Ribosomal RNA on the Surface of Ribosomes

Abstract. *Ribonuclease from pancreas releases nucleotide from* Escherichia coli *ribosomes while not altering significantly the base composition of the total ribosomal RNA. Ribonuclease hydrolyzes ribsomal RNA without destroying the structure of 70S or 30S and 50S ribosomes. The RNA in 30S and 50S ribosomes appears more sensitive to the action of ribonuclease. The data suggest that RNA may be a surface component of the ribosome.*

Escherichia coli ribosomes are composed of approximately 60 percent RNA and 40 percent protein. The 30*S* ribosomes contain one molecule of 16*S* RNA and about ten molecules of protein while 50*S* ribosomes contain either one 23*S* or two 16*S* RNA molecules and 20 molecules of protein (*1*). Although it is possible to suggest a model of a ribosome in which the protein can enclose the RNA, there is no evidence to support this idea. The evidence presented in this report suggests that ribosomal RNA is a surface component accessible to the action of ribonuclease, and that it is not buried in a protein "coat" like the RNA of tobacco mosaic virus.

Cultures of *E. coli* K10 were grown on a medium containing glucose, phosphate, and salts (*2*), and harvested in the log phase. Ribosomes were isolated by the procedures of Tissières *et al.* (*3*) with either $10^{-2}M$ Mg^{++} or 10^{-4} Mg^{++} ions to obtain 70*S* or 30*S* plus 50*S* ribosomes.

The RNA from ribosomes was purified by the phenol method (*4*) without the addition of detergent. It was dissolved in a mixture of 0.1*M* NaCl and 0.01*M* acetate, *p*H 4.6 (*5*), dialyzed against 500 volumes of the same buffer for 1 hour, and diluted in the same buffer for sedimentation-coefficient determinations which were made in a model E Spinco ultracentrifuge with ultraviolet optics (*5*). The pictures obtained were analyzed with the Joyce-Loebl densitometer. Sedimentation coefficients were corrected for viscosity and density to water at 20°C. Base compositions of RNA were determined by paper chromatography of nucleotides after alkaline hydrolysis (*6*). The ribonuclease from pancreas (5× crystallized) (*7*), was dissolved in the 0.01*M* tris buffer, *p*H 7.8, containing 0.02*M* KCl, and $10^{-2}M$ or $10^{-4}M$ Mg acetate.

The experiments of Tissières *et al.* (*3*) and Gierer (*8*) demonstrated that the structural integrity of ribosomes is not destroyed by ribonuclease since ribosomes after ribonuclease treatment are still in the 70*S* form, in the case of *E. coli*, and in the 80*S* form in the case of rabbit reticulocyte ribosomes. My experiments (*9*) on the effect of rabbit serum on ribosomes suggested that the ribonuclease might not be without some effect on ribosomal RNA; normal serum as well as immune serum (prepared against whole ribosomes) hydrolyzed the RNA contained in ribosomes without altering significantly the sedimentation properties of the ribosomes. On this basis, the effect of ribonuclease on *E. coli* ribosomes was examined. Material showing ultraviolet-absorption at 260 mμ, and which is acid soluble, is liberated almost completely after only 30-minutes incubation at room temperature. At the end of various time periods the base composition of the ribonuclease-treated ribosomes was determined (Table 1). It appears that the base composition is changed slightly (if at all) by ribonuclease treatment. Since the sedimentation properties of ribosomes is not altered by ribonuclease (*1*, *6*) it seems likely that the material showing ultraviolet absorption comes primarily from the hydrolysis of messenger RNA molecules and transfer RNA molecules associated with ribosomes (Fig. 1).

Ribosomal RNA from ribosomes which had been treated with ribonuclease was purified and its sedimentation properties were examined; the results are presented in Fig. 2.

The RNA derived from ribonuclease-treated ribosomes is not a two-component system made up of 16*S* and 23*S* RNA, but it is a heterogeneous collection of molecules. It appears that ribonuclease has attacked the ribosomal-contained RNA without releasing it from the ribosome.

These experiments were carried out at $10^{-2}M$ Mg^{++} ion concentration which maintains ribosomes in the 70*S* and 100*S* form. This configuration might interfere with more extensive hydrol-

Table 1. Base composition of ribonuclease-treated ribosomes. Ribosomes (equivalent to 1.0 mg of RNA) were incubated with 10 μg/ml of ribonuclease. At the end of each time period ribosomes were precipitated with 1*M* HCl, resuspended in 0.3*M* NaOH, and incubated at 37°C. After 24 hours, protein was precipitated by the addition of a few drops of 1*N* HCl and the supernatant was used for the separation and quantitative determination of nucleotides by paper chromatography.

