THE EFFECT OF RIBONUCLEASE ON RIBOSOMAL RNA AND SUBSEQUENT POLYPEPTIDE SYNTHESIS

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Received September 13, 1973

SUMMARY: In order to ascertain the importance of intact ribosomal RNA to ribosomal function, mouse brain ribosomes were treated with pancreatic RNase for various lengths of time. After exposure to RNase the rRNA was analyzed by SDS-polyacrylamide electrophoresis and the ability of the ribosomes to incorporate phenylalanine was measured. Even though dilute concentrations of RNase extensively hydrolyzed the 28S and 18S RNA components, ribosomal protein synthesis was little affected. RNase treatment of intact ribosomes for 20 to 30 minutes left only 2 percent of the rRNA intact although 80 to 90 percent of the ribosomal protein synthetic activity remained.

RNA comprises more than 50 percent of the dry weight of ribonucleoprotein particles (1) and thereby plays an integral role in the structure of both prokaryotic and eukaryotic cell ribosomes. However, the functional role of the rRNA molecules, if any, in the various steps involved in polypeptide elongation is uncertain. Nomura (2) and others have clearly demonstrated that intact rRNA is necessary for the assembly and maturation of active ribosomes. Although protein-protein interactions appear to play an essential role in the maintenance of ribosomal stability (3,4), it is also possible that RNA-protein and RNA-RNA interactions are instrumental in preserving the integrity and function of ribosomal particles.

A considerable amount of information has been gained concerning the conformation of rRNA and its interaction with proteins within the ribonucleoprotein particles by techniques that involve their exposure to RNase (1,5,6,7). The results from these experiments revealed that intact rRNA was not mandatory for the maintenance of ribosomal structural integrity (1,5). However, less information is presently available regarding the possible requirement of

intact rRNA for ribosomal function. Several studies that have included RNase treatment of ribosomes in order to measure any functional roles of rRNA have led to conflicting conclusions (1,5,8,9). In the present study we have taken advantage of the extreme sensitivity of brain ribosomes (10) to dilute RNase to examine the effects of RNA hydrolysis on subsequent ribosome-directed polypeptide synthesis.

MATERIALS AND METHODS: Ribosomes were isolated from mouse brain tissue essentially as described by Lerner and Johnson (11). Enzymes for protein synthesis were obtained from brain post-microsomal supernatant fractions which were dialyzed against 10 mM Tris-HCl, pH 7.8; 10 mM $MgCl_2$; and 6 mM 2-mercaptoethanol overnight at 4°.

The sensitivity of rRNA in intact ribosomes to RNase was tested by incubation of solutions (100 ul) which contained 5 mM Tris-HCl, pH 7.4; 5 mM KCl; 0.75 mM $MgCl_2$; 1.0 A_{260} unit of ribosomes; and, where appropriate, 0.02 ug/ml of pancreatic RNase. The reactions were incubated at 22° and RNase action was terminated by the addition of 50 ul of 3X electrophoresis buffer to provide a final concentration of 40 mM Tris-HCl, pH 7.2; 20 mM sodium acetate; 1 mM EDTA; and 0.2% SDS. After the addition of crystalline sucrose to the samples, the extent of hydrolysis of rRNA was determined by SDS-polyacrylamide electrophoresis essentially as described by Bishop, Claybrook and Spiegelman (12). Electrophoresis with 3.1% polyacrylamide gels (0.4 x 6.5 cm) was carried out at room temperature for 90 min at 5 ma/gel. After electrophoresis the RNA was immediately analyzed spectrophotometrically at 260 nm with a Gilford, Model 2400, spectrophotometer adapted with a linear transport (10).

The ability of RNase-treated ribosomes to incorporate amino acids into polypeptides was tested in a reaction system which used poly U as a template RNA (13). After incubation the reaction products were collected on Millipore filters (0.45 um) and the amount of radioactivity was determined (13).

RNase-free sucrose was purchased from Schwarz/Mann. Pancreatic RNase A was obtained from Sigma Chemical Co. E. coli K12 tRNA was purchased from Gallard-Schlesinger Corp.

