplemented with 0.4 M $MgSO_4$.

Chemicals. Poly-L-ornithine was obtained from Sigma Chemical Co., St. Louis, Mo. and cycloheximide from Nutritional Biochemicals Co. Cleveland, Ohio. Nystatin was a generous gift of E. R. Squibb & Sons, New Brunswick, N.J., and Brij 58 was kindly provided by Atlas Powder Co. (Wilmington, Del.). Radioactive compounds were obtained from Schwarz Bio Research Inc., Orangeburg, N.Y.

Analytical techniques. The techniques used to study the incorporation of radioactive precursors into macromolecular material was described previously (3). The incorporation of glucose into macromolecular material was determined in the same manner as that used for determining the incorporation of adenine.

Polyribosome analysis. Generally, 40 ml of spheroplast culture (prepared from approximately 4×10^8 cells) was harvested for each gradient by adding cycloheximide to a final concentration of 10^{-3} M and rapidly chilling the culture in an ice bath. The spheroplasts were collected by centrifugation at 4600 × *g* for 5 min and lysed in 1 ml of buffer containing 0.01 M tris(hydroxymethyl)aminomethane, 0.1 M NaCl, 0.03 M $MgCl_2$, 20 μg of poly-L-ornithine per ml, *p*H 7.4 (lysing buffer). An 0.1-ml amount of 5% sodium deoxycholate was added, and the mixture was held at 5 C for 5 min; after this time, 0.15 ml of 5% Brij 58 was added, and the mixture was held for another 5 min at 5 C before it was layered on 31 ml of a 10 to 60% (w/v) linear sucrose gradient in lysing buffer minus poly-L-ornithine. Centrifugation was carried out in a Spinco SW 25.1 head at 60,000 × *g* for 3 to 5 hr at 5 C.

Genetic techniques. Techniques used in the mating of haploids and the selection of the resulting diploids were described previously (3).

RESULTS

Protein synthesis and viability at the restrictive temperature. Sixteen temperature-sensitive mutants of yeast were picked for this study because of their rapid cessation of protein synthesis at the restrictive temperature. Protein synthesis was studied by the incorporation of radioactive amino acids into acid-precipitable material. In Table 1, we recorded the amount of radioactivity incorporated by cultures of the mutants growing in YM-5 medium over a period of 3 hr relative to a culture of the parent strain A364A after a shift to the restrictive temperature, 36 C. The parent strain had a doubling time of 1.5 to 2 hr at this temperature. All of the mutants incorporated 14% or less of the amount of radioactivity incorporated by the parent strain. The mutants were grouped into four classes, IA, IB, IIA, and IIB (Table 1). The significance of this grouping will be made apparent.

TABLE 1. *Protein synthesis and viability in temperature-sensitive mutants*

Class	Mutant	Protein synthesis during 3 hr at 36 C		Viability after 3 hr at 36 C[c]
		Mutant A364A[a]	Increase[b]	
			%	%
IA	136	0.13	17	120
	171	0.03	9	120
	187	0.03	−12	130
IB	241	0.04	11	90
	275	0.10	34	90
	296*	0.07		70
	341	0.08	28	100
IIA	278	0.08	4	80
	331	0.06	14	50
	347	0.08	15	70
	364	0.05	27	80
	434	0.05	21	90
	437	0.06	24	80
IIB	132	0.14	35	10
	134	0.14	38	20
	441	0.05		<10

[a] Amount of ^{14}C-protein hydrolysate incorporated by a culture of the mutant divided by the amount incorporated by the parent strain A364A during 3 hr after a shift from 23 to 36 C in YM-5 medium.

[b] Percentage of increase in total protein in a culture of the mutant during 3 hr after a shift from 23 to 36 C in YM-5 medium.

[c] Percentage of the initial colony-forming ability of a culture of the mutant 3 hr after a shift from 23 to 36 C.