Incubation time (min)	Nucleotide			
	A*	C	U	G
	(moles per 100 moles of all nucleotides)			
0	26	25	20	29
10	28	26	16	30
30	26	25	17	32
60	28	23	18	31
1200 †	29	26	15	30

* A, adenylic acid, C, cytidylic acid; U, uridylic acid; G, guanylic acid. † Incubated at 4°C after 60 min at room temperature.

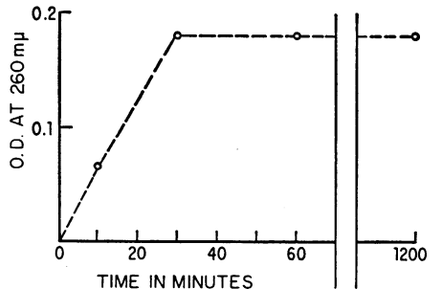

Fig. 1. Acid soluble, ultraviolet-absorbing material produced by ribonuclease treatment of *E. coli* ribosomes. A number of equal portions of washed ribosomes (equivalent to 1.0 mg to RNA) were suspended in 0.01*M* tris buffer, *p*H 7.8, and $10^{-2}M$ Mg acetate plus 10 μg of ribonuclease. The total reaction volume was 1 ml which was incubated at 25°C. At the end of each time period the reaction mixture was acidified with HCl and the resulting precipitate was collected. The ultraviolet absorption of supernatant was read at 260 mμ.

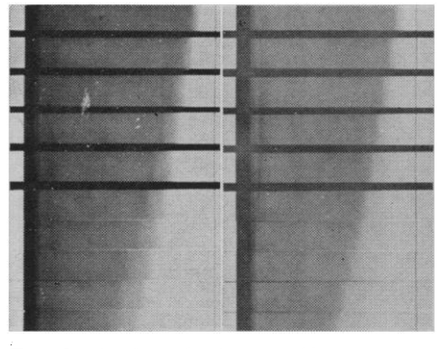

Fig. 2. Analytical ultracentrifuge pattern of RNA from ribonuclease treated and control ribosomes. Ribosomes were suspended in tris, KCl, Mg ($10^{-2}M$) buffer, *p*H 7.8, and treated with 5 μg/ml of ribonuclease for 40 min at 25°C. Control ribosomes were incubated for the same period of time and subsequently ribonuclease and phenol were added simultaneously. The rotor speed was 39,460 rev/min; the first picture was taken 9 min after rotor attained speed, at an interval of 4 min between pictures; the solvent was 0.1*M* NaCl plus 0.01*M* acetate, *p*H 4.6, and RNA concentration, 15 μg/ml. Left, two-component system of 23*S* and 16*S* RNA. Right, RNA from ribonuclease treated ribosomes.

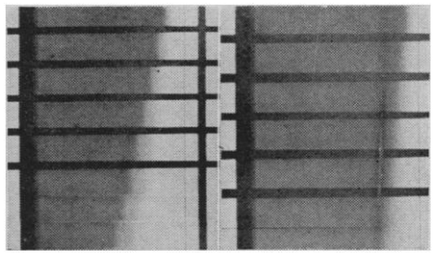

Fig. 3. Analytical ultracentrifuge pattern of RNA from ribonuclease treated and control 30*S* and 50*S* ribosomes. Ribosomes, suspended in tris, KCl, Mg (10^{-4}) buffer, *p*H 7.8, were treated with 5 μg of ribonuclease per milliliter. Control ribosomes treated as in Fig. 2. Rotor speed 39,460 rev/min; first picture taken 9 min after rotor attained speed; interval between pictures, 4 min; solvent 0.1*M* NaCl plus 0.01*M* acetate, *p*H 4.6, and RNA concentration 15 μg/ml. Left, two-component system of 23*S* and 16*S* RNA. Right, RNA from ribonuclease treated ribosomes.

ysis of the RNA by ribonuclease. Ribosomes in the 30*S* and 50*S* form were therefore incubated with ribonuclease for the same time period that 70S and 100S ribosomes were treated. The purified RNA was examined in the model E ultracentrifuge and the results of this experiment are shown in Fig. 3. It is obvious that even more extensive

