The Control of Ribonucleic Acid Synthesis in Bacteria

THE SYNTHESIS AND STABILITY OF RIBONUCLEIC ACID IN RIFAMPICIN-INHIBITED CULTURES OF *ESCHERICHIA COLI*

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(Received 11 September 1970)

A study was made of the kinetics of labelling of the stable ribonucleic acids (rRNA+tRNA) and the unstable mRNA fraction in cultures of *Escherichia coli* M.R.E.600, inhibited by the addition of 0.1g of rifampicin/l. Labelling was carried out by adding either [2-¹⁴C]- or [5-³H]-uracil as an exogenous precursor of the cellular nucleic acids. From studies using DNA-RNA hybridization, the kinetics of the synthesis and degradation of mRNA was followed in the inhibited cultures. Although a considerable proportion of the mRNA labelled in the presence of rifampicin decayed to non-hybridizable products, about 25% was stabilized beyond the point where protein synthesis had finally ceased. It therefore seems unwise to extrapolate the results of studies on mRNA stability in rifampicin-inhibited cultures to the situation existing in the rate of steady growth, where there appears to be little, if any, stable messenger. The kinetics of labelling of RNA in inhibited cultures indicated that the elapsed time from the addition of rifampicin to the point at which radioactivity no longer enters the total cellular ribonucleic acids is a measure of the time required to polymerize a molecule of rRNA. At 37°C, in culture grown in broth, glucose-salts or lactate-salts media, exogenous [2-¹⁴C]uracil entered rifampicin-inhibited cells and was incorporated into RNA for 2-3 min after the antibiotic was added. Taking this time as that required to polymerize a complete chain of 23S rRNA, the polymerization rate of this fraction in the three media was 25, 22 and 19 nucleotides added/s to the growing chains. Similar experiments in cultures previously inhibited by 0.2g of chloramphenicol/l showed virtually identical behaviour. This confirmed the work of Midgley & Gray (1971), who, by a different approach, showed that the polymerization rate of rRNA in steadily growing and chloramphenicol-inhibited cultures of *E. coli* at 37°C was essentially constant at about 22 nucleotides added/s. It was thus confirmed that the rate of polymerization of at least the rRNA fraction in *E. coli* is virtually unaffected by the nature of the growth medium and therefore by bacterial growth rate.

Bactericidal antibiotics of the rifamycin family, and the synthetic derivative rifampicin, are known to act by binding irreversibly to DNA-dependent RNA polymerase and inhibiting all enzyme molecules that, at the moment of addition, are not engaged in polymerizing new chains of RNA on the DNA cistrons (Mizuno, Yamazaki, Nitta & Umezawa, 1968; Sippel & Hartmann, 1968; Tocchini-Valentini, Marino & Colvill, 1968; Wehrli, Nüesch, Knüsel & Stachelin, 1968). Thus the initiation of synthesis of new RNA chains is effectively prevented, and any residual synthesis of RNA *in vivo* occurs only over the period needed for the completion of those chains that were then in the process of polymerization. This situation is

potentially ideal for the study of the synthesis and breakdown of mRNA molecules that have only a transient life in the bacterial cells, as protein biosynthesis is unaffected by the antibiotic and continues for as long as the appropriate machinery remains operative. In *Escherichia coli*, the incorporation of exogenously supplied radioactive amino acids into protein continues for about 15 min after the addition of rifampicin (Lancini & Sartori, 1968). At the end of this period, the inability of the cells to promote further synthesis is assumed to be the result of the degradation of the mRNA templates.

Depending on the kinetics of mRNA labelling and degradation after the addition of rifampicin,

the labelling patterns in bacterial cells may provide some information on the rate of chain elongation of certain RNA species that are being completed during inhibition. Over any short period, in growing bacterial cultures, most of the exogenous labelled bases such as [5-³H]uracil, enter rapidly labelled RNA as precursors of rRNA (Bolton & McCarthy, 1962; Midgley, 1962; Midgley & McCarthy, 1962; Pigott & Midgley, 1968). To a first approximation, the time after which labelled precursors no longer enter the RNA pool *in vivo* in rifampicin-inhibited cells may be a measure of the time needed to form the largest stable RNA (i.e. 23S rRNA). Indeed, in *E. coli*, the formation of 23S rRNA exercises some 50% of the total RNA-synthesizing capacity of the cells (Bolton & McCarthy, 1962; Pigott & Midgley, 1968; Gray & Midgley, 1970).

