RIBOSOME SYNTHESIS DURING UNBALANCED GROWTH

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The thymineless mutant of Escherichia coli (15 T^-) can synthesize ribonucleic acid (RNA) and protein even though the synthesis of deoxyribonucleic acid (DNA) is prevented by lack of thymine (Barner and Cohen, 1954; Hanawalt, 1958). We have used this mutant to determine whether the absence of DNA synthesis caused any alteration in the distribution of RNA among the soluble RNA and the different classes of ribonucleoprotein particles (ribosomes). Ultracentrifugation and column chromatography were used to separate the various types of RNA and P^{32} labeling served to distinguish newly formed nucleic acid from that originally present.

Methods

A culture of E. coli 15 T^- growing exponentially in Tris-glucose medium (Roberts, et. al. 1957) containing 3 µg thymine/ml and 0.7 µmol $PO_4^{\equiv}$/ml was harvested, then washed with and resuspended in, the Tris-$PO_4^{\equiv}$ medium lacking thymine. Glucose was added and the culture was then aerated at 37° after being divided into two portions, one with and the other without thymine (3 µg/ml). P^{32} was added immediately to observe the entire time course of incorporation or at appropriate times to observe the incorporation after different periods of incubation without thymine. Samples of the culture were taken for chemical fractionation (Roberts, et. al. 1957), for centrifugal fractionation and chromatography (Roberts, Britten and Bolton 1958), for measurement of bacterial

mass (optical density at 650 mμ) and nucleic acid content (optical density at 260 mμ), and for plate counts. The nucleic acid content of the various fractions was measured by absorption at 260 mμ and the protein content was measured by Lowry's modification of the Folin method (Lowry, et. al. 1951). The P^{32} of trichloracetic acid (TCA) precipitable compounds was measured by adding TCA, filtering on membrane filters, and counting the material on the filter (Britten, Roberts and French 1955).

Results

In the control culture with thymine present the growth was exponential during the period of observation (two hours) and there was no change in the relative proportions of the various components.

The culture lacking thymine exhibited these previously observed features (cf. Hanawalt 1958). The growth (measured by OD at 650 mμ) was linear; RNA was synthesized at a rate which was approximately constant; protein and phospholipids were synthesized at increasing rates; the incorporation of P^{32} into DNA was drastically reduced; and the number of cells capable of forming colonies dropped by more than a factor of ten after 60 minutes' incubation without thymine.

The characteristics of the RNA produced during this period of unbalanced growth were then examined. Three methods were used which had been shown to be capable of distinguishing the abnormal RNA which is produced in the presence of chloramphenicol (Pardee, Paigen and Prestidge, 1957; Aronson and Spiegelman, 1958; Carnegie, 1958). (1) Samples of cells taken after 90 minutes' growth without thymine were disrupted and analyzed in the Spinco Model E ultracentrifuge. More than one half of the RNA of these cells had been formed during unbalanced growth. The pattern of particles (which showed ribosomes having sedimentation constants of approximately 20S, 30S, 50S, 70S and 85S)

was typical of those commonly observed in exponentially growing E. coli B. The control culture of 15 T^- growing with thymine showed a slightly smaller proportion of the 30S and 50S particles. (2) Samples were taken periodically from both cultures and the cells were harvested and washed. The cells were broken and the walls and membranes removed by a short period of centrifugation. The ribosomes were then pelleted from the wall-free extracts by centrifugation in the angle head rotor of the Spinco Model L centrifuge (2 hours, 40,000 RPM). 55 ± 5 per cent of the ultraviolet absorbing material was sedimented from eight samples taken (at 15 minute intervals) from the culture lacking thymine. The same proportion sedimented in four samples taken from the control culture. (3) Chromatography on DEAE-cellulose of a ribosome pellet obtained from cells which had grown 90 minutes without thymine gave an elution pattern typical of ribosome pellets obtained from E.coli B. (cf. Roberts, Britten and Bolton 1958). Thus the RNA formed during unbalanced growth is initially distributed in the usual proportions among soluble RNA and the various classes of ribosomes.

As the total RNA increased linearly with time and the ribosomes made up a nearly constant proportion of the total, the ribosomes must have also been synthesized at a constant rate. Direct measurements of the nucleic acid content or the P^{32} of the ribosome pellets also indicated a constant rate of synthesis but this technique was less accurate because of variations in the efficiency of breaking the cells.

In contrast the soluble RNA seems to be synthesized at an increasing rate. The increasing rate was most readily observed by measuring the incorporation of P^{32} into the soluble and ribosome fractions during 15 minute intervals after various periods of incubation without thymine. This technique showed a doubling of the rate of synthesis of soluble RNA after 75 minutes of incubation without

thymine whereas the rate of ribosome synthesis did not increase. As the newly formed soluble RNA contributes only 15-20% of the total ultraviolet absorption, its increasing rate of synthesis cannot be demonstrated if only the total is measured.

Discussion

The RNA formed during the unbalanced growth caused by chloramphenicol is abnormal as can be shown by its lower sedimentation rate, by its changed ultracentrifuge pattern, and by its chromatography. In contrast these techniques fail to show any abnormality in the RNA which is formed during the unbalanced growth of 15 T^- caused by the absence of thymine. Accordingly the synthesis of ribosomes does not require the concurrent synthesis of DNA. The rate of ribosome synthesis, however, no longer increases after synthesis of DNA stops suggesting that the quantity of DNA may be a rate limiting factor in the synthesis of ribosomes.

References

1. Aronson, A. I. and S. Spiegelman, Biochim. et Biophys. Acta, 29, 214, 1958.
2. Barner, H. D. and S. S. Cohen, J. Bacteriol., 68, 80, 1954.
3. Britten, R. J., R. B. Roberts, and E. F. French, Proc. Nat. Acad. Sci., 41, 863, 1955.
4. Carnegie Inst. Wash. Year Book 57, 129, 1958.
5. Hanawalt, P. C., Yale Univ. thesis, 1958.
6. Lowry, O. H., N. J. Rosenbrough, A. Furr, A. Lewis, and R. J. Randall, J. Biol. Chem., 193, 265, 1951.
7. Pardee, A. B., K. Paigen, and L. S. Prestidge, Biochim. et Biophys, Acta, 23, 162, 1957.
8. Roberts, R. B., P. H. Abelson, D. B. Cowie, E. T. Bolton, and R. J. Britten, Carnegie Inst. Wash. Publ. 607, second printing 1957.
9. Roberts, R. B., R. J. Britten, and E. T. Bolton, In Microsomal Particles and Protein Synthesis, 84, Pergamon Press, New York, 1958.