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Temperature-sensitive Yeast Mutant Defective in Ribonucleic Acid Production

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A single, recessive mutation in a nuclear gene confers a temperature-sensitive growth response in a mutant of *Saccharomyces cerevisiae*, *ts*⁻ 136. The mutant grows normally at 23 C, but exhibits a rapid and preferential inhibition of ribonucleic acid (RNA) accumulation after a shift to 36 C, demonstrating a defect in stable RNA production. Cultures of the mutant which were shifted from 23 to 36 C display the following phenomena which indicate that messenger RNA (mRNA), as well as stable RNA production, is defective. The entrance of pulse-labeled RNA into cytoplasmic polyribosomes is even more strongly inhibited than is net RNA accumulation. The rate of protein synthesis, at first unaffected, decreases slowly; this decrease is paralleled by the decay of polyribosomes to monoribosomes with a half-time of 23 min. The polyribosomes which remain after a 30-min preincubation of the mutant at 36 C are active in polypeptide synthesis in vivo, whereas the monoribosomes which accumulate are not. Furthermore, ribosomes isolated from a culture of the mutant preincubated for 1 hr at 36 C are inactive in polypeptide synthesis in vitro, but can be restored to full activity by the addition of polyuridylic acid as mRNA. We conclude that mutant *ts*⁻ 136 is defective either in the synthesis of all types of cytoplasmic RNA, or in the transport of newly synthesized RNA from the nucleus to the cytoplasm, and that the mRNA of a eucaryotic organism (yeast) is metabolically unstable, having a half-life of approximately 23 min at 36 C.

Conditional lethal mutants provide valuable objects for the study of essential gene functions. Not only does the conditional nature of the defect allow one to maintain a mutant which normally would not survive, but also an investigation of the behavior of the mutant at the restrictive condition allows one to examine the function of the defective gene in cellular physiology. The behavior of a distinct group of temperature-sensitive mutants of *Saccharomyces cerevisiae*, those which exhibit a rapid inhibition of protein synthesis at the restrictive temperature, was previously examined (3). Sixteen mutants were divided into four classes based upon certain physiological properties; three mutants in class IA (*ts*⁻ 136, *ts*⁻ 171, *ts*⁻ 187) displayed properties consistent with the hypothesis that they were defective in the initiation of protein synthesis, or in ribonucleic acid (RNA) synthesis. A subsequent report (4) demonstrated that two of these three mutants (*ts*⁻ 171, *ts*⁻ 187) were defective in the initiation of polypeptide chains. Here we present evidence that the third mutant, *ts*⁻ 136, is defective in RNA production.

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MATERIALS AND METHODS

Strains and media. Mutant *ts*⁻ 136 was obtained from strain A364A (a ad_1 ad_2 ur_1 ty_1 hi_7 ly_2 ga_1) by mutagenesis with nitrosoguanidine (2). Strain 2262-2A (α ad_1 ur_1 hi_5 ly_{11} le_2 ga_1) was obtained from D. C. Hawthorne, University of Washington, Seattle. All experiments with whole cells were carried out in YM-5 medium, and those with spheroplasts were carried out in YM-5 supplemented with 0.4 M $MgSO_4$ (2). Spheroplasts were prepared and incubated under growing conditions for 3 hr prior to the beginning of each experiment (6).

Genetic analysis. Techniques used for the isolation, sporulation, and tetrad dissection of hybrids, and the techniques used for scoring the segregation of various genes, including intergenic complementation and the composition of diagnostic media, were taken from the methods of Hawthorne and Mortimer (5) and Mortimer and Hawthorne (9). Only data from complete tetrads are reported.

Macromolecule synthesis, cell fractionation, and polyribosome analysis. Techniques used to quantitate

the incorporation of radioactive precursors into macromolecules were previously reported (2).

Spheroplasts were fractionated into crude nuclear and cytoplasmic fractions by lysing as in polyribosome preparation. This was followed by zone sedimentation in 11.5 ml of a 10 to 50% (w/w) linear sucrose gradient in lysing buffer [minus poly-L-ornithine (3)] in a Spinco model SW41 ultracentrifuge for 40 min at 190,000 × *g*. The gradient was drawn off the nuclear pellet to obtain the cytoplasmic fraction; the nuclear pellet was dissolved in 0.5% sodium dodecyl sulfate.