The net increase in protein in cultures of the mutants at the restrictive temperature was also determined by growing cultures for several generations in YM-5 medium at the permissive temperature, 23 C, in the presence of ^3H-lysine, and then by determining the net increase in radioactivity after 3 hr at the restrictive temperature. The mutants are all auxotrophs for lysine. Since there is only a small increase in protein, this measurement is not as accurate as the former one. Nevertheless, the net increases were small and, in most cases, in line with what one would expect from the previous experiment (Table 1). The largest increase was 38%, and approximately one-half of the mutants showed a net increase in protein of less than 20%.

The viability of the mutants after an exposure to

the restrictive temperature was determined by diluting samples of cultures which had been incubated at 36 C for 3 hr and by plating for colony-forming ability on YEPD plates. Only the three mutants in class IIB exhibited a significant loss of viability at the restrictive temperature (Table 1).

Complementation patterns. Since all the mutants belong to mating type *a*, complementation studies were performed by crossing each mutant with an α ts^+ strain and by obtaining from the resulting diploid a segregant which was both ts^- and α in mating type. Diploids heterozygous for two ts^- mutations were then constructed by mating the *a* and α ts^- haploids in all possible pair combinations, and the diploids were then tested for their ability to grow at 36 C on YEPD plates (Table 2). There are 10 complementation groups represented among the 16 mutants. Two complementation groups contained more than one mutant: mutants 171 and 187 comprise a complementation group; and mutants 278, 331, 347, 364, 434, and 437 comprise the second complementation group.

Further complementation tests were carried out between one member of each of the complementation groups from classes IA, IB, and IIA, and the entire group of 400 ts^- mutants. The mutants in group IIB were not tested in this manner. The purpose of this study was to determine whether our selection of these mutants, based upon their rapid cessation of protein synthesis at the restrictive temperature, singled out a unique group of mutants. This search did uncover a few additional members of the complementation groups in classes IB and IIA (Table 3). Several of these mutants synthesized large amounts of protein at the restrictive temperature, unlike the original 16 mutants chosen for this investigation.

Glucose incorporation in the parent strain in the presence of various inhibitors. Cycloheximide is a well-characterized inhibitor of protein synthesis which prevents the incorporation of amino acids from aminoacyl-transfer RNA (tRNA) into growing polypeptide chains (10). At a concentration of 40 μg of cycloheximide per ml, protein synthesis in cultures of A364A was rapidly and completely inhibited, as measured by the incorporation of ^{14}C-amino acids into macromolecular material (Fig. 1). However, at the same concentration cycloheximide had little effect upon the initial rate of ^{14}C-glucose incorporation into macromolecular material.

An inhibition of energy metabolism would be expected to inhibit strongly both protein synthesis and glucose incorporation. This expectation was confirmed by the use of arsenate and sulfite; both prevented the production of adenosine triphosphate (ATP) by the glycolytic pathway. Arsenate competed with phosphate resulting in the arsenolysis of glyceraldehyde-3-phosphate and other compounds (7); sulfite acted as a trap for acetaldehyde which accumulates in the form of its bisulfite derivative (13). The concentration of arsenate and sulfite, just sufficient to produce a relatively complete inhibition of protein synthesis, was determined by preliminary experiments to be 0.01 and 0.05 M, respectively. These same concentrations caused an immediate and complete inhibition of glucose incorporation (Fig. 1). It was apparent that the ability of the cell to incorporate glucose into macromolecules following an inhibition of protein synthesis is diagnostic of the site of action of the inhibitor. Inhibitors whose pri-

TABLE 2. *Complementation patterns among the 16 temperature-sensitive mutants*[a]

Class	Mutant	Temperature-sensitive mutant														
		444	134	132	437	434	364	347	331	278	341	296	275	241	187	171	136
IA	136	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	171	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	
	187	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	
IB	241	+	+	+	+	+	+	+	+	+	+	+	+	+	−		
	275	+	+	+	+	+	+	+	+	+	+	+	+	−			
	296	+	+	+	+	+	+	+	+	+	+	+	−				
	341	+	+	+	+	+	+	+	+	+	+	−					
IIA	278	+	+	+	−	−	−	−	−	−							
	331	+	+	+	−	−	−	−	−								
	347	+	+	+	−	−	−	−									
	364	+	+	+	−	−	−										
	434	+	+	+	−	−											
	437	+	+	+	−												
IIB	132	+	+	−													
	134	+	−														
	441	−															

[a] A plus sign indicates that the diploid formed by mating the two haploid mutants is able to grow at 36 C; a minus sign indicates that the diploid does not grow at 36 C.

