RAPIDLY LABELLED RIBOSOMAL RNA ASSOCIATED WITH MEMBRANE GHOSTS OF STREPTOCOCCUS FECALIS

L. Nielsen* and A. Abrams

Department of Biochemistry, University of Colorado
School of Medicine, Denver, Colorado

Received September 23, 1964

In Streptococcus fecalis a small portion of the RNA appears to be specifically associated with the membrane ghosts produced when protoplasts are lysed (Abrams et al., 1964). This conclusion was based on experiments showing that the RNA in the membrane fraction was labelled with P^{32} orthophosphate faster than the RNA in free ribosomes or soluble RNA. The relative rates of labelling were determined by degrading the RNA in each cell fraction with alkali and measuring the specific radioactivity of their constituent mononucleotides. Although the molecular size of the rapidly synthesized membrane RNA was not determined, its base composition, as indicated by the distribution of P^{32} among the four nucleotides, suggested that it was of the ribosomal type rather than messenger RNA or transfer RNA. These results were obtained with stationary phase aged cells labelled for a short time in a very dilute growth medium, i.e. cells in the lag phase of growth. It is significant that such conditions resemble the "shift up" conditions which lead to preferential synthesis of ribosomal RNA in E. Coli. (Mitsui et al., 1963). Actually ribosome-like particles were observed in electron micrographs of the S. fecalis membrane ghosts, but no evidence was obtained to show that these particles contained the rapidly labelled RNA. Thus there was considerable information which suggested, but did not show directly, that the rapidly labelled RNA in membrane ghosts was ribosomal RNA.

* Pre-doctoral trainee supported by USPHS Training Grant No. TI GM 51-06.

In the present communication we present the results of a sedimentation analysis of labelled RNA isolated from membrane ghosts, from free ribosomes and from whole protoplasts after incubating intact cells with P_i^{32} as previously described. The results clearly show that the membrane ghosts contain RNA of the ribosomal type and that this RNA is more rapidly labelled than the RNA extracted from free ribosomes. In addition a highly labelled RNA component sedimenting at a slower rate than ribosomal RNA was present in the membrane ghosts but absent in the free ribosomes.

Methods. The procedures used for preparing cells (S. fecalis ATCC #9790), protoplasts, free ribosomes and membrane ghosts were as described previously except that 90 µg lysozyme per ml was used instead of 180 µg per ml (Abrams et al., 1964, 1962, 1960). The regular growth medium contained 1% Tryptone, 1% glucose, 1% K_2HPO_4 and 0.5% yeast extract. The procedure for labelling cells is described in the legend of Fig. 1. P^{32} labelled RNA was extracted by a procedure somewhat modified from that of Scherrer and Darnell (1962). Whole protoplasts, membrane ghosts, and free ribosomes were each suspended in 15 ml of solution A (0.01 M sodium actate, pH 5, containing 0.5% sodium dodecyl sulfate). An equal volume of solution B (90% phenol containing 0.1% 8-hydroxyquinoline) was added, the mixture heated to 55°C, and stirred for 3-5 min. The mixture was quickly cooled to 10°C in an ice bath, centrifuged, and the aqueous phase drawn off with a syringe. The phenol phase was washed with 15 ml of solution A by repeating the heating and stirring process. The aqueous phases were combined and re-extracted with solution B once or as many times as was necessary to eliminate all interphase material. RNA was precipitated by the addition of 2 volumes of 95% ethanol containing 2% sodium actate at 4°C. To remove contaminating inorganic P^{32} the precipitates were collected, washed with 75% ethanol, dissolved in 5 ml .01 M Sodium acetate-.01 M K_2HPO_4, and re-precipitated twice with 2 vols. 95% ethanol-2% NaAc. The final precipitates were washed with 75% ethanol, drained free of ethanol, and stored as pellets in a freezer. The RNA

isolated by this hot phenol-detergent method from whole protplasts contained less than 1% DNA as determined with diphenylamine (Schneider, 1945). A total yield of 77 mg RNA was obtained from protoplasts derived from 1 gram dry weight of cells. Of the total RNA, 10% was recovered in the washed membrane ghosts.