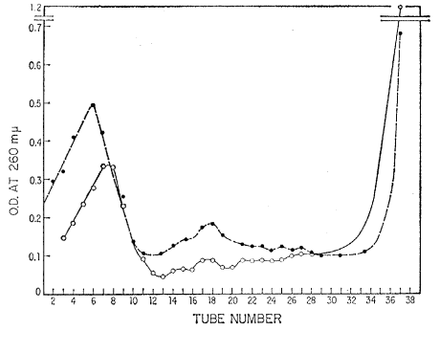

Fig. 4. Sucrose-gradient analysis of ribonuclease treated and control 30*S* and 50*S* ribosomes. Extract of *E. coli* was prepared in a 0.01*M* tris buffer at *p*H 7.8 containing 0.02*M* KCl 10^{-4}*M* Mg acetate and 1 μg deoxyribonuclease (*7*) per ml. Ribosomes were collected and resuspended in the same buffer and incubated at room temperature for 40 min with ribonuclease at a concentration of 5 μg/ml. At the end of the incubation period 0.1 ml portions were placed on top of a sucrose linear gradient (5- to 20-percent) prepared in the above buffer, and centrifuged in the SW39 rotor for 2.5 hours at 39,000 rev/min. Six drops per fraction were collected. To each fraction, 2 ml of water was added; the fractions were then read at 260 mμ. The bottom of the tube is at the left. Open circles, optical density (OD) values for ribonuclease treated 30*S* and 50*S* ribosomes. Solid circles, OD values for untreated ribosomes.

degradation of the ribosomal-contained RNA has taken place; no 23*S* or 16*S* RNA has remained. The 30*S* and 50*S* ribosomes which had been treated with ribonuclease were centrifuged on a 5 to 20 percent sucrose linear gradient in the SW39 swinging-bucket rotor to determine if extensive degradation of the ribosomes had taken place (Fig. 4). The ribonuclease-treated 50*S* and 30*S* ribosomes sediment in essentially the same position as control ribosomes, however, the total ultraviolet absorbing material is significantly reduced in the 50*S* and 30*S* components. Most of this ultraviolet-absorbing material appears where messenger RNA molecules would appear, toward the top of the sucrose gradient, and the rest is recovered at the top of the gradient probably as polynucleotides and mono- and dinucleotides of small molecular weight.

When ribonuclease is added to ribosomes under conditions of high magnesium ion concentration, the following sequence of events is suggested by the results of this study and the known action of ribonuclease. Ribonuclease attacks the 3′, 5′ phosphodiester linkages of those portions of ribosomal-contained RNA which are on the "surface" of the ribosome. At the moment, it is impossible to know how far the hydrolysis of the ribosomal RNA proceeds. It seems clear in any case that all 23*S* and 16*S* molecules are hydrolyzed to some extent. Under conditions where the 30*S* and 50*S* ribosomes are not associated, it appears that more RNA becomes accessible to ribonuclease attack. The point of attachment of 30*S* and 50*S* ribosomes may be a surface rich in RNA components or the structure of the ribosomes is altered by the lower magnesium concentration.

An analogous situation may be inferred from the experiments of Darnell reported by Jacob (*10*). Cells of *E. coli* which were heavily labeled with P^{32} and allowed to undergo extensive radioactive decay were still active in supporting phage growth, indicating that the structure of the ribosome, as a 70*S* monomer, was maintained in spite of breaks in the RNA chains. Phenol extraction of ribosomes which had undergone radioactive phosphorus decay did not yield 23*S* and 16*S* RNA.

The suggestion that RNA appears as a surface component in ribosomes raises the question of its functional significance in this position. It may be that the ribosomal RNA participates in the attachment of messenger RNA to the ribosome which would require having ribosomal RNA as a surface component. In addition, the association of 30*S* and 50*S* ribosomes may depend partially on their surface RNA components.

Although it appears from the data cited by Jacob that the primary structure of the RNA is not necessary for ribosome function, the integrity of the ribosome must nevertheless be preserved for protein synthesis. This may be due to the necessity of maintaining the appropriate surface configuration of those sections of ribosomal RNA which function in protein synthesis (*10*; *11*).

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Reductive Dechlorination of DDT to DDD by Yeast

Abstract. *Labeled DDD [1,1-dichloro-2,2-bis(p-chlorophenyl)-ethane] was formed from C^{14}-labeled DDT in the presence of yeast. The formation of DDD from DDE [1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene] was not observed, indicating that a reductive dechlorination of DDT occurs.*

Commercial yeast cakes (*1*) were used in an investigation of the action of yeast on DDT (Fig. 1) that was labeled with C^{14} in the phenyl group. In most experiments, 1 g of yeast cake was added to an autoclaved medium consisting of 1 liter of water, 90 g of sucrose, 1 g of K_2HPO_4, and 2 g of $(NH_4)_2SO_4$. The *p*H of this solution was 7.05. About 8 μg of DDT, with