TABLE 3. *Complementation patterns among certain mutants in classes IA, IB, and IIA, and 400 other temperature-sensitive mutants*

Mutation	Noncomplementing mutations
136	136
187	171, 187
241	241
275	275, 232, 483
296	296
341	341, 443
437	437, 139, 199, 271, 278, 309, 323, 331, 347, 352, 364, 434

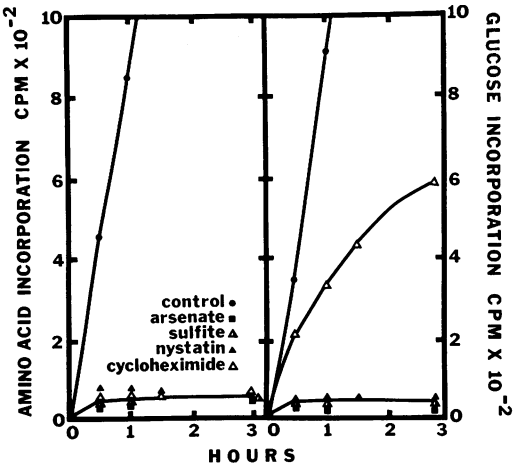

FIG. 1. *Protein synthesis and glucose incorporation in the presence of arsenate, sulfite, nystatin, and cycloheximide. Whole cells growing in YM-5 medium containing glucose at a concentration of 0.3% were incubated at 36 C in the presence of potassium arsenate (0.01 M), or sodium sulfite (0.05 M), or nystatin (10 μg/ml), or cycloheximide (40 μg/ml), or with no inhibitor added (control). D-Glucose-^{14}C (0.1 μc/ml) or reconstituted protein hydrolysate-^{14}C (0.05 μc/ml) was added, and samples were removed at various times and analyzed for the amount of radioactivity incorporated into macromolecular material. CPM (counts/min) × 10^{-2} signifies that an observed datum of 100 CPM has been recorded on the figure as 1 CPM.*

mary site of action is the cell's energy supply system would be expected to inhibit both processes, whereas an inhibitor whose primary site of action was the cell's protein synthetic system would be expected to allow the cell to continue glucose incorporation. Nystatin, a polyene antibiotic which alters the permeability of the yeast cell membrane (6), also inhibited strongly both the synthesis of protein and the incorporation of glucose (Fig. 1).

Glucose incorporation in the mutants. Next, we wished to examine protein synthesis and glucose incorporation in cultures of the mutants at the restrictive temperature in an attempt to distinguish mutants with primary defects in their protein synthetic system from mutants with primary defects in energy metabolism and membrane permeability. However, a direct comparison of the results obtained with mutant cultures and the data presented in Fig. 1 is not possible, since many of the mutants do not show an immediate and complete inhibition of protein synthesis after a shift to the restrictive temperature. Consequently, a calibration curve was constructed by determining the level of glucose incorporation in cultures of the parent strain A364A under conditions of various degrees of protein synthesis inhibition produced by various concentrations of cycloheximide. The amount of glucose incorporated into macromolecular material during a 3-hr period is plotted (Fig. 2) as a function of the amount of amino acids incorporated into protein; both numbers are recorded as the fraction of the amount incorporated by an uninhibited culture. The same data were then obtained for each of the mutants at the restrictive temperature in the absence of cycloheximide; again, the data were normalized to an uninhibited culture of the parent strain A364A. The data for those mutants considered to display significantly less glucose incorporation than expected for a defect in protein synthesis are plotted, and the mutants were placed in class II. The data for the mutants considered to be