Problems in the interpretation of the kinetics of RNA labelling may arise in rifampicin-inhibited cells from the fact that a small proportion of the mRNA fraction in bacterial rapidly labelled RNA may consist of giant polycistronic molecules of a larger size than the stable 23S fraction (Imamoto & Yanofsky, 1967; Venetianer, 1969). As these probably take longer to be completed by transcription than the shorter 23S rRNA molecules, it may be that these define the time at which the net incorporation of labelled precursors into RNA ceases in inhibited cultures. Alternatively, as the degradation of these larger mRNA molecules may begin before their synthesis is complete (Morikawa & Imamoto, 1969; Morse, Mosteller, Baker & Yanofsky, 1969), their ultimate effects on the RNA-labelling patterns may be negligible.

In this paper, we have studied the kinetics of labelling the stable and unstable RNA fractions in rifampicin-inhibited cultures of *E. coli* M.R.E. 600. We have shown that, in spite of complications caused by the labelling of mRNA, the kinetics of labelling the total RNA in inhibited cells allows a fairly accurate estimation of the rate of chain polymerization of the 23S rRNA. In confirmation of the work of Midgley & Gray (1971), it appears that the rate of RNA chain polymerization is unaffected by the nature of the growth medium or by inhibition by chloramphenicol.

MATERIALS AND METHODS

Organism. *Escherichia coli* M.R.E. 600 was used.

Bacterial growth and preparation of RNA. Bacterial cultures were grown at 37°C under forced aeration, in the three media described by Gray & Midgley (1970). The harvesting of cultures, the method of cell breakage, RNA extraction and labelling were as described by Pigott & Midgley (1968).

DNA-RNA hybridization. The method was essentially that of Gillespie & Spiegelman (1965) and Pigott &

Midgley (1968), nitrocellulose membrane filters being used as an inert support for denatured DNA.

Assay of RNA and DNA labelling in bacterial cells. Labelling of the total nucleic acids in bacterial cultures was assayed by the usual technique of precipitating 1 ml samples in 4 ml of ice-cold 10% (w/v) trichloroacetic acid. Simultaneously, 1 ml samples were injected into 2 ml of m-KOH, and the samples were incubated at 37°C for 18 h. In this time, the cellular RNA had been digested, leaving labelled DNA. This was then precipitated by the addition of 6 ml of ice-cold 20% (w/v) trichloroacetic acid. The radioactive precipitates were collected by filtration on membrane filters (Sartorius Membranfilter MF50, 2.5 cm diam.; V. A. Howe and Co. Ltd., London W.11, U.K.), with three washes with 5 ml of the 10% acid.

To check that this technique effectively estimated the labelling of the bacterial DNA only, a culture of *E. coli* B3005 (a thymine-requiring mutant of *E. coli* B) was grown in the presence of [2-¹⁴C]thymidine to label the DNA specifically. At intervals, 1 ml samples were removed and were either precipitated immediately with trichloroacetic acid as described above, or were first incubated with KOH before precipitation. The resulting labelling curves are shown in Fig. 1. The intervening period of incubation in m-KOH had no effect on the precipitability of the ¹⁴C-labelled DNA.

A further check was made to ensure that during the prolonged period of incubation in m-KOH, all the labelled RNA in the bacteria was digested to products not precipitable in trichloroacetic acid. A culture of *E. coli* M.R.E. 600 was inhibited by 0.2 g of chloromphenicol/l for 90 min at 37°C. In this time, DNA synthesis had effectively ceased, though RNA synthesis and turnover was still proceeding. The inhibited culture was given [2-¹⁴C]uracil, and at intervals 1 ml samples were withdrawn and either precipitated immediately in ice-cold 10% trichloroacetic acid, or were digested in m-KOH as described above, before acid precipitation. The resulting labelling curves are shown in Fig. 2. Virtually all the radioactive material initially

Fig. 1. Time-course of incorporation of [2-¹⁴C]thymidine into the DNA of *E. coli* B3005. O, ¹⁴C radioactivity (c.p.m./ml of culture) as measured by trichloroacetic acid precipitation. ●, ¹⁴C radioactivity (c.p.m./ml of culture) as measured by acid precipitation after an intervening period of 18 h at 37°C in 0.7M-KOH.