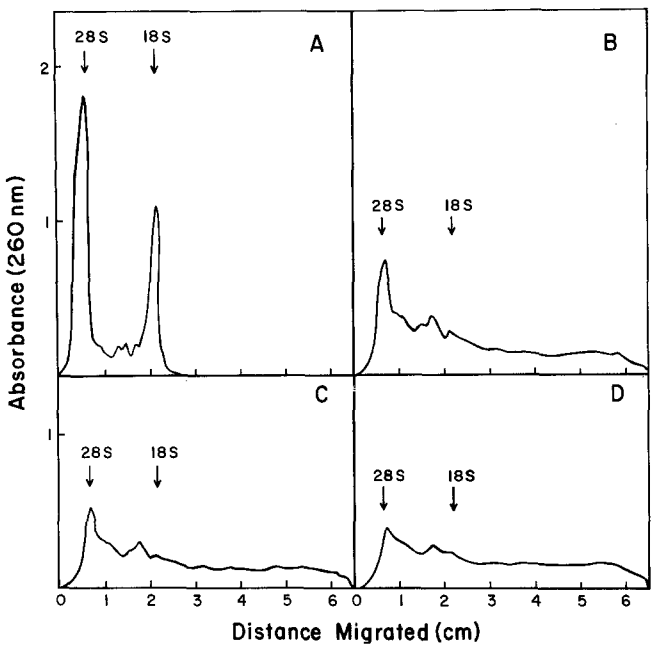

Fig. 1. Resolution of rRNA from native and RNase-treated ribosomes. Approximately 1.0 A_{260} unit of brain ribosomes was preincubated with RNase for 10 min (B), 20 min (C) or 30 min (D) and the RNA was resolved by electrophoresis as described in the Materials and Methods. The direction of migration was from left to right and the arrows indicate the normal migration of 28S and 18S RNA of native (non-treated) brain rRNA (A). The concentration of 28S RNA, exclusive of any hydrolyzed fragments, was calculated with the aid of a compensating polar planimeter.

RESULTS AND DISCUSSION: Several experiments were carried out with intact brain ribosomes to determine the sensitivity of the rRNA to hydrolysis by pancreatic RNase. The exposure of brain ribosomes to 0.02 ug/ml of RNase at 22° resulted in the rapid degradation of the rRNA molecules (Fig. 1). The resolution of rRNA by SDS-polyacrylamide electrophoresis, subsequent to preincubation at 22° in the presence and absence of RNase, allowed an estimation of the proportion of ribosomes that contained intact RNA molecules. Although the RNA of the small brain ribosomal subunit has been shown to be more sensitive to RNase hydrolysis than the RNA of the large ribosomal subunit (10), degradation products from the 28S RNA molecule obscured any measurements of intact 18S RNA molecules (Fig. 1). However, an accurate apprais-

al of the maximal number of ribosomes that contain rRNA can be obtained by the measurement of the area associated with the 28S RNA (9). The extreme sensitivity of brain ribosomes to the action of pancreatic RNase was clearly evident in that less than 2 percent of the 28S RNA was intact after 20 or 30 minutes of hydrolysis (Fig. 1). These calculations actually represent the maximal number of ribosomes with intact rRNA since the 18S has been shown previously to be considerably more sensitive to enzymatic degradation.

A series of experiments were conducted to assure us that the apparent hydrolysis of rRNA was the result of pancreatic RNase action. Prolonged incubation of ribosomes at 22°, without the addition of exogenous RNase, did not result in any detectable hydrolysis of rRNA. In addition, when SDS was added to ribosomes prior to the addition of pancreatic RNase, there was no evidence of rRNA hydrolysis. Therefore, the presence of 0.2 percent of SDS was sufficient to inhibit any detectable enzymatic action (6). Although brain rRNA was rapidly degraded by RNase, in a related study we found that RNase-treated ribosomes appeared to maintain their structural integrity since their sucrose gradient profiles were similar to those of control, non-enzyme treated ribosomes. This finding is consistent with the observations of Cox(5) who found that even higher concentrations of RNase did not appreciably alter the sedimentation properties of reticulocyte ribosomes.

