THE RELEASE OF 5S RNA FROM RETICULOCYTE RIBOSOMES

T. Zehavi-Willner

Section of Biological Ultrastructure

The Weizmann Institute of Science, Rehovot, Israel

Received February 26, 1970

Summary: Reticulocyte ribosomes were dissociated under various conditions and the release of 5S RNA was assayed. The 5S RNA was released from ribosomes suspended in Tris buffer, pH 9.5, containing EDTA or in Tris buffer, pH 9.5, after dialysis against the same buffer. Only negligible amounts of protein were solubilized during the removal of the 5S RNA. The particles devoid of 5S RNA had a slightly lower sedimentation coefficient than particles retaining 5S RNA formed through treatment of ribosomes with Tris buffer, pH 9.5, or Tris buffer, pH 7.4, with EDTA or high salt concentrations. The 5S RNA-free particles, when dialyzed against high Mg Tris buffer, pH 7.4, refolded to a more compact form with a sedimentation coefficient close to the 60S subunit. During refolding of the latter particle there was no specific reattachment of 5S RNA.

In recent years 5S RNA was detected on the ribosomes of many organisms including bacteria, Fungi, echinoderms and vertebrate (1,2,3,4). The widespread existence of this low molecular weight RNA on the larger subunit of the ribosomes suggests that it is a universal constituent of living cells. In many aspects regarding chemical structure (Molecular weight, minor bases) or biological properties the 5S RNA of different origin seems to be alike (5,6,7,8,9). The differences found between 5S RNA of bacterial and mammalian origin were in the base sequence of those molecules (8,10,11), and in the inability to release 5S RNA from mammalian ribosomes by techniques appropriate for ribosomes of bacterial or fungal origin (7).

In the present work conditions are described for the release of 5S RNA from reticulocyte ribosomes. The conditions suitable to release 5S RNA from the ribosomal particles cause structural changes in the subunits. Ribosomal particles free of 5S RNA when dialyzed against high Mg Tris buffer, pH 7.4

refold into a more compact form with a sedimentation coefficient close but not identical to the 60S subunit. This transformation occurs without a specific reattachment of 5S RNA to the refolded particle.

EXPERIMENTAL

Conditions for the release of 5S RNA from reticulocyte ribosomes: In order to establish optimal conditions for release of 5S RNA from reticulocyte ribosomes, various methods were employed, described for releasing 5S RNA from microorganisms (12,13,14) as well as conditions for dissociation of reticulocyte ribosomes into subparticles (15,16,17).

^{32}P-labelled reticulocytes were prepared as described before (18). The reticulocytes were lysed as described by Warner et al. (19) and ribosomes prepared. The ribosomes were washed twice with low Mg-Tris buffer (0.05M KCl, 0.01M Tris-HCl, pH 7.4, 0.03mM $MgCl_2$) and resuspended in the same buffer to a final concentration of 150-200 A_{260} units per ml. The treatments were performed by suspending the washed ribosomes in different salt solutions in a final concentration of 10 A_{260} units per ml unless otherwise stated. After one hour at 0°C, the ribosomes were centrifuged at 50,000 rpm for 5 hours. The supernatant was removed and used for estimation of released 5S RNA, t-RNA and protein. The amounts of ^{32}P-labelled 5S RNA and t-RNA were determined by means of G-100 Sephadex chromatography (5). The protein content was determined by the method of Lowry et al. (20).

The data presented in Table 1 demonstrate that all the treatments tested in this study for their ability to release 5S RNA from the reticulocyte ribosomes primarily dissociated them into two subunits. During the different dissociation processes most of the bound t-RNA as well as small amounts of proteins were released. This was not the case with 5S RNA. EDTA treatment of reticulocyte ribosomes at neutral pH as well as suspension of the ribosomes in 0.1M NaCl appeared to be ineffective in causing the release of 5S RNA. Treatment of reticulocyte ribosomes with high salt concentration