Fig. 2. Time-course of incorporation of [2- 14 C]uracil into the nucleic acids of a culture of *E. coli* M.R.E. 600 previously inhibited by 0.2 g of chloramphenicol/l for 90 min. The labelling experiment was carried out on a culture of *E. coli* that had been inhibited for 90 min by chloramphenicol, when DNA synthesis had ceased. Rifampicin (0.1 g/l) was added (at the time indicated by the arrow) to arrest RNA synthesis and to give conditions that were comparable with those in other experiments where rapid sampling was to be carried out to assay RNA and DNA labelling. ●, 14 C radioactivity (c.p.m./ml of culture) as measured by trichloroacetic acid precipitation, after 18 h incubation at 37°C in 0.7 M-KOH. O, 14 C radioactivity (c.p.m./ml of culture) measured by acid precipitation alone.

precipitable in trichloroacetic acid was rendered soluble by KOH incubation. These experiments showed that the techniques employed gave accurate estimations of the labelling of RNA or of DNA.

Inhibition of cultures by antibiotics. Cultures (50 ml) of *E. coli* were inhibited by the addition of chloramphenicol in ethanol to a final concentration of 0.2 g/l. Rifampicin was used at a concentration of 0.1 g/l, and was added in methanolic solution.

Source of radiochemicals and other compounds. [5- 3 H]-Uracil (sp. radioactivity 1 Ci/mmol), [2- 14 C]uracil (sp. radioactivity 54.5 mCi/mmol) and [2- 14 C]thymidine (sp. radioactivity 54.5 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Chloramphenicol was obtained from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. Rifampicin was a kind gift from Lepetit Pharmaceuticals Ltd., Maidenhead, Berks., U.K.

RESULTS

Inhibitory effects of rifampicin on bacterial cultures.

(a) Assessment of the concentration needed for effective inhibition of *E. coli*. Cultures of *E. coli* M.R.E. 600 were grown in glucose-salts media to a cell turbidity E_{650} 0.5. To samples (approx. 50 ml) of the cultures, various amounts of rifampicin were added. At 2 min before the addition of the antibiotic, 5 μ Ci of [2- 14 C]uracil was injected, and the labelling patterns for the RNA and DNA were

obtained by rapid sampling (see the Materials and Methods section). The results are shown in Fig. 3. A concentration of 0.1 g of rifampicin/l is adequate to ensure the most rapid inhibition of the cultures. Further, the kinetics of labelling of the RNA indicate no change in slope in the curves when the rifampicin is injected. Thus, if the antibiotic is effectively preventing the initiation of new RNA chains, it has no initial effect on the rate of completion of partly finished chains.

(b) Rapidity of inhibition of RNA chain initiation by 0.1 g of rifampicin/l. If estimations of the rate of chain growth of RNA molecules are to be made successfully in rifampicin-inhibited cultures, it is essential to know the time required for the antibiotic to act after its injection into growing cells. A satisfactory assessment of this time was made by experiments designed to show how quickly the induction of β -galactosidase could be inhibited by the addition of rifampicin.

