POLYRIBOSOMES AND RIBOSOMAL SUB-UNITS OF BACTERIAL PROTOPLASTS

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Metabolically active protoplasts yielded lysates rich in polyribosomes with only 10-15% of the total ribosomal material present as sub-units. Treatment of such lysates with pancreatic ribonuclease (RNase) converted the polyribosomes virtually quantitatively to 70s material. When protoplasts were incubated with actinomycin D, polyribosomes were broken down with the accumulation of 50s and 30s sub-units, not 70s ribosomes. Similar results were obtained in the presence of low concentrations of chlortetracycline and also when protoplasts were allowed to "run-down" due to cooling and lack of aeration. These results suggest that the products of run-off from polyribosomes accumulate 50s and 30s sub-units and not 70s ribosomes, at least under the conditions prevailing in these experiments.

Methods

The methods used have been described with full details elsewhere (Cundliffe 1967, 1968) and will be given here only in outline. Bacillus megaterium KM was steady-state labelled with (^{32}P) orthophosphate and converted to protoplasts, suspensions of which were incubated until the optical density at 600 mμ was increasing exponentially. "Control" samples were then taken and incubation of the remaining protoplasts was continued in the presence of drugs and sampled

as indicated in the text. Samples were promptly chilled and lysed (with detergent) and analysed on 15-40% (w/v) sucrose density gradients. After centrifugation gradient fractions (40 in total) were collected, precipitated with TCA, filtered on glass fibre discs and counted in a liquid scintillation counter. The distribution of [^{32}P] radioactivity in gradients was found to be a sensitive indicator of the distribution of ribosomes etc. The methods used here differed from those in the papers cited above in one respect only: here sucrose gradients containing 5mM Mg^{++} were used whereas previously they contained 10mM Mg^{++}. It should also be noted that the concentration of Mg^{++} during incubation of cells and protoplasts and during lysis was 20mM.

Results and Discussion

Figure 1 shows a typical polyribosome profile together with the effect of a mild treatment with RNase at 0°. Sedimentation coefficients were not measured directly, rather it was assumed that the major peak after RNase-treatment of control polyribosomes sedimented at 70s. In other experiments control lysates were analysed on gradients containing 5 or 10mM Mg^{++} and the polyribosome profiles were seen to be essentially identical in both cases.

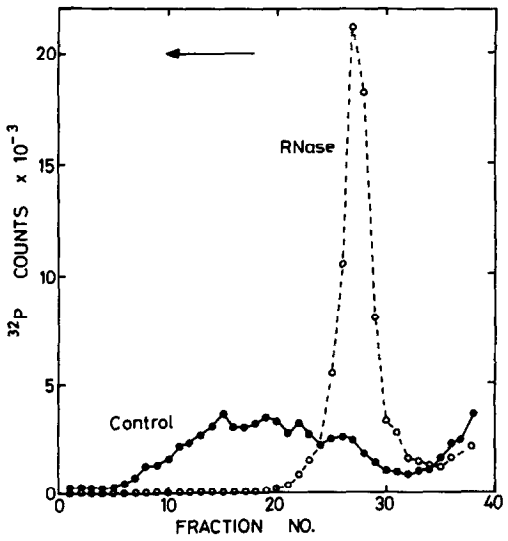

FIG.1. Effect of RNase on polyribosomes. Gradients centrifuged at 38,000 rpm for 75 min. at 0°. In all Figures sedimentation is right to left.