This procedure was tested by growing the parent strain (an adenine auxotroph) from a small inoculum in both ^{14}C-*8*-adenine to uniformly label the deoxyribonucleic acid (DNA) and RNA, and in ^{14}C-reconstituted protein hydrolysate to label the cellular proteins. Spheroplasts were prepared, grown for 3 hr in the same labeled medium, and fractionated as described above. The nuclear pellet contained 91% of the cellular DNA, 18% of the RNA, and 8% of the protein, as determined by the distribution of radioactivity; the cytoplasmic fraction contained 9, 82, and 92% of the DNA, RNA, and protein, respectively.

Polyribosomes were prepared from spheroplast cultures, fractionated on sucrose gradients, and analyzed as described previously (3); a slight variation was introduced in one experiment by continuous monitoring of the OD_{260} (optical density at 260 nm) profile through a flow cell in a Zeiss PMQ II spectrophotometer.

In vitro protein synthesis. Cell-free extracts for in vitro protein synthesis were obtained from spheroplasts pregrown at 23 C for 3 hr in YM-5 medium containing 0.4 M $MgSO_4$, and were then grown for 1 hr at either 23 or 36 C. A 500-ml amount of a spheroplast culture was collected by centrifugation and was lysed by homogenization in lysing buffer. The particulate matter was removed by centrifugation at 10,000 × *g* for 10 min; the supernatant fluid then constituted the S10 fraction. Ribosome pellets were prepared from the S10 supernatant fluid by centrifugation at 100,000 × *g* for 60 min. Ribosome pellets and S10 fractions were stored at −70 C until use.

Protein synthesis was monitored by the incorporation of ^{14}C-amino acids into material precipitable in 5% trichloroacetic acid. Each assay tube contained (in a total volume of 0.25 ml): KCl, 0.5 μmoles; magnesium acetate, 0.14 μmoles; tris-hydroxymethylaminomethane, 1.25 μmoles (pH 7.6); adenosine triphosphate, 2.25 μmoles; guanosine triphosphate, 0.0075 μmoles; β-mercaptoethanol, 0.3 μmoles; phosphoenolpyruvate kinase, 2 μg; phosphoenolpyruvate, 375 μg. Assays with endogenous mRNA also contained ^{14}C-reconstituted protein hydrolysate, 1 μc. Assays with added polyuridylic acid also contained 0.05 μmole of each of the amino acids except phenylalanine; ^{14}C-phenylalanine, 0.015 μmoles (50 μc/μmole); polyuridylic acid, 18 μg; and spermine, 40 μg. S10 extracts (0.80 to 0.88 mg of protein) or ribosomes (0.18 to 0.24 mg of protein) supplemented with S100 (0.18 mg of protein) previously obtained from A364A.

RESULTS

Genetic characterization. Mutant *ts*$^-$ 136 is one of many temperature-sensitive yeast mutants which have been isolated for their ability to grow normally at 23 C, but not at 36 C, even on highly enriched medium. As with most of these mutants, the mutation harbored by mutant *ts*$^-$ 136 is recessive (2).

