Decay of Ribosomal Ribonucleic Acid in *Escherichia coli* Cells Starved for Various Nutrients*

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Decay of pre-existing ribonucleic acid was studied in *Escherichia coli* cells subjected to high temperature or to starvation for nitrogen, phosphate, amino acids, or a carbon source. In these studies, a series of mutants affected in ribonuclease I (RNase I, EC 3.1.4.22) polynucleotide phosphorylase (EC 2.7.7.8) or ribonuclease II (RNase II, EC 3.1.4.23) were used.

Degradation of total RNA and the disappearance of 23 S and 16 S rRNA were followed. The results obtained indicated that, by and large, decay of 23 S and 16 S RNA parallels that of total RNA. Decay of RNA depended on the nuclease content of the cells as well as on the treatment applied. It was most pronounced during carbon starvation and least in cells deprived of phosphate ions. It was most effective in strains containing all three nucleases and least in the strain defective in all three. The exo- and endonucleases RNase I and RNase II did not seem to affect the extent of 23 S and 16 S RNA disappearance. Strains with modified exonucleases did accumulate low molecular weight RNA species during treatments which induced considerable degradation of 23 S and 16 S RNA.

Based on the above data and previous observations, we suggest that during various starvations a similar mechanism is operative. The 23 S and 16 S RNAs are degraded endonucleolytically, and this is the rate-limiting step during starvation. The exo- and endonucleases RNase I and RNase II seem to participate primarily in the decay of the low molecular weight RNA species formed by the endonuclease(s), not as yet identified.

**EXPERIMENTAL PROCEDURES**

Materials—[5-3H]Uracil (18 Ci/mmol) was purchased from Schwarz/Mann. Carrier-free [32P]phosphoric acid in solution was obtained from Mallincrodt. Sucrose (special grade gradient) was obtained from Naftone, Inc. Lysozyme grade I was a Sigma product and deoxyribonuclease was the RNase-free preparation from Worthington Biochemical Corp.

Bacterial Strains—AB501: RNase I+, PNPase+, RNase II+; AB537: RNase I+, PNPase+, RNase II-; AB5: RNase I-; RP: RNase I-; PNPase+, RNase II+; AB100: RNase I-, PNPase-, RNase II+; PR13: RNase I-, PNPase-, RNase II+; N7080: RNase I-, PNPase-, RNase II- (for further details concerning these strains see Refs. 5-8).

**Growth and Starvation Media**—Casamino acid medium was a mineral medium (9) supplemented with 0.2% glucose, 0.2% casamino acids and 5 μg/ml of uracil. Low phosphate-casamino acid medium resembled the above medium except that it contained only 20 μg/ml of PO₄³⁻, was buffered with 0.06 M Tris (pH 7.4), and was supplemented also with 20 μg/ml of thiamine. Carbon starvation medium was the mineral medium supplemented with 50 μg/ml of uracil. For experiments on amino acid starvation, the mineral medium was supplemented with 0.2% glucose and 50 μg/ml of uracil. For experiments on nitrogen starvation, the medium was supplemented with 20 μg/ml of uracil. For experiments on

It is well known that bacteria such as *Escherichia coli* turn over ribosomes during famine (1-6) and this process is adaptive (6). Information regarding this process is rather scarce. We had earlier studied the process of ribosome decay in *E. coli* subjected to carbon starvation (5, 6) and suggested that endonucleolytic degradation of rRNA is the first step in the degradative process; after the endonucleolytic attack, which probably occurs in the ribosomal subunits (6), the particle falls apart and the RNA pieces generated by the endonuclease are degraded to acid-soluble material by the exo- and endonucleases RNase I and RNase II phosphorylase. Decay of total RNA depends on the nuclease content of the cell as well as on the treatment applied. All of the experiments are consistent with the concept that under all conditions of starvation...
nitrogen starvation, ammonium chloride was omitted from the mineral medium, and for experiments on phosphate starvation, 0.05 M Tris, pH 7.4, replaced the phosphate buffer. The three last mentioned media also were supplemented with 0.2% glucose and 50 μg/ml of uracil. Complete minimal medium was the mineral medium, with 0.2% glucose, 50 μg/ml of uracil, 20 μg/ml of L-methionine, 20 μg/ml of L-tyrosine, 20 μg/ml of L-threonine, 30 μg/ml of L-leucine, and 2 μg/ml of thiamine.

Buffers—Tris-magnesium buffer was 0.01 M Tris, pH 7.6, and 0.01 M magnesium acetate. Sucrose-Tris-magnesium buffer contained 30% sucrose, 0.01 M Tris (pH 7.75), and 0.01 M magnesium acetate.

Measurement of Total RNA Degradation—For details see Kaplan and Apirion (5).