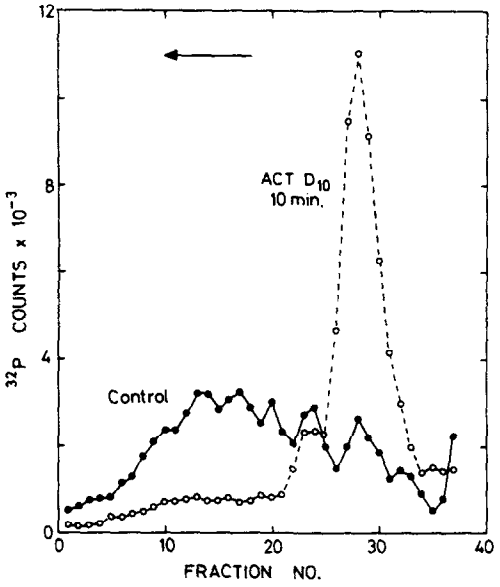

FIG.2. Effect of actinomycin D (10µg per ml.) on polyribosomes. Gradients centrifuged at 38,000 rpm for 75 min. at 0°. "ACT D_{10}" means Actinomycin D 10µg per ml. (final concentration).

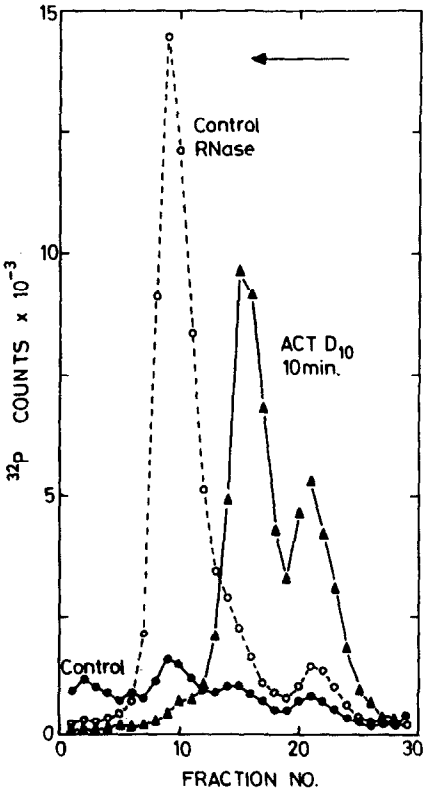

FIG.3. Products of run-off in the presence of actinomycin D. Gradients centrifuged at 45,000 rpm for 150 min. at 0°.

Incubation of protoplasts with actinomycin D resulted in breakdown of polyribosomes (Fig. 2) with a corresponding accumulation of material in the 70s region of the gradient - defined as above (see also Cundliffe, 1968). However when lysates similar to those shown in Figs. 1 and 2 were subjected to prolonged centrifugation, it became apparent (Fig.3) that the products of "run-off" in the presence of actinomycin were not 70s ribosomes but sub-units (assumed to be 50s and 30s). It is also clear from Fig. 3 that not more than 10-15% of the total ribosomal material of undegraded control lysates was present as sub-units and that treatment with RNase caused only a slight increase in this amount.

Essentially similar results were obtained when protoplasts, previously incubated with shaking at 37°, were allowed to stand at 20° without aeration for 15 min. before sampling (Fig. 4). Under these conditions run-off occurred due to the decreased temperature and inadequate aeration and resulted in the accumulation of 50s and 30s sub-units.

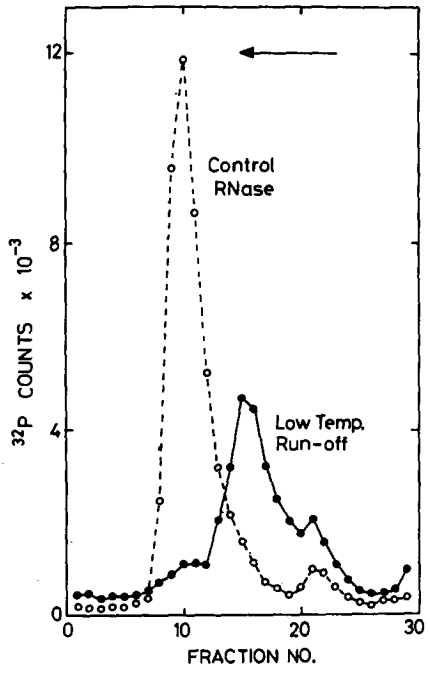

FIG.4. Products of run-off at room temperature. Gradients centrifuged at 45,000 rpm for 150 min. at 0°.

