SPECIFIC DEGRADATION OF RIBOSOMAL RNA IN RABBIT RETICULOCYTE MEMBRANE-BOUND RIBOSOMES

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1. Introduction

In a previous work we demonstrated that a membrane-linked component from rabbit reticulocytes inhibits incorporation of amino acids into polypeptides in a cell-free protein synthesis system. We concluded that the block was at the elongation level and was not due to a simple degradation of mRNA by an RNAase [1], as tested by polysomal breakdown.

Recently, however, we found [2] that the factor isolated from the membranes of reticulocytes ghosts (ghost factor) acted by causing one or two nicks at specific loci along the 28 S rRNA. It was thus important to ascertain the functionality of this effect and to see whether this membrane component played a role of a specific endonuclease in the intact reticulocyte. In this work we analyse the rRNA profile of membrane bound and free polysomes and show that there is a specific degradation of the rRNA species from plasma membrane bound ribosomes.

2. Materials and methods

Reticulocytes and free ribosomes preparation has been described elsewhere [1]. Bound ribosomes were prepared by the following steps: Reticulocytes were lysed in 1 vol. buffer containing 0.01 M NaCl, 0.005 M $MgCl_2$ and 0.001 M Tris–HCl, pH 7.4 and isotonicity was resumed by addition of 0.1 vol. 1.5 M NaCl. This operation was repeated till white ghosts were obtained. The last wash with 3 vol. lysis buffer was not accompanied by a return to isotonicity. White ghosts were mixed with an equal vol. 1% Triton X-100 in 0.003 M Tris–HCl, pH 7.4 (TT buffer) at 0°C and homogenized with 10 strokes of a Teflon-homogenizer. The mixture was then stirred at 0°C for 35 min and centrifuged at 15 000 rev/min for 20 min in a Sorvall RC2B centrifuge with a SS34 Rotor in order to eliminate most of the membrane components. The supernatant was collected and centrifuged again in a Spinco L_3–$_{50}$ centrifuge for 2 h at 100 000 × *g* in a Ti 50 Rotor through a 1 M sucrose-cushion containing 0.005 M Tris–HCl, pH 7.4, 0.001 M DTT and 0.0001 M EDTA. The pellet contained small membrane vesicles and ribosomal particles as observed by electron microscopy (see Results). The supernatant constitutes the crude ghost factor described in a previous work [1]. The ribosomal fraction constituted 1–2% total ribosomes and is the ribosomal population the most tightly bound to plasma membrane.

Ribosomal RNA was extracted by chloroform–phenol [3] and analysed in an isokinetic sucrose-gradient between 15% and 40% sucrose in a buffer containing KCl 0.1 M, Tris–HCl pH 7.4, 0.01 M, EDTA 0.001 M. After 1 h equilibration, samples were loaded onto the gradient and run for 14 h at 36 000 rev/min in a SW 41 rotor in a L_3–$_{50}$ Spinco centrifuge at 4°C. The optical density profile at 260 nm was analysed using a Gilford spectrophotometer with direct recording.

Samples for electron microscopy were processed either by negative staining using 1% uranylacetate in water on collodion carbon coated grids, or by sectioning of glutaraldehyde, OSO_4-fixed preparation embedded in Epon block, onto uncoated 400 mesh grids. Samples were visualized using a JEM electron microscope.