Measurement of 23 S and 16 S RNA Degradation; Polyacrylamide Gel Electrophoresis—Cells were grown for three generations in low phosphate-casamino acids medium supplemented with 2 μCi/ml of [3H]thymidine. When the culture attained an absorbance (A660) of 0.5, the cells were collected by centrifugation and washed at room temperature with the appropriate starvation medium. Starved cultures were resuspended in prewarmed starvation medium and incubated for 1 to 2 hours at 50°C. At zero time and at the end of the starvation period, 0.5-ml samples were acidified and the total trichloroacetic acid-precipitable radioactivity was determined. The percentage of total degradation for each sample was calculated from the data.

Controls and starved cultures were washed at 4°C, once with Tris-magnesium buffer and once with sucrose-Tris-magnesium buffer, and the resulting pellets were frozen. RNA was prepared in a final volume of 1.0 ml of preparation buffer (i.e., sucrose-glycerol buffer). The resulting supernatant EDTA (1 mM) sucrose (100 mM), and 300 μg of bromphenol blue were added.

RNA was analyzed on 3.2% polyacrylamide sodium dodecyl sulfate gels. A slab gel system (100 x 145 x 1.5 mm) was used. Approximately 5000 cpm were applied in each gel. Electrophoresis was stopped when the bromphenol blue marker was 1 cm from the end of the gel. The resulting supernatant EDTA (1 mM) sucrose (100 mM), and 300 μg of bromphenol blue were added.

The total radioactivity (in counts per min) in each gel was taken as 100%. The percentage of counts in gel areas in which no radioactive compounds had been separated, were subtracted. The total radioactivity (in counts per min) in each gel was calculated and multiplied by the percentage of degradation of total RNA. Each determination is an average of at least four runs (two experiments each in duplicate).

RESULTS

Degradation of pre-existing RNA in bacterial mutants deprived of different nutrients was compared. The strains used in this study were AB301 (RNase I+, RNase II+), PR100 (RNase I-, PNPase+, RNase II+), PR13 (RNase I-, PNPasemod, RNase II+), and N7060 (RNase I+, PNPase+ mod RNase II mod). (Strains PR100 and PR13 are isogenic.) Previous studies on RNA degradation during carbon starvation (5, 6) have shown that rRNA degradation is qualitatively similar at 45°C and at 50°C. Due, however, to the preferential inactivation of polynucleotide phosphorylase and RNase II in the mutants studied here at 50°C (7, 8, 11, 12), the differences between the strains were better manifested at this elevated temperature. Therefore all of the studies described here were performed at 50°C.

In order to see what effect the high temperature itself has on the degradative process, we analyzed also the rate of RNA degradation at 50°C in cultures suspended in mineral medium supplemented with glucose, thiamine, and all of the necessary amino acids (L-methionine, L-tyrosine, L-threonine, and L-leucine).

Two techniques were used in the studies reported here: (a) total RNA degradation was measured by monitoring the [3H]uracil-containing acid-precipitable material in starved cells; (b) the disappearance of 23 S and 16 S RNA molecules was measured by quantitative analysis of total RNA on sodium dodecyl sulfate polyacrylamide gels.

Total RNA Degradation—In a typical experiment of this kind, cells grown to log phase were harvested at 4°C. The cells were centrifuged and resuspended in the appropriate starvation medium. Starved cultures were washed at 4°C, once with Tris-magnesium buffer, and finally resuspended in the appropriate starvation medium. At zero time and at different time intervals thereafter, aliquots were removed into trichloroacetic acid and the percentage of remaining acid-insoluble radioactivity was calculated.

The rate of RNA degradation in mutants incubated at 50°C in minimal medium is summarized in Fig. 1A. It can be seen that strain AB301, which contains RNase I, RNase II, and polynucleotide phosphorylase, degrades its RNA at the fastest rate. All the other strains which are lacking in RNase I degrade their RNA at a considerably slower rate.

The data obtained with these mutants during phosphate, nitrogen, carbon, or amino acid starvation are depicted in Fig. 1, B, C, D, and E, respectively. Under all these conditions RNA degradation is always most pronounced in the RNase I-containing strain AB301. The amount of RNA degraded by all the mutants depends on the treatment used. It is lowest during phosphate starvation and highest during carbon or nitrogen deprivation.

During phosphate or amino acid starvation, all the strains but AB301 (RNase I+) show similar levels of degradation of RNA, while during carbon starvation each of the four strains has a characteristic behavior: the less nucleic acid content, the lower the decay. This was comparable to the behavior of similar strains studied previously (5). During nitrogen starva-

![Fig. 1. Degradation of long term labeled RNA during different treatments. A, incubation at 50°C in complete minimal medium; B, phosphate starvation; C, nitrogen starvation; D, carbon starvation; E, amino acid starvation. Strains AB301 (●), PR100 (■), PR13 (▲), and N7060 (▼) were grown with [3H]uracil at 37°C and submitted to the different treatments at 50°C. The per cent of acid-precipitable [3H]-uracil after different starvation period was determined.](http://www.jbc.org/figure/3175/Fig.1.png)
tion, three patterns of RNA decay were observed. Strain AB301 was the most active, while the triple negative strain N7060 was the least active and the two other strains PR100 and PR13 showed intermediate activity (Fig. 1).