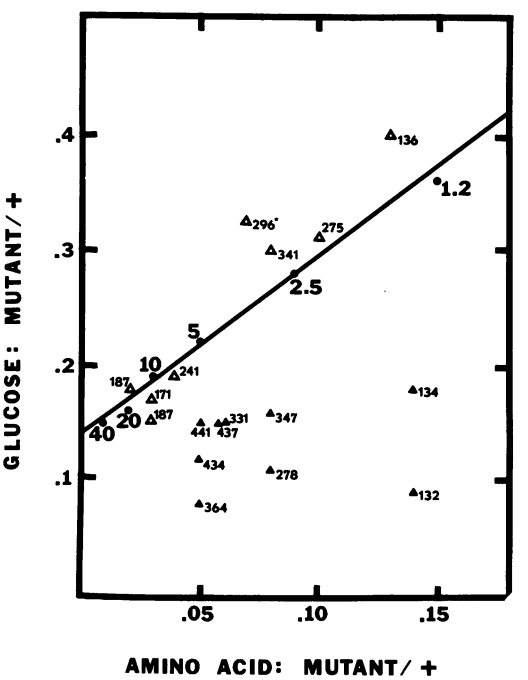

FIG. 2. *Protein synthesis and glucose incorporation in the parent strain in the presence of various concentrations of cycloheximide and in the mutants at the restrictive temperature. The experiment was carried out as described in the legend to Fig. 1, except that cycloheximide was used with the parent strain at concentrations of 1.2, 2.5, 5, 10, 20, and 40 μg/ml as indicated in the figure, and the mutants were tested in the absence of any inhibitor at 36 C. (△) Mutants considered to be in reasonably close agreement with the parent strain in the presence of cycloheximide; (▲) mutants not in agreement; (●) parent strain in the presence of cycloheximide. Large numbers indicate the concentration of cycloheximide expressed as micrograms per milliliter.*

in close agreement with the calibration curve are recorded (Fig. 2); these latter mutants are candidates for mutants with defects in the protein synthetic system and were placed in class I.

Polyribosomes in the parent strain. Figure 3 displays a typical polyribosome preparation from the parent strain A364A. Roughly 90% of the ribosomes were present as large polyribosomes in lysed preparations of spheroplasts growing at 23 C; for cells growing at 36 C, the percentage was less, about 70%. All of the polyribosomes were converted to monoribosomes by incubation for 5 min with 1 μg of ribonuclease per ml at 5 C.

Extracts of the parent strain were examined for their content of polyribosomes after the inhibition of protein synthesis in spheroplasts by a variety of means. An inhibition of polypeptide chain elongation by cycloheximide addition or by tyrosine starvation resulted in a retention of greater than 80% of the ribosomes in polyribosome structures even after 2 hr of inhibition (Fig. 4). Lower concentrations of cycloheximide that resulted in only a partial inhibition of protein synthesis also brought about a retention of polyribosomes (Table 4). On the contrary, an inhibition of protein synthesis due to a limitation of energy supply by glucose starvation, arsenate, or sulfite addition resulted in a conversion of polyribosome to monoribosomes (Fig. 4 and Table 4). Glucose starvation caused a very rapid initial loss of polyribosomes with a half-time of about 4 min; there was a subsequent slight increase in polyribosome content probably caused by the mobilization of internal cellular energy reserves. Nystatin has an intermediate effect, resulting in a retention of about 50% of the ribosomes as polyribosomes.