Tetrad analysis was undertaken to determine whether this mutant contained more than one temperature-sensitive lesion and to determine whether the lesion was in a nuclear or cytoplasmic gene. A hybrid (H28) was constructed by mating mutant *ts*$^-$ 136 with strain 2262-2A (*ts*$^+$), and isolating the diploid by prototrophic selection. The diploid was sporulated; dissection of the resulting tetrads by micromanipulation was then undertaken. The dissections were impeded, however, because few aggregates containing four spores were present after the enzymatic digestion of the ascus wall, in spite of the fact that many four-spored asci were noted in the original preparation. Observation of asci during enzymatic digestion revealed that although spores are customarily very resistant to digestion by the enzyme preparation employed, some of the spores from this hybrid were digested. Since other hybrids prepared with strain 2262-2A have not exhibited this phenomenon, it is apparent that mutant *ts*$^-$ 136 carries an additional mutation which renders spores subject to enzymatic digestion. Random spore preparations of this hybrid produce approximately equal numbers of *ts*$^-$ and *ts*$^+$ haploids after enzymatic digestion, indicating that the lesion rendering the spores subject to digestion is not closely linked to the temperature-sensitive mutation. By carefully controlling the digestion conditions, it was possible to obtain 22 tetrads for dissection. Six of these tetrads segregated in a nonmendelian fashion for known nuclear genes; they were not included in the analysis. These cases were probably not true tetrads, but resulted from the fortuitous association of spores, a phenomenon which has been termed "false tetrads" (7); false tetrads would be enriched by conditions in which one or more spores from true tetrads were digested. The other 16 tetrads segregated $2^-:2^+$ spores for known nuclear genes and segregated $2ts^-:2ts^+$ spores. One of the segregating *ts*$^-$ haploids was back-crossed to 2262-2A, and the hybrid was sporulated. The resulting spores were not digested by enzyme treatment, and 21 complete tetrads were examined, all of which segregated $2ts^-:2ts^+$ spores. A centromere-linked marker, le_2, was included in these crosses

to determine whether the *ts*⁻ lesion was centromere-linked. The pattern of segregation for the gene pair *ts*⁻:le_2 was 4 parental ditype asci: 7 nonparental ditype asci: 26 tetratype asci, indicating that the *ts*⁻ lesion is not closely centromere-linked. These results indicate that the temperature-sensitive lesion is probably due to a single mutation in a nuclear gene.

Macromolecule synthesis. An examination of the incorporation of radioactive precursors into protein and RNA by cultures of mutant *ts*⁻ 136 revealed a very rapid cessation of RNA production at the restrictive temperature (Fig. 1); the rate of protein synthesis, however, was not immediately affected, but gradually decreased. A detailed investigation of the early kinetics of protein and RNA syntheses after the shift to 36 C is presented in Fig. 2A and 2B. The rate of RNA production begins to decrease within 5 min after the shift to 36 C, relative to a culture remaining at 23 C; the rate of protein synthesis is initially accelerated at 36 C and does not begin to decrease significantly until about 25 min after the shift. The kinetics of DNA synthesis at 23 and 36 C was examined in a separate experiment and was found to be similar to the kinetics of protein synthesis (Fig. 2C). The rate of DNA synthesis in the culture which was shifted to 36 C was at first faster than that in the culture remaining at 23 C and then began to decrease after approximately 25 min.

Previous results have demonstrated that the incorporation of ^{14}C-glucose into macromolecular material, most of which is polysaccharide, was even less inhibited in cultures of this mutant incubated at the restrictive temperature than was protein synthesis (3).

Thus, when a culture of mutant *ts*⁻ 136 is shifted to the restrictive temperature, RNA production is quickly and preferentially inhibited in comparison to the synthesis of the other macromolecular components of the cell, protein, DNA, and polysaccharide. Evidently, the function normally performed by the temperature-sensitive component in mutant *ts*⁻ 136 is required for RNA production.

Distribution of newly synthesized RNA between nucleus and cytoplasm. The fractionation of yeast cells into nuclear and cytoplasmic components, the isolation of polyribosomes, and the analysis of RNA metabolism are conveniently carried out in yeast spheroplasts growing in an osmotically stabilized medium (6). To examine the distribution of newly synthesized RNA between nucleus and cytoplasm, growing cultures of parent and mutant spheroplasts were

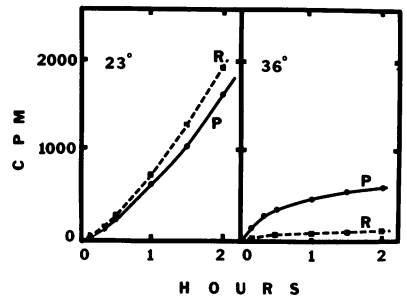

FIG. 1. *RNA and protein synthesis in cultures of mutant ts⁻ 136. One-half of a culture of mutant ts⁻ 136 growing at 23 C was shifted to 36 C and the other half remained at 23 C. ^{14}C-8- adenine (0.035 μc/ml) or ^{14}C-reconstituted protein hydrolysate (0.1 μc/ml) was added at the time of the shift, and 1-ml samples were removed and analyzed for the amount of radioactivity in RNA (R) or protein (P).*