A culture of *E. coli* was grown in sodium lactate-salts medium to a turbidity E_{650} 0.5. A sample (30 ml) was removed, and 0.5 ml of 0.04 M-isopropyl- β -D-thiogalactopyranoside (Sigma) was added, as a gratuitous inducer of β -galactosidase. After 3 min, 0.1 g of rifampicin/l was added to both the preinduced sample and to the uninduced culture. This prevented further initiation of new RNA chains, but did not prevent the translation of existing mRNA molecules (Lancini & Sartori, 1968). After a further 15 s, 0.5 ml of 0.04 M-isopropyl- β -D-thiogalactopyranoside was added to 30 ml of the uninduced culture, and 15 s, later, inducer was added to an additional 30 ml sample. All three samples were then incubated at 37°C for 40 min to allow translation of the mRNA specific for β -galactosidase production. After this time, 0.05 ml of toluene was added to 3 ml of each sample and the mixture was shaken at intervals over a further incubation period of 30 min. To 1 ml of this mixture, 2 ml of 0.1 M-sodium phosphate buffer, pH 6.7, was added, followed by 0.25 ml of 0.03 M-O-nitrophenyl- β -D-galactopyranoside as a substrate for any enzyme molecules present. The mixture was incubated at room temperature for 10 min, the E_{420} of the solution being read at 1 min intervals. The time-course for enzyme hydrolysis of the nitrophenyl galactopyranoside in cultures induced before and after the addition of 0.1 g of rifampicin/l is shown in Fig. 4. The results show that the inhibition of β -galactosidase induction by rifampicin is more than 90% effective after 15 s in the presence of the antibiotic. Since the induction of β -galactosidase is preceded by the derepression of the appropriate mRNA (Kepes, 1963), this indicates that the inhibitory effect of rifampicin on the initiation of new RNA chains is almost immediate (Mosteller & Yanofsky, 1970).

Fig. 3. Time-course of incorporation of $[2-^{14}\text{C}]$ uracil into the RNA and DNA of *E. coli* inhibited by various concentrations of rifampicin. $\circ$, RNA labelling; $\bullet$, DNA labelling. Rifampicin was added at the time indicated by the arrow at the following concentrations: (a) 0.001 g/l; (b) 0.005 g/l; (c) 0.01 g/l; (d) 0.05 g/l.

Molecular size of RNA formed in rifampicin-inhibited cultures. Roberts (1969) has shown that the molecular size of RNA molecules produced in *E. coli* is controlled by a protein that causes specific termination of growing RNA chains on the DNA cistrons. As our studies were directed towards measurements of the rate of elongation of the rRNA chains in this organism, it was decided to see whether rifampicin had any effect on the control of RNA termination. Accordingly, 0.1 mCi of $[5-^3\text{H}]$ -uracil and 0.1 g of rifampicin/l were added simultaneously to 250 ml of a growing culture; samples were taken and quickly chilled, and the RNA was extracted in the usual way. The labelled RNA was displayed on sucrose density gradients (Avery & Midgley, 1969) (Fig. 5). The results show that 16S and 23S rRNA are completed as normal-sized molecules and the specific radioactivities of

each fraction are equal, indicating that there is no differential disturbance in the labelling pattern for the two species. There seems to be no reason to suspect that the RNA chain-termination factor is in any way affected by rifampicin inhibition in *E. coli*.

Kinetics of RNA and DNA labelling in rifampicin-inhibited cultures. A culture of *E. coli* grown steadily in glucose salts medium was injected with $5 \mu\text{Ci}$ of $[2-^{14}\text{C}]$ uracil and, 2 min later, with rifampicin to a concentration of 0.1 g/l. Radio-sampling was carried out to obtain kinetic labelling curves for total RNA and for DNA. Figure 3 shows the results. Simultaneously, samples of inhibited cultures were poured on crushed glass and ^{14}C -labelled RNA was extracted and prepared for DNA-RNA hybridization. The labelled RNA was challenged with homologous DNA at a D

Fig. 4. Production of β -galactosidase by induction of *E. coli* cultures before and after the addition of rifampicin. Details of the experiment are given in the text. ●, Un-induced cells; ▲, cells induced 30s after the addition of rifampicin (0.1g/l); ○, cells induced 15s after addition of rifampicin; △, cells induced 3min before addition of rifampicin. In all experiments, the background extinction caused by the rifampicin was corrected by the use of a blank of antibiotic-inhibited culture without nitrophenyl galactoside. $E_{420} = 0.410$.

RNA ratio 5:1 (w/w), to hybridize the mRNA fraction preferentially (Pigott & Midgley, 1968). The labelling kinetics for the mRNA fraction is included in Fig. 6.