In view of the differential effects of pancreatic RNase on the RNA and structural integrity of the brain ribosomes, it was of interest to test the effects of enzyme treatment on subsequent ribosomal polypeptide synthesis. Although pancreatic RNase rapidly degraded the RNA of ribosomes (Fig. 1), pretreatment under these conditions had little effect on their ability to participate in protein synthesis. Exposure of ribosomes to RNase for 10, 20 and 30 minutes resulted in a loss of 0, 14 and 17 percent of the control activity, respectively (Table I). In order to minimize ribosomal incativation which occurs rapidly at 37° (Grove, Johnson and Gilbert, submitted for publication), all preincubation procedures were carried out at 22°. Even incubation at 22°

Table I

Effect of RNase treatment on ribosomal protein synthesis.

Time of Preincubation (min)	RNase	Phenylalanine Incorporation (pmoles/A_{260})	RNase-treated/ Control
0	-(native)	4.91	-
10	-(control)	4.18	
	+	4.23	1.01
20	-(control)	4.25	
	+	3.64	0.86
30	-(control)	3.77	
	+	3.14	0.83

Brain ribosomes were preincubated at 22° for the times indicated in the presence and absence of pancreatic RNase as described in the Materials and Methods. Ribosomes not preincubated, but maintained at 0°, were considered native. Protein synthesis was carried out in reaction mixtures (0.2 ml) that contained 10 mM Tris-HCl, pH 7.4; 50 mM KCl; 10mM $MgCl_2$; 6 mM 2-mercaptoethanol; 0.8 mM GTP; 10 mM phosphoenolpyruvate; 8 ug of pyruvate kinase; 40 ug poly U; 0.26 mg of post-microsomal enzyme protein; 0.44 A_{260} unit of ribosomes and 6 pmoles (4,200 DPM) of ^{14}C-phenylalanyl-tRNA (E. coli K12). Reactions were incubated at 37° for 10 min and the amount of incorporation was determined as described in the Materials and Methods.

resulted in a loss of approximately 25 percent of the original ribosomal activity within 30 minutes (Table I). In order to be assured that any changes in ribosomal activity were not the result of thermal denaturation, controls without the addition of RNase were included for each time interval. However, the loss in protein synthesis, which could be attributed to the effects of RNase action, may actually be the result of an increased thermal lability of the enzyme treated ribosomes (4). We also considered the possibility that template RNA or tRNA in the protein synthesis reaction mixture might be degraded by the introduction of RNase (0.8 ng) with the addition of RNase-treated ribosomes. However, the addition of this amount of RNase directly to the reaction mixtures was found to have no effect of measured phenylalanine incorporation.

These results suggest that intact rRNA is not a requisite for ribosomal participation in protein synthesis and that extensive hydrolysis of the molecules can occur without inhibiting ribosomal activity. It is possible that once intact rRNA has been utilized as the backbone in ribosomal assembly and maturation, the RNA-protein and/or protein-protein interactions are adequate to maintain the ribosomes in the proper configuration for protein synthesis even though the rRNA is extensively cleaved. These results are in contrast to several previous studies where ribosomal activity was reported to be sensitive to RNase (1,5,8). However, the discrepancies may be explained by several reasons that are not directly related to rRNA and ribosome function. For instance, some investigators have examined the protein synthetic activity of RNase-treated polyribosomes. In these cases, the loss in protein synthesis may have been wholly due to the hydrolysis of the endogenous mRNA. Another variable may have been introduced by sedimentation of RNase-treated ribosomes, during procedures to free them of added RNase, which may have damaged to ribonucleoprotein particles and decreased their activity. Some investigators have failed to account for the loss in ribosomal activity due to thermal inactivation. Our results are consistent with those of Szer (9) and Kuechler et al. (14) who found that bacterial ribosomes, with extensively cleaved RNA, appeared to retain a major proportion of their original activity. It should also be noted that the limitations of these techniques did not allow a consideration of the possible functional role of 5S RNA which has been shown to be an integral component of the large ribosomal subunit (1,15).

ACKNOWLEDGEMENT: This investigation was supported by Public Health Service research grant NS 06853 from the National Institute of Neurological Diseases and Stroke and 130-13-RD from the Illinois Department of Mental Health. BKG is a predoctoral trainee (5 T01 GM-00724) from NIGMS.

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