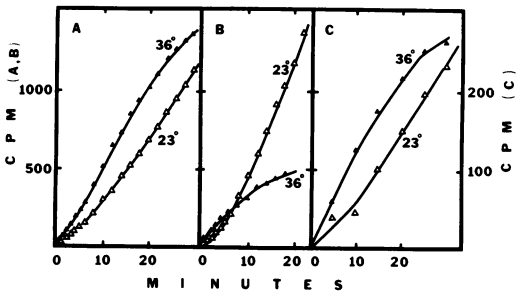

FIG. 2. *Early kinetics of protein, RNA, and DNA synthesis in mutant ts⁻ 136, after a shift from 23 to 36 C. One-half of a culture of mutant ts⁻ 136 growing at 23 C was shifted to 36 C and the other half remained at 23 C. Reconstituted ^{14}C-protein hydrolysate, 0.1 μc/ml, (A) or ^{14}C-8-adenine, 0.1 μc/ml, (B) was added at the time of the shift, and the radioactivity incorporated in 1-ml samples was determined. DNA synthesis (C) was examined by centrifuging a culture of ts⁻ 136 grown at 23 C and suspending at 10^7 cells/ml in YM-5 medium containing 0.5 μc of ^{14}C-adenine per ml without carrier adenine. After a 15-min incorporation period at 23 C to allow equilibration of nucleotide pools, one-half of the culture was shifted to 36 C and the other half was left at 23 C. Samples of a 1-ml amount were removed at various times and analyzed for radioactivity in DNA; the data are plotted as the increase in radioactivity after the time of the shift (time zero).*

shifted to 36 C, and ^{14}C-adenine was added. Samples were collected by centrifugation at various times, and were lysed to prepare crude nuclear and cytoplasmic fractions. In the parent culture, the majority of the labeled RNA was found in the nucleus at early times (less than 7 min), whereas later the majority was found in the

cytoplasm (Fig. 3A). Since most of the RNA is synthesized in the nucleus of eucaryotic cells and then transported to the cytoplasm, this was the expected result. Nuclear RNA labeling followed roughly the same time course in the mutant, and the amount of radioactivity was only 2.5-fold less than in parent nuclei (Fig. 3B). In the mutant, however, a much smaller proportion of the newly synthesized RNA appears in the cytoplasm; even after 30 min of incorporation, the amount of radioactivity in the mutant cytoplasm does not equal that in the nucleus.

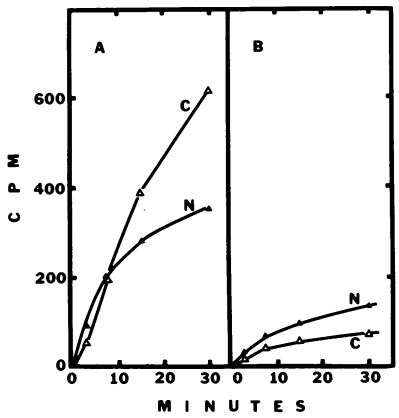

FIG. 3. *Distribution of newly synthesized RNA between nucleus and cytoplasm of A364A and mutant ts⁻ 136 at 36 C. Spheroplast cultures of A364A (A) and mutant ts⁻ 136 (B) growing at 23 C were shifted to 36 C and ^{14}C-8-adenine (0.2 μc/ml) was added. Samples (5 ml) of each culture were collected, lysed, and centrifuged to prepare crude nuclear (N) and cytoplasmic fractions (C). Counts/min in 0.5 ml of sample are recorded.*