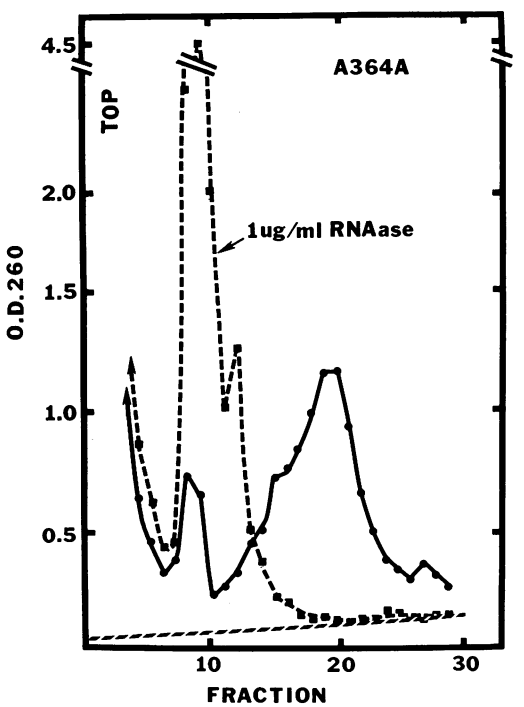

FIG. 3. *Sucrose gradient analysis of polyribosomes from a lysate of the parent strain incubated in the presence and absence of pancreatic ribonuclease. The spheroplast lysate was incubated at 5 C with and without 1 μg of pancreatic ribonuclease per ml for 5 min before it was layered on the gradient and then centrifuged for 3 hr.*

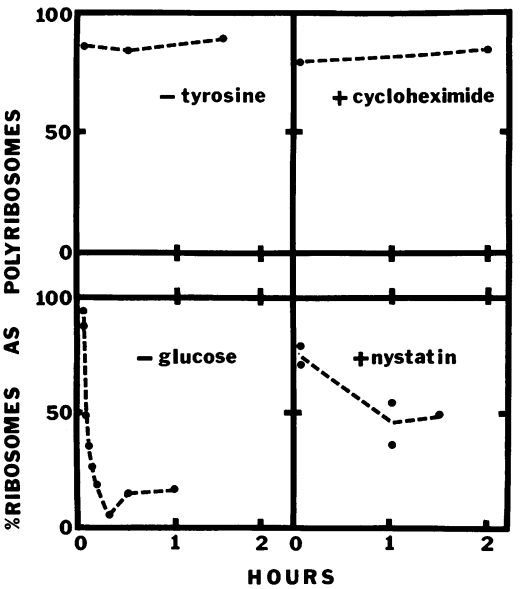

FIG. 4. *Polyribosome content in the parent strain under conditions of glucose starvation, tyrosine starvation, cycloheximide addition, or nystatin addition. For the experiments involving tyrosine starvation, the spheroplasts were incubated in the defined medium at 23 C; the other experiments were carried out in YM-5 medium at 23 C. The glucose and tyrosine starvation conditions were established by centrifuging spheroplasts out of the complete medium at room temperature for 3 min at 2,500 × g and suspending the pellet gently in the starvation medium. The zero-time sample in each experiment was taken after the centrifugation step.*

Polyribosomes in the mutants. Each of the mutants was examined for its content of polyribosomes after 1 and 2 hr of incubation at the restrictive temperature. Some mutants lost all polyribosomes at the restrictive temperature, whereas others retained significant levels (Table 5). To interpret these data, one must realize that the rate of protein synthesis in the mutants after 1 hr at the restrictive temperature was only about 10% or less of that of the parent strain. Thus, the presence of greater than 20% of ribosomes as

TABLE 4. *Effect of inhibitors upon polyribosomes in the parent strain A364A*

Inhibitor	Concn	Time after addition of inhibitor (min)[a]					Inhibition protein synthesis
		0	30	60	90	120	
	μg/ml						%
Cycloheximide[b]	0.1	80				85	83
Cycloheximide[b]	0.4					89	92
Cycloheximide[b]	1.0					88	97
Cycloheximide[b]	4.0					88	97
Cycloheximide[b]	40					86	99
Tyrosine starvation[c]		87	85		91		95
Nystatin[b]	10	79		55	50		100
Arsenate[b]	(0.01 M)			17			99
Sulfite[b]	(0.05 M)			18			94
Glucose starvation[c]		94	16	18			92

[a] Expressed as percentages of ribosomes appearing as polyribosomes.
[b] Cells growing in YM-5 medium at 23 C.
[c] Cells growing in defined medium at 23 C.

polyribosomes is considered a significant stabilization of polyribosomes above that level necessary to carry out the continuing rate of protein synthesis (subclass A); a polyribosome content of less than 20% is interpreted as a lack of polyribosome stabilization (subclass B). In fact, the distinction between the two subclasses is rather clear, since all the mutants in subclass A had 29% or more of their ribosomes present as polyribosomes, and all the mutants but one of subclass B contained 10% or less of their ribosomes as polyribosomes.