TABLE 1

Soluble material released from ribosomes by different treatments

Buffer used for dissociation	Sedimentation coefficient of main subunits- S_{20}	% of component solubilized during treatment		
		Protein	t-RNA	5S RNA
0.1M NaCl 0.01M Tris, pH 7.4 - 0.03mM $MgCl_2$	*40, 60		70	-
0.05M Tris, pH 7.4 - 0.01M EDTA - 0.05M KCl	30, 52	14	90	5
0.5M KCl	30, 47	12	85	20
0.05M Tris, pH 9.5 - 0.05M KCl	32, 50	8	75	25
0.05M Tris, pH 9.5 - 0.05M KCl followed by dialysis against the same buffer	24, 42	9	100	85
0.05M Tris, pH 9.5 - 0.05M KCl-0.001M EDTA	26, 44	9.5	100	80

Samples of 20 A_{260} units of ^{32}P-labelled ribosomes (22,000cpm/A_{260} unit ribosomes) were treated with different buffers as indicated. After treatment, the ribosomes were removed by centrifugation and the solubilized material was estimated in the supernatant as described in Experimental. For determination of the sedimentation coefficient, unlabelled ribosomes were treated with the different buffers for one hour in the cold. They were then analyzed at 4°C using a model E ultracentrifuge and their S values calculated.

solutions, which are known on one hand to release 5S RNA of bacterial origin (13,14) and on the other hand to dissociate reticulocyte ribosomes into subunits (17) released only small amounts of 5S RNA. The same was also true for the treatment of ribosomes with Tris buffer pH 9.5. However, combinations of this basic buffer with 0.001M EDTA resulted in the release of 60-80% of 5S RNA from the reticulocyte ribosomes. Treatment of the ribosomes with Tris

buffer, pH 9.5, followed by dialysis for 24 hr at 0°C vs 2 liters of the same buffer released also about 80% of the 5S RNA.

In order to establish the changes taking place in subunits obtained by treatment of ribosomes with Tris buffer, pH 9.5 as compared to subunits free of 5S RNA obtained by using a buffer which included 0.001M EDTA, the differently treated ribosomes were analyzed by their centrifugation on the same sucrose gradient with one of the samples labelled. Figure 1 shows that there

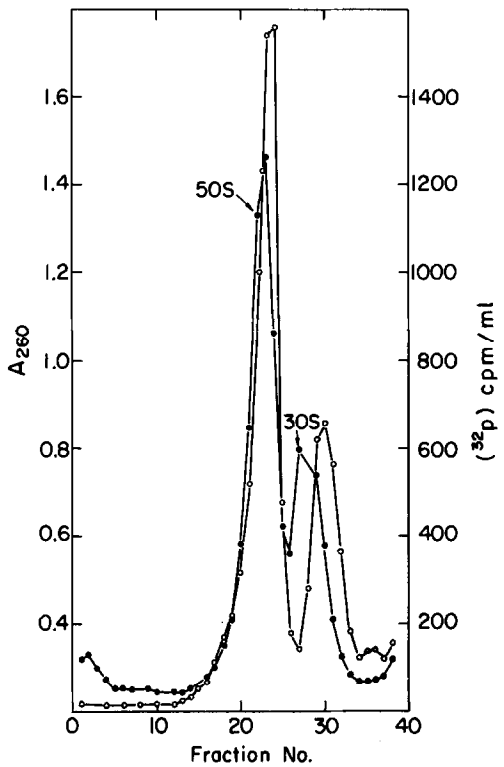

Fig. 1.

Comparison of reticulocyte ribosomal 44S and 26S particles (devoid of 5S RNA) to 50S and 30S particles.

^{32}P- ribosomes (16,400cpm/A_{260} units) were treated with Tris buffer pH 9.5 - EDTA, whereas unlabelled ribosomes were treated with Tris buffer, pH 9.5 only. 0.9 A_{260} units of the labelled ribosomes were mixed with 8 A_{260} units of the unlabelled ribosomes and were layered on a 5-20% sucrose gradient supplemented with Tris buffer pH 9.5. The samples were centrifuged in the Spinco SW 25 Rotor for 5 hr at 25,000 rpm and fractions of 0.8ml were collected and their absorption at 260mμ and radioactivity measured.

o——o unlabelled ribosomes
o——o ^{32}P-ribosomes

are slight differences in sedimentation coefficient between the large subunit deprived of 5S RNA and that still having 5S RNA attached to it. The difference between the two small subparticles is even more prominent.