Entrance of newly synthesized RNA into polyribosomes. The entrance of newly synthesized RNA into the cytoplasmic components was further examined by sucrose density-gradient centrifugation. Parent and mutant spheroplasts were labeled with ^{14}C-8-adenine for 15 min, after a shift to 36 C; as an additional control, mutant spheroplasts were labeled for 15 min at 23 C. Cytoplasmic extracts were prepared and displayed on sucrose density gradients (Fig. 4). Newly synthesized RNA appears in the polyribosomal region (fractions 11 to 24), in the monoribosomal region (8 to 10), and in three peaks which sediment more slowly than the monoribosome; by analogy with studies in mammalian cells and in bacteria, it is likely that the latter three peaks represent transfer RNA and the two ribosomal subunits. The radioactivity of the cytoplasmic components prepared from the mutant culture at 36 C is much less than that of the parent culture at 36 C, or the mutant culture at 23 C (Table 1). Whereas the spheroplast culture of the mutant incorporated 36% as much radioactivity into newly synthesized RNA during the 15-min period at 36 C as did the parent culture, the mutant polyribosomes incorporated only 12% as much radioactivity as did the poly-

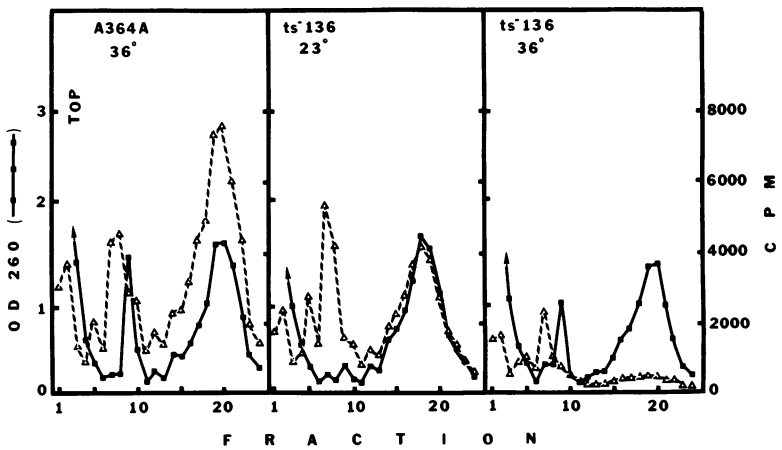

FIG. 4. *Entry of newly synthesized RNA into the polyribosomes of strain A364A and mutant ts⁻ 136. Spheroplast cultures of A364A and ts⁻ 136 growing at 23 C were centrifuged and suspended at 10⁸ cells/ml in medium containing 2 μg of adenine per ml. The spheroplasts were preincubated in this medium for 5 min at 23 C and shifted to 36 C (or left at 23 C); ^{14}C-8-adenine (1 μc/ml) was added at the time of the shift. Samples of 0.1-ml were removed and analyzed for total radioactivity incorporated into RNA. After 15 min of incubation, polyribosomes were prepared from 4 ml of each culture and centrifuged for 3 hr in the Spinco model SW25 ultracentrifuge at 24,000 rev/min. Fractions were collected and analyzed for absorbancy and radioactivity; the top of each gradient is at fraction 1.*

TABLE 1. *Radioactivity in RNA of sphereoplasts, cytoplasm, and polyribosomes after 15 min of ^{14}C-8-adenine incorporation*[a]

Strain and temp	Radioactivity[b]		
	Total in culture	Total in gradient (cytoplasm)	Polyribosomal region
A364A, 36 C	168,000	77,200	50,300
ts⁻ 136, 23 C	141,000	53,200	29,500
ts⁻ 136, 36 C	60,600	18,500	5,840

[a] Data have been normalized for small differences in total ribosomal OD_{260} recovered.
[b] Expressed as counts/min in 4 ml of culture.

ribosomes from the parent strain. Similarly, the spheroplast culture of the mutant incorporated 43% as much radioactivity into newly synthesized RNA at 36 as at 23 C, but only 20% as much into polyribosomes at 36 as at 23 C.