In conclusion, under all experimental conditions tested, RNA degradation is most pronounced in the strain which contains RNase I (AB301). Modified exoribonucleases, on the other hand, affect the capacity of the cell to degrade its pre-existing RNA only under conditions which favor extensive RNA degradation such as carbon source deprivation or nitrogen starvation (Fig. 1).

Decay of 23 S and 16 S rRNA—Three of the five treatments were chosen for further analysis. The treatments were: (a) carbon source deprivation, where degradation of RNA is most extensive and differences are easily observed among all strains; (b) phosphate starvation, where RNA degradation is at its lowest; and (c) heating at 50° in minimal medium, which is the common feature of all the different treatments.

For these experiments the four mutants were grown in low phosphate-casamino acid medium supplemented with [32P]orthophosphate. After at least three doublings, the cells were collected, washed, and starved. RNA was prepared from control cultures and from cells submitted to either 1 or 2 hours of the different starvations and was separated on 3.2% polyacrylamide slab gels. The gels were dried and autoradiographed as detailed under “Experimental Procedures.”

Fig. 2 depicts the electrophoretic separation of RNA prepared from strain PR100 submitted to carbon starvation (A), phosphate starvation (B), or incubation at 50° in minimal medium (C). The qualitative features of RNA degradation during carbon starvation are as follows: 23 S and 16 S RNA disappear, while a high molecular weight degradation product, d16S (6), and small RNA fragments near the bottom of the gel can be observed. No other degradation products appear. The data obtained with strain PR100 subjected to carbon deprivation resemble our previous results obtained with strain A19 (6). The RNA degradation products of the latter strain had been analyzed in more detail. A mass of 5 x 10^6 daltons had been assigned to the molecule which runs below 16 S RNA (d16 S RNA), whereas the small degradation products were found to have a mass of 2.6 to 1.7 x 10^4. The d16 S RNA molecule is most likely derived from 16 S rRNA (6).

 Autoradiograms of RNA prepared from cells deprived of phosphate and from cells treated at 50° in minimal medium are depicted in Fig. 2, B and C, respectively. The high molecular weight degradation product, d16 S RNA, appears as a shaded area in autoradiograms of RNA prepared from cells starved for phosphate (Fig. 2B) and is slightly more distinct in RNA prepared from cells kept at 50° in minimal medium (Fig. 2C). As during carbon starvation no other high molecular weight degradation products can be discerned. Low molecular weight degradation products accumulate only to a limited extent under those conditions (Fig. 2B and C).

Quantitative data on 23 S and 16 S RNA decay in all four strains subjected to carbon starvation are summarized in Fig. 3. It can be seen that the rate of disappearance of 23 S RNA is most pronounced in strain AB301 (Fig. 3A) and is identical, although less, in all the other strains which lack RNase I. The degradation of 16 S RNA is similar in all four strains tested (Fig. 3B). The four strains differ mainly in the accumulation of products smaller than 16 S RNA (Fig. 3C). No such products accumulate in strain AB301; they accumulate slightly in strain PR100, and to a much larger extent in strains PR13 and N7060.

The quantitative analysis of the disappearance of 23 S and 16 S RNA during phosphate starvation and treatment of cells at 50° is shown in Figs. 4 and 5, respectively. During phosphate deprivation, both 23 S RNA and 16 S RNA are degraded at an identical rate by the three strains which are lacking in RNase I, and at a slightly faster rate in the strain containing RNase I (AB301). No significant amounts of low molecular weight breakdown products accumulate in the four strains even after 2 hours of phosphate starvation (Fig. 4C).

In cells incubated at 50° the degradation of 23 S RNA and 16 S RNA proceeds at an intermediate rate, less than the degrada-
tion observed during carbon starvation and more than during phosphate starvation (compare Fig. 5 to Figs. 3 and 4). The degradation is most pronounced in the RNase I-containing strain and lower in the other strains. Low molecular weight degradation products do accumulate in cells heated at 50° (Fig. 5C) but only to a limited extent. Thus after 2 hours at 50° strain N7060 accumulates about 30% more of these products than strain AB301.

In our previous studies on carbon starvation (6) we found that it was difficult to extract polysomes from starved cells, and the amounts extracted especially after prolonged starvation periods varied from experiment to experiment. The same was true in the studies carried out here and therefore we did not compare the fate of the ribosomes themselves in the various starvation experiments employed here.