Structural changes in 5S RNA free subparticles upon reversal to primary conditions: The ^{32}P-labelled subunits obtained on treatment of ribosomes with Tris buffer, pH 9.5 - EDTA were dialyzed for 24 hours against high Mg-Tris buffer (0.05M KCl, 0.01M Tris-HCl, pH 7.4 - 1.5mM $MgCl_2$) at 0°C. They were mixed with unlabelled 60 and 40S reticulocyte subunits and layered on a 5-20%

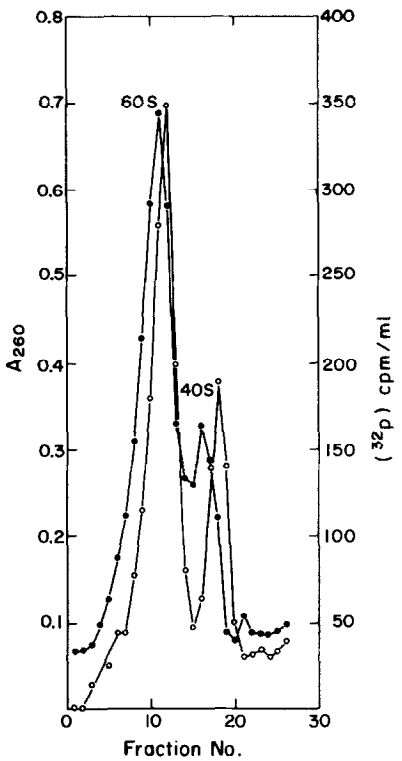

Fig.2.

Comparison of reticulocyte particles devoid of 5S RNA dialyzed against high Mg Tris buffer, pH 7.4 to 60S and 40S ribosomal subunits

^{32}P reticulocyte ribosomes were treated with Tris buffer, pH 9.5-EDTA followed by dialysis against high Mg-Tris buffer, pH 7.4. 0.6 A_{260} units (3,500cpm/A_{260} units) of ribosomes were mixed with 5.2 A_{260} units of unlabelled ribosomes suspended in 0.01M Tris buffer, pH 7.4 - 0.1M NaCl - 0.03mM $MgCl_2$. The mixed ribosomes were layered on a 5-20% sucrose gradient containing the last mentioned buffer. One ml fractions were collected.

o——o unlabelled ribosomes

o——o ^{32}P ribosomes

sucrose gradient containing 0.1M NaCl, 0.01M Tris-HCl pH 7.4, 0.03mM $MgCl_2$. From Fig. 2 it can be seen that although the rearranged subunits move on the sucrose gradient very close to the 60 and 40S subunits, they are not identical.

To determine whether the change of the particle deprived of 5S RNA (44S) into a more compact form (55S) is dependent on rebinding of the 5S RNA to it, reticulocyte ribosomes unlabelled and labelled with ^{32}P were treated with Tris buffer, pH 9.5-EDTA, after centrifugation at 50,000 rpm in a Spinco Type Rotor for 5 hr, the pellet obtained from the unlabelled ribosomes was suspended in part of the labelled supernatant. Rebinding of 5S RNA to the 5S RNA free particles was carried out by dialysis against high Mg-Tris buffer, pH 7.4 for 24 hours at 0°C, followed by centrifugation for 5 hours to precipitate the ribosomes. The amounts of 5S RNA, t-RNA and soluble protein were determined in the supernatant fraction before the suspension of the pellet in it and in the supernatant after the resuspended ribosomes were dialyzed and removed. It appears that 50-80% of the 5S RNA was removed from the supernatant on dialysis of the resuspended ribosomes against high Mg-Tris buffer -pH 7.4, whereas only 15-30% t-RNA was removed. Those values were confirmed by extracting RNA from the unlabelled ribosomes treated with the labelled supernatant and estimating the amount of 5S RNA and t-RNA bound to them by means of Kiselguhr-methylated albumin chromatography (6). However, when the unlabelled ribosomes suspended in ^{32}P-labelled supernatant and dialyzed against high Mg-Tris buffer, pH 7.4, were layered on a sucrose gradient containing the same buffer, almost no radioactivity was detected in the monosomes and the larger subunit (Fig.3). Small amounts of label appeared in the smaller subunit region. The labelled RNA bound to the 30S subunit when analyzed on a Sephadex column, appeared to be a mixture of a small amount of t-RNA and an unidentified RNA of a higher molecular weight which might be m-RNA (21). When the top of the gradient was analyzed, it appeared that almost all of the 5S RNA and t-RNA stayed there. This observation may imply that the refolding of 44S particle into a denser form is accompanied with the