Decay of polyribosomes. The rapid inhibition of RNA production in cultures of mutant *ts*⁻ 136 and the preferential effect upon the entrance of newly synthesized RNA into polyribosomes suggest an explanation for the slow decay in the rate of protein synthesis which occurs in cultures of this mutant at 36 C (*see* Fig. 1). If the mRNA of yeast is metabolically unstable as it is in bacteria, then an inhibition of mRNA synthesis, or its transport into the cytoplasm, would result in a decay of cytoplasmic polyribosomes with a time constant which is characteristic of the instability of mRNA (11). Therefore, polyribosome preparations from spheroplasts of mutant *ts*⁻ 136 were examined at various times after a shift to 36 C for any change which might be correlated with the decreasing rate of protein synthesis. The percentage of ribosomes in polyribosome structures decreases progressively as a function of time after the shift to 36 C (Fig. 5). The results obtained in a detailed examination of the kinetics of polyribosome decay are presented in Fig. 6. Ribosomes present in polyribosome structures decrease from 90% at time zero to about 10% at 2 hr. The decay is approximately exponential, exhibiting a half-time of 23 min; the kinetics correlate well with the rate of decay of protein synthetic capacity (half-life about 26 min; Fig. 1).

Protein synthesis in vivo. If the decay of polyribosomes is due to a deficiency in mRNA production, then the decreasing rate of protein synthesis reflects a decreasing number of ribosomes attached to mRNA, rather than a decline in the synthetic activity per ribosome. The hypothesis of a deficiency in mRNA predicts that the accumulating monoribosomes will be inactive in polypeptide synthesis, but that the remaining polyribosomes will be as active as those at the permissive temperature. Spheroplast cultures of mutant *ts*⁻ 136 were preincubated for 30 min at 23 and 36 C, and then were pulse-labeled for 2 min with reconstituted ^{14}C-protein hydrolysate to

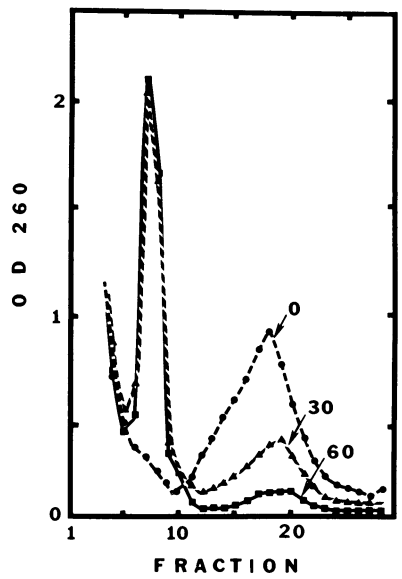

FIG. 5. *Decay of polyribosomes in mutant ts⁻ 136 at 36 C. Polyribosomes were prepared from 40 ml of a spheroplast culture of ts⁻ 136 and then centrifuged for 3 hr in the Spinco model SW25 ultracentrifuge at 24,000 rev/min. The spheroplasts were harvested at 0, 30, and 60 min after a shift from 23 C to 36 C.*

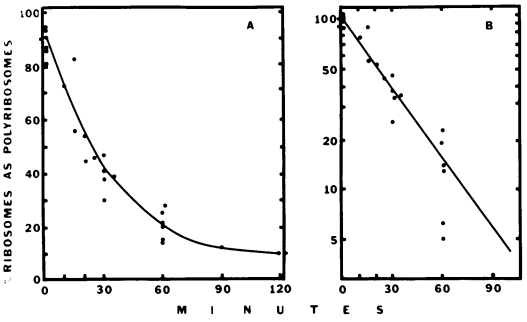

FIG. 6. *Kinetics of polyribosome decay in mutant ts⁻ 136 at 36 C. Polyribosomes were prepared and analyzed in several experiments. The percentage of ribosomes contained in polyribosomes from each gradient is plotted as a function of the time after the shift from 23 C to 36 C on a linear scale (A) and a logarithmic scale (B). To obtain the data for part B, the plateau value of 10% was subtracted from each point in part A, and the data were then normalized to 100% at zero time.*

