INCORPORATING ACTIVITY OF RIBOSOMES AND INTEGRITY OF RIBOSOMAL RNA

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Received October 15, 1970

Summary

The interrelationship between the amino acid incorporating ability and the state of ribosomal-RNA in ribonuclease treated rat liver ribosomes was studied. It was shown that ribosomes containing no or only negligible amounts of 28 S ribosomal-RNA retained at least 30 per cent of their original incorporating activity /measured without addition of any exogenous messenger-RNA/. On the basis of these results we conclude that the integrity of the 28 S ribosomal-RNA molecule is not required for ribosomal function.

The role of ribosomal-RNA in maintaining the ribosomal structure and function has been extensively studied but not yet elucidated. In the present paper changes in amino acid incorporating ability and in the state of ribosomal-RNA were followed after treating rat liver ribosomes with different amounts of pancreatic-RNase.

Methods

Preparation of ribosomes, measurement of incorporating activity and the sucrose gradient analysis of ribosomal-RNA were described earlier /1,2/.

The RNase treatment of ribosomes and the removal of RNase were performed essentially as described by Brentani et al. /3/ in the following way: ribosomes were treated with various amounts of pancreatic RNase /from Reanal, Budapest/ at 38° C for 20 minutes in a reaction mixture containing 0.05 M

Tris-HCl buffer pH 7.6; 0.025 M KCl; 0.005 M Magnesium acetate; 8.5 per cent sucrose /solution A/. After incubation the ribosomes were sedimented at 50.000 rpm in the Spinco rotor No. 50 at 0° C for 60 minutes, resuspended in solution A and sedimented repeatedly as described above.

Results and Discussion

In order to compare the state of ribosomal-RNA and the amino acid incorporating ability of RNase treated ribosomes, the ribosomes incubated with RNase and sedimented as described in Methods were divided into two parts, one of which was used for measurement of incorporating ability, and the other portion for analysis of ribosomal-RNA by sucrose density gradient centrifugation.

Since the ribosomes purified by sedimentation may be contaminated with traces of RNase which may cause an extensive degradation of ribosomal-RNA during the time of preparing the samples for RNA analysis /2/ a very potent RNase inhibitor - diethyl-pyrocarbonate was added to the samples used for RNA analysis at the same time when the radioactive amino acid was added to the amino acid incorporating system containing the other aliquots. In this way the RNA sedimentation profile corresponds to the state of RNA at the starting time of the incorporation, i.e. the RNA in the aliquots taken for ribosomal-RNA analysis is equally or less degraded than in other aliquots taken for examining incorporating ability.

Fig.1. shows the incorporating ability of RNase treated ribosomes. It can be seen that the time curves of incorporation of RNase treated samples decline earlier than that of the control one. This fact suggests that some traces of RNase may remain associated with ribosomes in spite of the repeated sedimentation.

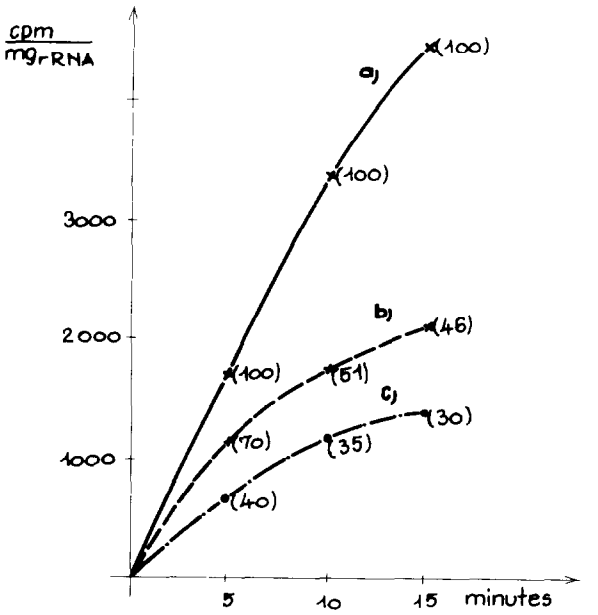

Fig.1. - Incorporating activity of RNase treated ribosomes. Ribosomes were treated with a/ 0.0; b/ 0.13; c/ 1.3 ug RNase/mg RNA for 20 minutes at 38° C, sedimented and then incorporating ability was tested. The values are corrected for samples incubated in energy free system. The numbers in parantheses indicate incorporating ability expressed in percentage of control samples.

Comparing the cpm amount of incorporated radioactive amino acid during the first ten minutes of incubation of the incorporating system, 51 and 35 per cent respectively, of the original incorporating ability were retained after treatment with 0.13 and 1.3 ugRNase mg RNA respectively. These values are certainly lower than the initial rates of incorporation which could be directly correlated to the RNA sedimentation profiles.