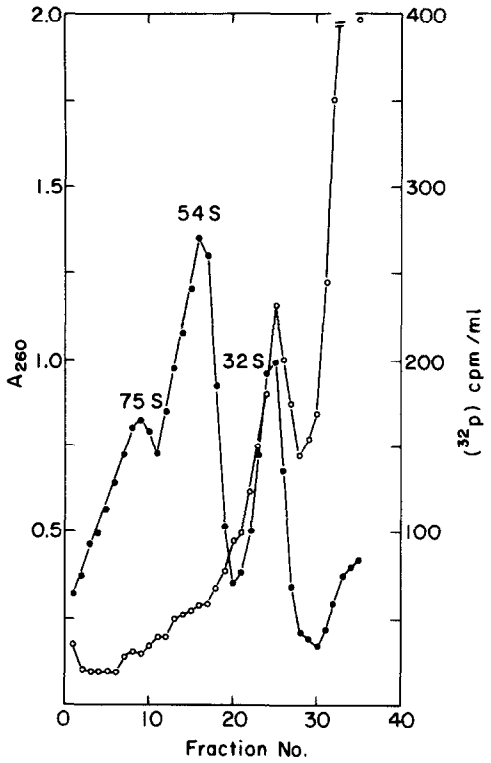

Fig. 3.

Rebinding of solubilized RNA at conditions favoring refolding particles

20 A_{260} units of ^{32}P-labelled ribosomes and 20 A_{260} units of unlabelled ribosomes were treated with Tris-buffer, pH 9.5 - EDTA. The ribosomes were centrifuged, the pellet of the unlabelled ribosomes was suspended in the labelled supernatant (4,700cpm), and the suspension was dialyzed against high Mg-Tris buffer, pH 7.4 at 0°C for 24 hrs. After dialysis the ribosomes were layered on a 5-20% sucrose gradient supplemented with the high Mg-Tris buffer, pH 7.4.

o——o unlabelled ribosomes

o——o solubilized ^{32}P-RNA from supernatant

binding of 5S RNA to it in a loose form or a non-specific form and upon centrifugation on sucrose gradient the 5S RNA is dissociated from the particles.

DISCUSSION

The 5S RNA of the reticulocyte ribosomes is firmly bound to the larger subunit (22). Removal of Mg ions by EDTA at neutral pH or treatment with high salt concentrations, conditions which cause the release of 5S RNA from ribosomes of bacterial origin (13,14,23,24) are not effective in the case of re-

ticulocyte ribosomes. Since Knight et al. (7) reported the same observation for HeLa cell ribosomes, this may be a property common to ribosomes of mammalian origin.

In this work, for the first time the release of 5S RNA from ribosomes of mammalian origin was accomplished by the removal of Mg ions at basic pH. Treatment of reticulocyte ribosomes with Tris buffer, pH 9.5 or with Tris buffer pH 7.4 including EDTA resulted in the formation of subunits with sedimentation coefficient of 50S and 30S. Those particles although having a partly extended conformation as compared to the more compact 60 and 40S subunits, still retained the 5S RNA. Only upon further treatment either with EDTA at basic pH or upon dialysis against Tris-buffer pH 9.5, was the 5S RNA released from the 50S particle. The release was accompanied by some further unfolding of the 50S particle to a 44S particle. These experiments do not provide information on the sequence of events, namely, whether release of 5S RNA causes further unfolding or vice versa. It should be stated that the release of 5S RNA occurred without significant removal of proteins, although the possibility of the release of only one or two proteins cannot be excluded here.