label nascent polypeptide chains. Most of the ribosomes were present as polyribosomes in the culture incubated at 23 C, whereas approximately 50% had decayed to monoribosomes in the culture incubated at 36 C (Fig. 7); the specific activity of the nascent polypeptide chains located on polyribosomes was approximately the same in the two cultures, being 224 count/min per OD_{260} unit on the 23 C polyribosomes, and 238 counts/min per OD_{260} unit on the 36 C polyribosomes. The specific activity of the monoribosomes which had accumulated in the 36 C culture was only 37 counts/min per OD_{260} unit; this amount might be due to newly synthesized ribosomal proteins which appear on ribosomes very quickly in yeast (4). This result indicates either that most of the monoribosomes do not carry nascent polypeptide chains, or that the nascent chains on the monoribosomes are much shorter on the average than those on the polyribosomes. It seems unreasonable to think that the temperature shift would induce a progressive change in the size distribution of the polypeptide chains being synthesized; we conclude, therefore, that polypeptide synthesis occurs to a significant extent only on polyribosomes. The correlation between the rate of polyribosome decay and the rate of decay of protein synthesis is, therefore, explained by the finding that the accumulating monoribosomes are inactive in protein synthesis.

In vitro protein synthesis. The foregoing results argue strongly that the monoribosomes which accumulate in cultures of mutant *ts*⁻ 136 after an incubation at the restrictive temperature are deficient in mRNA. This hypothesis was confirmed by an experiment which tested the ability of ribosomes prepared from mutant and parent cultures to stimulate protein synthesis in vitro. Extracts were prepared from spheroplasts of the mutant preincubated at 23 and at 36 C for 1 hr, after which 90 and 15%, respectively, of the ribosomes would be present as polyribosomes. Extracts were also prepared from spheroplasts of the parent strain incubated under the same conditions, after which 90% and 70%, respectively, of the ribosomes would be present as polyribosomes based on previous results. Extracts of S10 were prepared by homogenizing the spheroplasts in lysing buffer and then centrifuging out the particulate debris at 10,000 × *g* for 10 min; ribosome pellets and S100 supernatant fluids were in turn prepared from S10 extracts by centrifugation at 100,000 × *g* for 120 min. The assay mixture supplied all components necessary for polypeptide synthesis except ribosomes, supernatant factors, and mRNA. Extracts of S10 or ribosomes prepared from parent spheroplasts preincubated at 36 C were approximately the same in activity (when using either endogenous mRNA or polyuridylic acid) as the comparable extract, or ribosomes prepared from parent spheroplasts preincubated at 23 C (Table 2). On the contrary, the S10 extract prepared from mutant spheroplasts preincubated at 36 C was only 10% as active when using endogenous mRNA as the S10 extract prepared from mutant spheroplasts preincubated at 23 C. The ribosomes isolated from mutant spheroplasts were only one-third as active when using endogeneous mRNA as those from mutant spheroplasts preincubated at 23 C. The fact that ribosomes did not show as great a

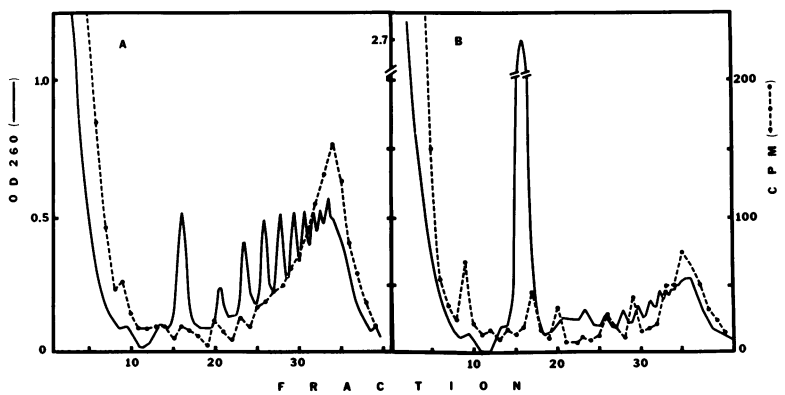

FIG. 7. *Pulse-labeling of nascent polypeptide chains on polyribosomes of mutant ts⁻ 136. A spheroplast culture of mutant ts⁻ 136 was divided in two parts, one of which was incubated at 23 C for 30 min (A); the other was incubated at 36 C for 30 min (B). The cultures were concentrated 10-fold by centrifugation, preincubated for 5 min at 23 and 36 C, respectively, and labeled for 2 min with reconstituted ^{14}C-protein hydrolysate (5 μc/ml), after which time the cultures were collected for polyribosome analysis.*

difference as did the S10 estract may be because the S100 supernatant liquid added to the mutant ribosomes perhaps contains some mRNA, since it was prepared from parent spheroplasts growing at 23 C. The addition of polyuridylic acid as an mRNA restored the activity of both the S10 extract and the ribosomes prepared from mutant spheroplasts preincubated at 36 C to levels equal to, or greater than, either the S10 extract or ribosomes prepared from spheroplasts preincubated at 23 C, demonstrating a deficiency in mRNA in the former perparations.