Results. The experiment described in Fig. 1 shows first of all that the bulk RNA extracted from membrane ghosts (Fig. 1C) contains the two

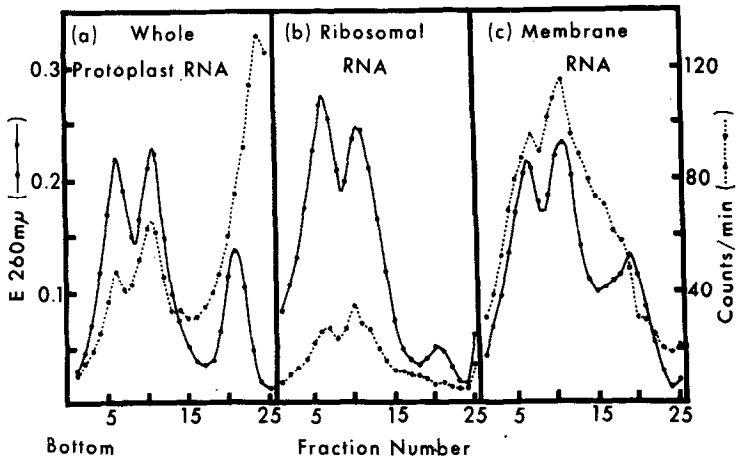

Fig. 1. Sedimentation patterns of P^{32} labelled RNA isolated from whole protoplasts (a), free ribosomes (b) and membrane ghosts (c) of Streptococcus fecalis. Cells were harvested in the stationary phase of growth from 800 ml of regular growth medium (ca 1 gm dry weight), then washed several times with cold H_2O and stored one day at 4°C in 48 ml H_2O. For labelling, the aged cells were suspended in 1000 ml of a 1:50 dilution of regular growth medium containing 10 mC P^{32} orthophosphate, incubated 10 min. at 25°C and cooled in ice bath. The labelled cells were then converted to protoplasts, some of which were lysed by osmotic shock in H_2O at 4°C in order to isolate free ribosomes and membrane ghosts (Abrams et al., 1964). RNA was extracted and purified as described under Methods. For sucrose gradient sedimentation analysis the P^{32} labelled RNA was dissolved in .01 M sodium acetate-.05 M NaCl, pH 5. One half mg in 0.2-0.4 ml buffer was layered onto 4.0 ml gradients containing 5-20% sucrose in the same buffer, and centrifuged at 38,000 rpm for 8 hours at 4°C in the SW 39 rotor in a model L Spinco. Ten drop fractions were collected (total of 25-26 fractions) into stainless steel planchets and counted directly to less than 5% error in a Nuclear-Chicago thin window gas flow counter. Each fraction was then taken up in 3 ml water and its absorbency at 260 mμ measured in Beckman DB spectrophotometer.

species of RNA characteristic of ribosomes since they are identical in sedimentation rate to the two RNA components extracted from free ribosomes (Fig. 1b). Most important, however, is the fact that the

specific radioactivity (cpm/optical density unit) of the membrane ribosomal RNA was 4.7 times greater than that of the RNA from free ribosomes. The specific radioactivity of the membrane ribosomal RNA was estimated from the heavier peak (fractions 1-8 in Fig. 1c) where it can be clearly seen that the CPM and optical density parallel each other almost exactly, thus excluding overlap with the highly labelled RNA in the vicinity of fraction 15. The specific radioactivity of the RNA from free ribosomes was also estimated from the corresponding region (fractions 1-8 Fig. 1b). The ribosomal RNA extracted from the whole protoplasts* (Fig. 1a) was intermediate in its specific radioactivity as expected since it consisted of a mixture of labelled RNA from the membranes and free ribosomes.