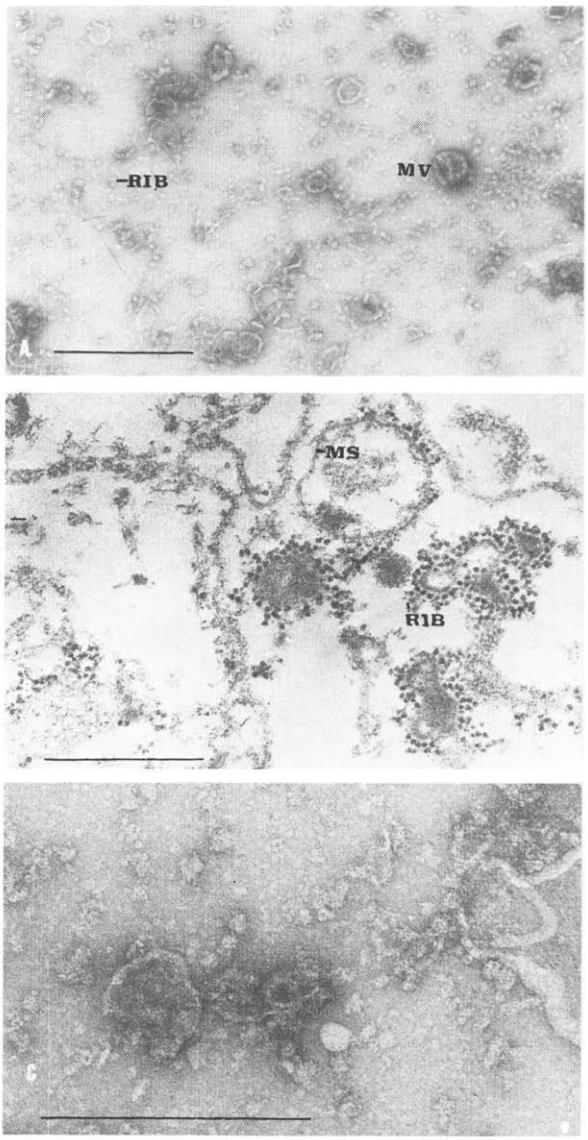

Fig.1(a) After Triton X-100 treatment, the 100 000 × *g* pellet was observed by negative staining. One can observe in this low magnification picture a typical field showing both membrane vesicles (MV) and ribosomes (RIB). (b) The white ghost membrane fraction was processed prior to Triton X-100 treatment for electron microscopy by glutaraldehyde OSO_4-fixation and embedding in Epon. Sections were stained with Uranylacetate and lead citrate. Both membraneous structures (MS) and ribosomes bound to them (RIB) are clearly visible. (c) Even after Triton X-100 treatment some of the ribosomes seem to stick to membrane structure as seen in this negatively stained picture. Magnification is indicated by the 0.5 μm bar in each picture.

3. Results

As mentioned in Materials and methods, the 100 000 × *g* pellet obtained from white ghost after Triton X-100 treatment consists mostly of ribosomes and small membrane vesicles as seen in fig.1b. These ribosomes are freed from the membrane by Triton X-100 and all were attached to membranes before treatment as can be seen in fig.1a which shows the white ghost fraction before Triton X-100 treatment. Even after solubilization by the detergent it seems that at least part of the ribosomes are still bound to vesicles as in fig.1c.

When ribosomal RNA was analysed by sucrose-gradient it was clear that while free ribosomes gave rise to a regular 28 S, 18 S profile (fig.2a) bound ribosomes revealed an almost complete destruction of the 28 S peak while the 18 S remained unchanged and new small species appeared. There was, however, no accumulation of oligonucleotides at the top of the gradient and no new RNA species smaller than 5 S (fig.2b) appeared.

As was described in Materials and methods, the same operation was applied to obtain both ghost factor and the ribosomal fraction. We could not then neglect the possibility that the ghost factor was acting on ribosomes during their preparation and thus degraded their RNA. In order to eliminate this possibility a reconstruction experiment was devised. In a first set of controls, purified GHF was incubated with cytoplasmatic ribosomes at 4°C and their rRNA analysed by sucrose gradient (fig.3c) and compared to rRNA extracted from cytoplasmatic ribosomes

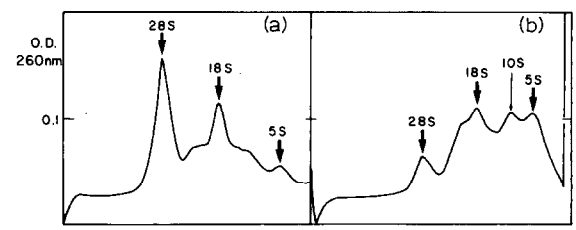

Fig.2. Purified RNA from either free (A) or membrane-bound (B) ribosomes were analysed by isokinetic sucrose-gradient as in Materials and methods. Notice the almost complete disappearance of the 28 S peak in (B) and the new species around 10 S. There is no accumulation of oligonucleotides in the top of the gradient.