**DISCUSSION**

In previous studies on carbon starvation (5, 6), we suggested that the primary event in ribosome degradation is an endonucleolytic attack on rRNA molecules in the ribosome. The products of this process, small pieces of RNA, are degraded to nucleotides by the enzymes RNase II and PNPase. In the studies reported here, we tried to find out to what extent the process of rRNA degradation during different starvations is similar to the process during carbon starvation. (The studies on carbon starvation were verified with the isogenic strains used in this study.)

This comparative analysis of the process of rRNA degradation indicates that there are several features common to the different treatments: (a) rRNA degradation is always most pronounced in the strain that contains RNase I; (b) except for d16 S RNA (the quantities of which depend on the treatment applied), no high molecular weight RNA intermediates are detected during the different treatments; (c) the treatments differ mainly in their effect on 23 S and 16 S RNA degradation.

![Fig. 4. Disappearance of 23 S RNA and 16 S RNA during phosphate starvation. For details and symbols see Legend to Fig. 3. The left-hand scale applies to A, B, and C.](image)

![Fig. 5. Disappearance of 23 S RNA and 16 S RNA during incubation at 50° in complete minimal medium. For details and symbols see Legend to Fig. 3. The left-hand scale applies to A, B, and C.](image)

At lower temperatures (37°) the effect of RNase I on viability and on rRNA degradation is rather small (5, 6). This and the fact that RNase I is a periplasmic enzyme (13, 14) suggest that the strong effects of RNase I on the degradation of rRNA at 50° are most likely due to rupture of membranes, which enables the enzyme to enter the cell and degrade rRNA endonucleolytically. It seems likely therefore that we can learn more about the physiological aspects of the process by studying strains in which this enzyme has been removed by a mutation.

The experiments presented here suggest that rRNA degradation during the various conditions studied resembles rRNA degradation during carbon starvation. This suggestion is supported by the observation that under conditions where total RNA was slowly degraded, the degradation of 23 S and 16 S RNA was also slow but similar in the three strains lacking in RNase I. This could happen if the same enzyme(s) is responsible for rRNA disappearance under the various conditions studied. Since we do not find RNA molecules intermediate in size in any of these starvations* (Fig. 2) and since the only degradation products observed are small pieces of RNA, it seems most likely that the initial event is endonucleolytic in all cases.

We previously suggested that the exonucleolytic enzymes RNase II and polynucleotide phosphorylase serve as scavengers of the RNA pieces formed by the endonuclease(s). This can be seen very clearly in cells subjected to carbon starvation. During the 1st hour of carbon starvation at 50° up to 50% of the 23 S RNA and 80% of the 16 S RNA are degraded. Such a huge endonucleolytic degradation of high molecular weight RNA gives rise to large quantities of low molecular weight RNA species; theoretically a 2.2- to 2.4-fold increase in low molecular weight RNA can be expected in the 1st hour and a 2-fold increase was observed (see Fig. 3). Strain PR100, which contains normal exonuclease, is capable of degrading most of the low molecular weight RNA species to acid-soluble products, whereas in strains with modified exonucleases (PR13 and N7060), low molecular weight RNA species accumulate (see Fig. 3C).

Only 15% of the 23 S RNA and 10% of the 16 S RNA (Fig. 4) are degraded during the 1st hour of phosphate starvation. Therefore only a 1.3-fold increase in low molecular weight RNA species can be expected. Since no accumulation of small RNA species was observed during the 1st hour, we suggest that either the exonucleases retain some degradative capacity or that the endonuclease(s) or some other enzyme take part in the conversion of the RNA pieces to acid-soluble material.

The slightly faster degradation rate of rRNA observed in cells kept in minimal medium at 50° as compared to phosphate-starved cells (Fig. 5, A and B), causes a slight accumulation of breakdown products in the strains with modified exonucleases (Fig. 5C).

One of the interesting problems, which is still unresolved, is the event(s) which triggers the endonucleolytic attack. Why is there more endonucleolytic attack on rRNA during carbon starvation than during phosphate starvation or heating? One possibility is that the level of the endonuclease(s) is higher during carbon starvation. This possibility is unlikely since there is virtually no protein synthesis at 50°, and therefore it is most likely that RNA degradation is carried out by preexisting

*Unpublished observations.
enzymes (6). If this is the case, then the extent of the endonucleolytic attack could be determined by the access of the endonuclease(s) to the rRNA in the ribosome. Our previous studies (6) indicated that the endonucleolytic attack is most likely directed against rRNA in ribosomal subunits. The subunits being attacked could be in a specific molecular conformation (15) in which the RNA is sensitive to endonucleolytic attack. If this is the case, it is possible that under the various starvations where there is a low level of endonucleolytic attack, the level of ribosomal subunits or of subunits in the specific conformation is lower than the level during carbon starvation.

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