DISCUSSION

The rapid and preferential inhibition of net RNA accumulation exhibited by cultures of *ts*⁻ 136 after a shift to 36 C indicates that the synthesis of stable RNA species is dependent upon the gene product that is defective in this mutant. The kinetics of net RNA accumulation, however, provide no information about the metabolism of unstable RNA species, since the synthesis and degradation of these forms may be accomplished by turnover of the intracellular nucleotide pools without the uptake of radioactive precursors from the medium (10). Thus, the data presented here do not distinguish between a cessation of nuclear RNA synthesis on the one hand and a continuation of nuclear RNA synthesis balanced by RNA degradation on the other. It is clear that a much smaller proportion of the RNA synthesized at 36 C reaches the cytoplasm of the mutant than is the case in the parent strain. One plausible model to explain these results is that the primary lesion in mutant *ts*⁻ 136 is in the transport of RNA to the cytoplasm and, as a result of this defect, nuclear RNA synthesis ceases or is balanced by degradation. On the other hand, a model can be postulated in which a primary defect in nuclear RNA synthesis results in the cessation of transport. In fact, some experimental evidence for such a model is provided by the observation that actinomycin, a well characterized inhibitor of RNA synthesis, inhibits both synthesis of new RNA and transport of previously synthesized RNA in mammalian cells (1).

Regardless of whether the primary lesion is in the synthesis or in the transport of RNA, the response of the protein synthetic system to the cessation of cytoplasmic RNA production strongly indicates that the mutational defect extends to the production of unstable cytoplasmic mRNA. This response includes a slow decay in the rate of protein synthesis, which is paralleled by the decay of cytoplasmic polyribosomes to monoribosomes. The polyribosomes which remain after 30 min at 36 C are fully active in polypeptide synthesis in vivo, but the monoribosomes which accumulate are inactive. The monoribosomes can be restored to full activity in vitro by the addition of polyuridylic acid, an artificial mRNA. These results are completely analogous to those obtained after the addition of actinomycin, a well-characterized inhibitor of RNA synthesis, to cultures of *Bacillus subtilis* (8, 11), and indicate that the mRNA of a eucaryotic organism (yeast) is metabolically unstable, having a half-life of about 23 min at 36 C.

TABLE 2. *Protein synthesis in vitro with S10 and ribosome fractions prepared from parent and mutant spheroplasts after preincubation for 1 hr at 23 or 36 C*

Strain	Kind of extract	Growth temp	Protein synthesis[a]	
			Endogeneous mRNA	Plus polyuridylic acid
A364A	S10	23	896	356
	S10	36	355	412
	Ribosome	23	6,609	12,793
	Ribosome	36	6,133	13,653
ts⁻ 136	S10	23	487	321
	S10	36	53	511
	Ribosome	23	5,528	16,985
	Ribosome	36	1,986	21,171

[a] Measured as counts/min incorporated into polypeptide per milligram of S10 or ribosome protein present in the assay tube.

In experiments not reported here, we have been unable to demonstrate any striking defect in an aggregate RNA polymerase preparation from this mutant, or in its ability to carry out nucleotide metabolism at 36 C. A negative result in this case is inconclusive, however, since in vitro conditions undoubtedly do not accurately mimic in vivo conditions. Although we are continuing our efforts to locate the lesion in mutant *ts*⁻ 136, it should be pointed out that the properties of this mutant offer some novel approaches for the investigation of RNA metabolism, regardless of the site of defect. For example, the rapid inhibition of RNA transport imposed by this mutation allows one to follow the movement or the maturation of prelabeled cytoplasmic RNA components uncomplicated by the entrance of newly synthesized RNA into the cytoplasm.

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