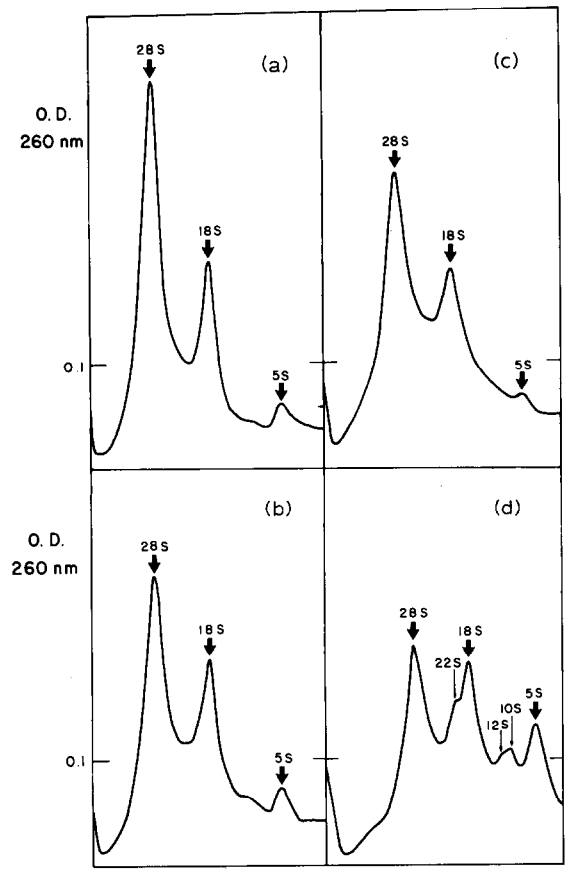

Fig.3. In this reconstruction experiment cytoplasmatic ribosomes were incubated with TT-buffer at 4°C (a) or at 37°C for 30 min (b) or incubated for 30 min with the ghost factor at 4°C, (c) and at 37°C (d) and their rRNA analysed by sucrose-gradient showing that only in the last case was the 28 S peak lowered while new species around 10 S appeared. In the three other cases the rRNA profile remained essentially unchanged.

incubated with the same amount of GHF but at 37°C (fig.3d). In a second set of controls, cytoplasmic ribosomes were incubated with TT-buffer either at 4°C (fig.3a) or at 37°C (fig.3b). One can clearly see from this reconstruction experiment that while incubation with GHF results in a lowering of the 28 S peak and the appearance of new smaller RNA species, no such effect is obtained in the other cases. This shows that the degradation of rRNA observed on membrane-bound ribosomes is not due to the action of GHF during ribosomal preparation which takes place at 4°C.

4. Discussion

The definition of membrane-bound ribosomes is still unclear and we chose to give this name only to those ribosomes which stay bound to plasma membrane even after a series of hypotonic shocks which liberates almost totally the hemoglobin content of the cell. These ribosomes can be released by Triton X-100 and pelleted as we showed here. However, even after the Triton treatment, some membrane vesicles are still present to which ribosomal structure tend to aggregate as in fig.1c. These plasma membrane-bound ribosomes were thought to synthesize different products than the free ribosomes [4]; however, it was shown by Lodish [5] that membrane proteins were synthesized both by free and membrane-bound ribosomes.

In the same line of research it was demonstrated that both membrane-bound and free reticulocytes ribosomes possessed the same mRNA profile when analysed by sucrose-gradient [6]. However, the profile presented by these workers do not show a clear-cut difference in the ribosomal RNA. This apparent contradiction must be due to two reasons; first, the aim of their work was to show differences if any, in the mRNA pattern and attention was not emphasized on rRNA and secondly, the technique used to prepare membrane-bound ribosomes are quite different. The population of bound ribosomes was up to 5% while in our cases the successive hypotonic shocks leave only 1% of the ribosomes bound.

The pattern of a rRNA degradation observed here on plasma membrane-bound ribosomes corresponds nicely to the degradation pattern obtained when intact ribosomes are incubated with ghost factor at 37°C. However, we show also that the degradation observed in bound ribosomes is not an artifact due to action of the ghost factor during fractionation, the ghost factor being inactive at 4°C (fig.3). From these results we conclude that the activity found previously [1] in purified ghost factor corresponds to a functional element existing in the membrane.

References

[1] Wreschner, D. and Herzberg, M. (1976) Eur. J. of Biochem. 64, 399.

[2] Wreschner, D. and Herzberg, M. (1977) submitted for publication.
[3] Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 1408.
[4] Bulova, S. I. and Burka, E. R. (1970) J. Biol. Chem. 245, 4907.
[5] Lodish, H. F. and Desalu, O. (1973) J. Biol. Chem. 248, 3520.
[6] Brennesel, B. A. and Goldstein, J. (1975) Biochim. Biophys. Acta 378, 73.