By combining the data obtained on glucose incorporation and polyribosome content, it was possible to group the mutants into four classes (Table 6). The data presented above on the behavior of the parent strain in the presence of certain inhibitors allow one to draw some tentative conclusions as to the possible site of the lesion in each of the four classes.

DISCUSSION

We investigated 16 temperature-sensitive mutants of yeast that exhibit a rapid cessation of protein synthesis after a shift to the restrictive temperature. The 16 mutants were grouped into four classes on the basis of glucose incorporation and polyribosome content at the restrictive temperature. The same determinations were performed with the parental strain in the presence of various inhibitors. Glucose incorporation into macromolecules, when normalized to protein synthesis, apparently distinguishes an inhibition of energy metabolism or membrane function from an inhibition of protein or RNA synthesis. Thus, arsenate and sulfite which inhibit energy metabolism and nystatin which changes the permeability properties of the yeast cell membrane all inhibit glucose incorporation and protein synthesis equally; cycloheximide, on the other hand, which inhibits polypeptide chain elongation, inhibits protein synthesis much more severely than it does glucose incorporation. These observations are not surprising, since glucose incorporation and protein synthesis both require a constant supply of ATP and undoubtedly a highly specific internal environment. Furthermore, it is known that glucose incorporation and protein synthesis are not obligatorily coupled in yeast, since stationary

TABLE 5. *Polyribosomes[a] in the temperature-sensitive mutants*

Class	Mutant	Time at 36 C (hr)		
		0	1	2
IA	136	87	15	10
	171	92	06	05
	187	93	<05	<05
IB	241	87	41	31
	275	75	52	46
	296*	92	25	30
	341	87	73	62
IIA	278	73	14	18
	331	93	<05	<05
	347			<05
	364	86	<05	<05
	434			
	437	85	<05	<05
IIB	132	84	61	—[b]
	134	98	29	—
	441	95	92	—

[a] Expressed as percentage of ribosomes.
[b] Could not be determined because of the lysis of the spheroplasts.

TABLE 6. *Summary of the properties of temperature-sensitive mutants that exhibit a rapid cessation of protein synthesis after a shift to the restrictive temperature*

Class	Mutant	Glucose incorporation	Polyribosome stabilization	Comments	Analogous inhibitor	Possible type of defect
IA	136	+	−	RNA synthesis[a]	None	RNA synthesis or polypeptide chain initiation
	171	+	−	Initiation factor[b]		
	187	+	−	Initiation factor[b]		
IB	241	+	+		Cycloheximide, amino acid starvation	Polypeptide chain elongation
	275	+	+			
	296*	+	+	Methionyl-tRNA synthetase[c]		
	341	+	+	Isoleucyl-tRNA synthetase (4)		
IIA	278	−	−	All in the same complementation group	Arsenate, sulfite glucose starvation	Energy metabolism
	331	−	−			
	347	−	−			
	364	−	−			
	434	−				
	437	−	−			
IIB	132	−	+	All lose viability and spheroplasts lyse	Nystatin	Membrane function
	134	−	+			
	441	−	+			

[a] H. T. Hutchison, C. S. McLaughlin, and L. H. Hartwell, *in preparation.*
[b] L. H. Hartwell and C. S. McLaughlin, *in preparation.*
[c] C. S. McLaughlin and L. H. Hartwell, *in press.*

phase cultures contain a much higher ratio of polysaccharide to protein than do exponentially growing cultures (9). We assume, therefore, that the mutants in classes IA and IB which incorporate large amounts of glucose relative to amino acids after a shift to the restrictive temperature are not defective in either energy metabolism or in membrane function but may be defective either in RNA or protein synthesis.