Further analyses verified the above results. The RNA in fractions 1-8 (Fig. 1b and c) was degraded in alkali and the P^{32} nucleotides were adsorbed onto charcoal (Abrams et al., 1964). The specific radioactivity (cpm adsorbed onto charcoal/optical density unit adsorbed onto charcoal) of the membrane RNA fractions 1-8 was found to be 4.6 times higher than the corresponding RNA fractions from free ribosomes. Finally, the nucleotides were eluted from the charcoal and subjected to paper electrophoresis (Davidson and Smellie, 1952). Only 2^1 (3^1) guanylic acid was resolved in amounts sufficient for a determination of specific radioactivity. The guanylic acid isolated from the membrane ribosomal RNA contained 723 cpm/μmole while the guanylic acid isolated from the RNA in free ribosomes contained 183 cpm per μmole giving a ratio of 4:1 in satisfactory agreement with the two results outlined above.

Discussion. The existence of membrane bound ribosomal RNA which is more rapidly labelled than the RNA in free ribosomes has not been demonstrated previously. It should be emphasized that we do not know as yet whether the rapidly labelled ribosomal RNA in the membrane ghosts was

*The RNA extracted from whole protoplasts contains a soluble RNA peak at fraction 21. A P^{32} peak (fraction 24) close to the top of the gradient has not been identified. It is probably not orthophosphate since it is not removed after repeated re-precipitation of the RNA preparation in the presence of unlabelled orthophosphate.

in mature or immature ribosomes, or if it was not yet associated with ribosomal protein as has been observed in E. Coli during "shift up" growth conditions (Kono and Osawa, 1964). The membrane fraction may also contain low molecular weight precursors to ribosomal RNA (McCarthy et al., 1962), and some messenger RNA (Fraction 15 region Fig. 1c).

A reasonable interpretation of these findings is that the apparatus involved in the synthesis of ribosomal RNA (or complete ribosomes) in bacteria is attached to membranous components. Such an apparatus may play a role analogous to the nucleolus in higher organisms (Perry, 1964; Brown and Gurdon, 1964). Thus the newly labelled ribosomal RNA (or complete ribosomes) would be expected to appear first in the membrane ghost fraction and later in the free ribosome fraction where the label would be diluted by the great mass of pre-existing unlabelled ribosomes. These findings are possibly related to the recent observations of B. Subtilis with the electron microscope showing a connection between the bacterial nucleus and the plasma membrane via the membranous mesosome structure (Ryter and Landman, 1964; Ryter and Jacob, 1963). In this regard it is of interest that a small amount of DNA is found in the washed S. fecalis membrane ghosts and that electron micrographs show a tubular structure emanating from the plasma membrane and containing ribosome-like particles (Abrams et al., 1964).

This work was supported by Grant No. RG 5810 from the National Institutes of Health, USPHS.

REFERENCES

Abrams, A., L. Nielsen and J. Thaemert (1964) Biochim. Biophys. Acta, 80, 325.
Abrams, A. and P. McNamara (1962) J. Biol. Chem., 257, 170.
Abrams, A., P. McNamara and B. Johnson (1960) J. Biol. Chem., 235, 3659.
Brown, D. D. and J. B. Gurdon (1964) Proc. Natl. Acad. Sci. 51, 139.
Davidson, J. N. and R.M.S. Smellie (1952) Biochem. J., 52, 594.
Kono, M. and S. Osawa (1964) Biochim. Biophys. Acta, 87, 326.
McCarthy, B. J., R. J. Britten and R.B. Roberts (1962) Biophys. J.,2,57.
Mitsui, H., A. Ishihama and S. Osawa (1963) Biochim. Biophys. Acta, 76, 401.
Perry, R. (1964) National Cancer Institute Monograph 14.
Ryter, A. and F. Jacob (1963) Compt. Rend., 257, 3060.
Ryter, A. and O. Landman (1964) J. Bact., 88, 457.
Scherrer, K. and J.E. Darnell (1962) Biochem. Biophys. Res. Communs., 7, 486.
Schneider, W. C. (1945) J. Biol. Chem., 161, 293.