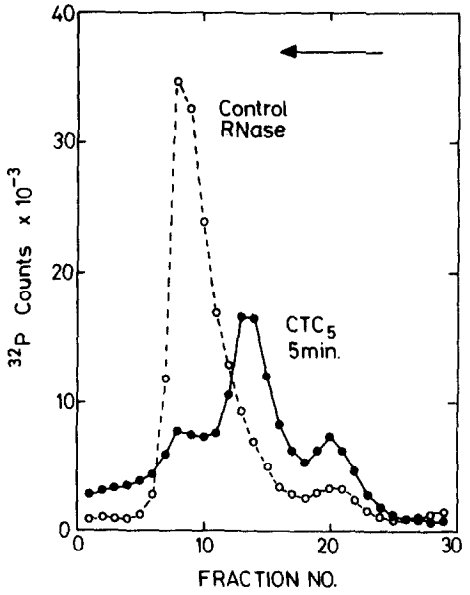

FIG.5. Products of run-off in the presence of chlortetracycline at low concentration. Gradients centrifuged at 45,000 rpm for 150 min. at 0°. "CTC_5" means chlortetracycline 5 µg per ml. (final concentration).

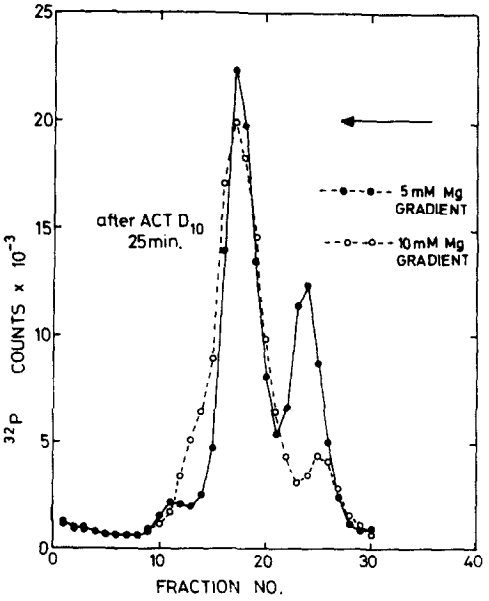

FIG.6. Effect on sub-units of varying concentration of Mg^{++} in gradients. Gradients centrifuged at 45,000 rpm for 150 min. at 0°.

In the previous report (Cundliffe, 1967) polyribosomes were observed to break down in the presence of low concentration of chlortetracycline (e.g. 5 μg per ml). It is now clear (Fig.5) that the products of breakdown under these conditions were ribosome sub-units and not 70s ribosomes as was assumed in that report.

Figure 6 shows that different gradient profiles were obtained when a run-off preparation (produced by incubating protoplasts with actinomycin D) was analysed on two different gradients one containing 5mM Mg^{++} (as in Fig. 3) the other containing 10mM Mg^{++}. In the latter case the 30s peak was greatly decreased and the 50s peak broadened with a shoulder on the leading edge. This seems to indicate aggregation of the small sub-unit under these particular ionic conditions and explains why essentially single peaks were found earlier (Cundliffe & McQuillen, 1967) under conditions where two peaks of sub-units might have been expected (namely, after removal of nascent peptides from ribosomes by puromycin).

These results confirm earlier ones (Mangiarotti & Schlessinger, 1966) with the important difference that in their control lysates up to about 50% of the total ribosomal material was recovered as sub-units whereas here the corresponding value is 10-15%. It is therefore apparent that in lysates of active protoplasts virtually all of the ribosomes present are active, at least to the extent that they become involved in polyribosomes (or polyribosome-like material).

Acknowledgement

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References

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