The mutants in class IA are distinguished from those in class IB on the basis of their polyribosome content at the restrictive temperature. The mutants in class IA lose their polyribosomes at 36 C. This is the behavior we would expect for a mutant in which protein synthesis was limited either by the supply of messenger RNA or by the ability of ribosomes to initiate new polypeptide chains. Indeed, actinomycin, an inhibitor of RNA synthesis, causes a loss of polyribosomes in bacterial cells (14); sodium fluoride, an inhibitor of polypeptide chain initiation, produces a loss of polyribosomes in cell-free preparations from reticulocytes (8). Unfortunately, these inhibitors have no effect on yeast cells. However, we have further evidence which indicates that one of the mutants in class IA, *ts*⁻ 136, is defective in RNA synthesis and that the other two mutants in this class, 171 and 187, are defective in the initiation of polypeptide chains (H. T. Hutchison, C. S. McLaughlin, and L. H. Hartwell; L. H. Hartwell and C. S. McLaughlin; *in preparation*).

The mutants in class IB retain significant levels of polyribosomes long after protein synthesis has been severely inhibited. This behavior is characteristic of a defect in polypeptide chain elongation as evidenced by the fact that both tyrosine starvation and cycloheximide addition bring about a retention of polyribosomes in the parent strain. Other investigators have shown that a variety of inhibitors which act at the level of polypeptide chain elongation such as cycloheximide (12), diphtheria toxin (1), and sparsomycin (11), all produce a stabilization of polyribosomes. Finally, two of the four mutants in this class have been definitely shown to be defective in aminoacyl-tRNA synthetases (4; C. S. McLaughlin and L. H. Hartwell, *in press*).

The mutants in classes IIA and IIB do not incorporate large amounts of glucose relative to amino acids after a shift to the restrictive temperature, and they may, therefore, be defective either in energy metabolism or in membrane function. The mutants in class IIA lose polyribosomes at 36 C. This behavior is consistent with a defect in energy metabolism, since the parent strain also loses polyribosomes under conditions of glucose

starvation or sulfite or arsenate addition. An inhibition of energy metabolism in *Escherichia coli* by glucose starvation has also resulted in a loss of polyribosomes (2). Complementation studies indicate that all six mutants in this class are in the same complementation group, and we have, therefore, only a single example of a defect corresponding to this class. Furthermore, complementation studies with the other 400 mutants revealed an additional six mutants of this complementation group, some with grossly different patterns of macromolecule synthesis at the restrictive temperature. The mutants of this complementation group represent somewhat of a puzzle both because of their frequent occurrence (representing about 3% of our total mutants) and because the defect can result in a variety of phenotypes with respect to macromolecule synthesis. Attempts to locate the site of the defect have thus far been unsuccessful. Results not reported here indicate that there is little if any inhibition of glycolysis or respiration for several hours after the shift to the restrictive temperature and the ATP pool in the cell remains at high levels.

The mutants in class IIB exhibit low levels of glucose incorporation and a retention of polyribosomes at the restrictive temperature. These properties are characteristic of the parent strain in the presence of nystatin, an inhibitor which acts on the yeast cell membrane. Furthermore, the mutants in this class exhibit two other properties not shared by the mutants in the other three classes; they lose viability rapidly at the restrictive temperature and, as spheroplasts, they lyse at the restrictive but not at the permissive temperature. Nystatin also produces a rapid loss of viability in yeast cells, whereas cycloheximide and glucose starvation do not. Thus, the properties of the mutants in this class are consistent with a defect in the cell membrane.

The data reported above and summarized in Table 6 provide physiological evidence for the type of biochemical defect present in these temperature-sensitive mutants of yeast. We are pursuing these leads by examining various components of the cell in vitro in an attempt to identify the site of the lesion in more of the mutants. The thermolabile protein has been identified in two of the mutants to be the isoleucyl-tRNA synthetase (4) and in a third to be the methionyl-tRNA synthetase (C. S. McLaughlin and L. H. Hartwell, *in press*).

ACKNOWLEDGMENTS

We thank Trudy Holland, Kristine Nelson, and Pamela Sutherland for competent technical assistance.

This investigation was supported by Public Health Service grant GM 13304-03 from the National Institute of General Medical Sciences and National Science Foundation grant GB 4828.

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