The different conditions needed in order to release 5S RNA from the reticulocyte ribosome as compared to ribosomes from bacterial origin (13,14, 23,24) may indicate a different way of attachment or additional binding points. The possibility of a different mode of interaction between 5S RNA and the larger subunit in mammalian ribosomes as compared to bacterial ribosomes may have well to do with the fact that 5S RNA of the mammalian ribosomes has a different base sequence from the bacterial 5S RNA (8,10,11) and as a consequence of it most probably the secondary structure is different (25) and the ribosome itself is also different in many aspects (26,27).

Ribosomal particles free of 5S RNA when dialyzed against high Mg-Tris buffer, pH 7.4, refolded into a more compact form with a sedimentation coefficient close but not identical to the 60S subunit.

This transformation occurred without a specific reattachment of 5S RNA to the refolded particle. The absence of 5S RNA might cause a conformation in those particles different from the 60S subunit, although many other reasons for non-specific refolding cannot be excluded.

Acknowledgments: My sincerest thanks go to Prof. David Danon for his constant interest and encouragement during the execution of this work. I thank Miss Ofra Stanger for excellent technical assistance.

REFERENCES

1. Rosset, R. and Monier, R., Biochim.Biophys.Acta, 68, 653 (1963).
2. Comb, D.G. and Katz, Z.S., J.Mol.Biol., 8, 801 (1964).
3. Brown, D.D. and Littna, E., J.Mol.Biol., 20, 95 (1966).
4. Galibert, F., Larson, C.J., Lelong, J.C. and Boiron, M., Nature, 207, 1039 (1965).
5. Schleich, T. and Goldstein, J., J.Mol.Biol., 15, 136 (1966).
6. Comb, D.G. and Zehavi-Willner, T., J.Mol.Biol., 23, 441 (1967).
7. Knight, E. and Darnell, J.E., J.Mol.Biol., 28, 491 (1967).
8. Forget, B.G. and Weissman, S.M., Science, 158, 1695 (1967).
9. Galibert, F., Lelong, J.C., Larson, C.J. and Boiron, M., J.Mol.Biol., 21, 385 (1966).
10. Brownlee, G.G., Sanger, F. and Barrell, B.G., Nature, 215, 735 (1967).
11. Williamson, R. and Brownlee, G.G., FEBS Letters, 3, 306 (1969).
12. Comb, D.G. and Sarkar, N., J.Mol.Biol., 25, 317 (1967).
13. Morell, P. and Marmur, J., Biochem., 7, 1141 (1968).
14. Siddiqui, M.A.Q. and Hosokawa, K., Biochim.Biophys.Res.Comm., 32, 1, (1968).
15. Philips, G.R., Z.Naturforschs., 206, 930 (1965).
16. Gould, H.J., Arnstein, H.R.V. and Cox, R.A., J.Mol.Biol., 15, 600 (1966).
17. Yang, P.C., Hamada, K. and Schweet, R., Arch.Biochem.Biophys., 125, 506 (1968).
18. Danon, D. and Cividalli, L., Biochem.Biophys.Res.Comm., 30, 717 (1968).
19. Warner, J.R., Knop, P.M. and Rich, A., Proc.Nat.Acad.Sci.Wash., 49, 122 (1963).
20. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., J.Biol.Chem., 193, 365 (1951).
21. Temmerman, J. and Lebleu, B., Biochim.Biophys.Acta, 174, 544 (1969).
22. Christman, J.K. and Goldstein, J., Biochim.Biophys.Acta, 179, 280 (1969).
23. Marcot-Queiroz, J., Monier, R., Bull.Soc.Chim.Biol., 49, 477 (1967).
24. Sarkar, N. and Comb, D.G., J.Mol.Biol., 39, 31 (1969).
25. Raacke, I.D., Biochem.Biophys.Res.Comm., 31, 528 (1968).
26. Ts'o, P.O.P. and Vinograd, J., Biochim.Biophys.Acta, 49, 113 (1961).
27. Tashiro, Y. and Siekevits, P., J.Mol.Biol., 11, 149 (1965).