Fig.2. shows the RNA profile of the same ribosomes, the incorporating ability of which was shown in Fig.1. As the peaks are shifted after RNase treatment to lower values, and as we cannot decide whether these shifted peaks are homogeneous or heterogeneous we cannot calculate the exact amount of 28 S RNA in RNase treated samples. We can, however, obtain a maximal value

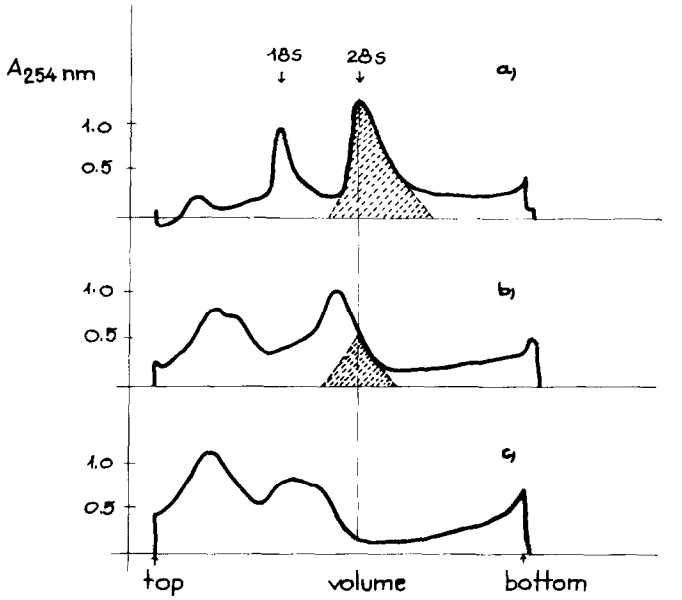

Fig.2. - RNA sedimentation profile of RNase treated ribosomes. Aliquots of ribosomes, the incorporation ability of which is shown in Fig.1. were treated with DEP before the liberation of ribosomal-RNA. Sedimentation was performed in linear sucrose density gradient /5 to 20 per cent w/w/ for 5.5 hours at 41.000 rpm in the Spinco rotor No. SW41. The gradients were analysed by an ISCO density gradient analysator type UA-2.

of the amount of the 28 S RNA present in RNase treated samples if we suppose that the position of the 28 S peak is the same in the RNase treated samples as in control ones, and that the peak is symmetrical. On the basis of these assumptions we have drawn the dotted area /Fig.2/b/ which surely overestimates the real value of intact 28 S RNA and thus gives a maximal value of the disintegrated 28 S ribosomal RNA.

On the basis of the above approximative calculation 28 per cent of 28 S RNA was found after treatment with 0.13 ug RNase/mg RNA, whereas 28 S RNA was undetectable by sucrose density gradient analysis when ten times as much RNase was added /1.3 ug RNase/mg RNA/.

This means that after RNase treatment the decrease in

amino acid incorporation is significantly lower than the decrease in the amount of intact 28 S RNA.

The following facts should be taken into consideration in connection with this statement: 1/ As mentioned above, our calculations give the maximal amount of intact 28 S ribosomal-RNA. 2/ The degradation of 18 S ribosomal-RNA and of messenger-RNA was not followed in these experiments but it is very probable that these ribonucleic acids are also degraded. Their degradation may also contribute to the lowering of incorporating ability.

As it was pointed out, our calculations overestimate the real amount of intact 28 S RNA and underestimate the real rate of incorporation after RNase treatment, but even these estimations show a significantly greater damage in 28 S ribosomal-RNA than in incorporating ability following RNase treatment.

The most likely interpretation of the above results is that the integrity of the 28 S ribosomal-RNA is not required for the function of ribosomes. Similar results were also found by Cahn et al. /4/ for E. coli 50 S native subunits. These results would suggest that the structure of ribosomes may be unchanged in spite of a break or breaks in the RNA, which is supported by our preliminary observation for rat liver ribosomes, and by Cox for rabbit reticulocyte ribosomes /5/.

One objection against the above interpretation might be that only a small, RNase resistant fraction of the original ribosome population was active in vitro, and in this case the RNase resistant ribosomes would be responsible for the incorporating activity. This objection seems to be very unlikely, since the original ribosome preparations generally contain at least 60 to 70 per cent of polysomes, i.e. the active form of ribosomes.

Our previous results /1/ have shown that after RNase treatment and precipitation of ribosomes by increasing the concentration of magnesium ions, the decrease in the incorporating activity was always somewhat greater than that in the amount of intact 28 S ribosomal-RNA. In the RNase treated samples, however, the RNA to protein ratio decreased after precipitation. As the excess proteins attached to ribosomes may interfere with the incorporating activity /3,6/ the lower incorporating ability of RNase treated ribosomes in our earlier work may partly have been due to this phenomenon. The ribosome sedimentation method used in the present work /3/ yielded ribosomes with normal RNA to protein ratio, thus avoiding the above complication.

On the basis of the presented results it can be concluded that RNA - protein and protein - protein interactions may play an essential role in maintaining the structural elements which are important for ribosomal function in the large subunit and probably in the small subunit of rat liver ribosomes. This conclusion is in agreement with those drawn from experiments on E. coli and reticulocyte ribosomes /4,5/ using different experimental techniques.

Acknowledgments

The interest in this work, the stimulating and valuable advices of Prof. F.B. Straub, as well as the helpful discussions of Prof. F. Antoni are gratefully acknowledged. The authors are also indebted to Mr. K. Kovács for his skilful technical assistance.

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Abbreviations used: RNase, pancreatic RNase /EC 2.7.7.16/
SDS, sodium dodecylsulfate