The results show that, as is usual for steadily growing cultures, radioactivity entered the mRNA and stable RNA fractions at relative rates of 1:2 (Gray & Midgley, 1970). However, after about 4min, the radioactivity in the mRNA fraction had reached its peak, and declined steadily thereafter. It did not completely disappear but reached a steady minimum value at about 9% of the stable RNA in the inhibited cultures, even as long as 40min after the addition of rifampicin. Moreover, the loss of hybridizable mRNA at later times was mirrored by a net loss of acid-precipitable radioactivity in the total RNA. About 20% of the label in RNA made in rifampicin-inhibited cells finally decayed to non-precipitable products. These findings indicate that, as about one-third of the rapidly labelled RNA is mRNA (Pigott & Midgley, 1968), all the labelled RNA that is finally degraded in antibiotic-inhibited cultures is mRNA. Further, if a labelling curve is devised for the stable RNA (Fig. 6), the time-point at which label no longer enters the total RNA pool is virtually the same as the time at which it no longer enters the pool of stable RNA alone. Thus, the labelling curve for total trichloroacetic acid-precipitable RNA in rifampicin-inhibited cultures is dominated by stable RNA, especially the 16S and 23S

Fig. 5. Sucrose-density-gradient sedimentation of RNA labelled with $[5\text{-}^3\text{H}]\text{uracil}$ in rifampicin-inhibited cultures. RNA was labelled for the following times after addition of 0.1g of rifampicin/l: (a) 2 min; (b) 5 min; (c) 10 min. Sedimentation was carried out as described by Avery & Midgley (1969). Only those parts of the gradients are displayed in which the 16S and 23S rRNA are present. ○, ^3H radioactivity in RNA; ● E_{260} .

rRNA species. If it is assumed that the 23S rRNA is the largest stable RNA molecule formed, with minimal alterations from slightly larger

Fig. 6. Time-course of labelling of total RNA and mRNA in rifampicin-inhibited cultures of *E. coli*. ●, mRNA labelling; ○, total RNA labelling; ■, stable RNA labelling. Rifampicin (0.1 g/l) was added at the time indicated by the arrow. The labelling of the DNA has been omitted for clarity. mRNA was estimated by hybridization at DNA/RNA ratio 5:1 (w/w) (see the text).

precursors (Adesnik & Levinthal, 1969) then the time-period after the addition of rifampicin required for radioactivity in total RNA to reach its maximum will be a measure of the rate of chain growth of the 23S fraction. In *E. coli*, the kinetics of labelling of 16S rRNA indicate that it is formed in the same time as a molecule of 23S rRNA (Mangiarotti, Apirion, Schlessinger & Silengo, 1968; Adesnik & Levinthal, 1969; Avery & Midgley, 1971). Thus, at least two-thirds of the rapidly labelled RNA will take the same time for chain completion in inhibited cells. From Fig. 6, this time is 2.0–2.2 min at 37°C, indicating that 23S rRNA molecules (with 3100 nucleotide residues; Kurland, 1960), are made at a rate of 23–26 nucleotides added/s.

As shown by others (Lancini & Sartori, 1968), the synthesis and labelling of the DNA is not affected by rifampicin over the time-period involved. However, as shown later, the net increase in DNA labelling ceased after about 40–50 min inhibition.

Midgley & Gray (1971) have shown that the rate of chain polymerization of the 23S rRNA in *E. coli* cultures is essentially independent of the nature of the bacterial growth medium and the growth rate, and is also unaffected by the inhibitory action of 0.2 g of chloramphenicol/l. Experiments were carried out as described above on cultures growing steadily at 37°C in enriched broth, glucose–salts and sodium lactate–salts media, in which the rates of bacterial growth are in the approximate ratio 4:2:1 (Gray & Midgley, 1970). Similarly, cultures grown in each of the three media and then inhibited

for 10 min by 0.2 g of chloramphenicol/l were inhibited with rifampicin in the presence of 5 μ Ci of [14 C]uracil. The labelling curves for DNA and for RNA in these conditions are shown in Fig. 7. The assessment of the time required for completion of 23S rRNA in each case is shown in Table 1. As shown by Midgley & Gray (1971), the rate of chain elongation of 23S rRNA is little affected by the bacterial growth rate or by the addition of chloramphenicol. This finding is essentially in agreement with the results of other workers (Bremer & Yuan, 1968; Manor, Goodman & Stent, 1969).

Protein synthesis in rifampicin-inhibited cultures of E. coli. A 50 ml culture of *E. coli* in glucose–salts medium was given 10 μ Ci of L-[14 C]leucine (sp. radioactivity 5 mCi/mmol) followed 5 min later by 0.1 g of rifampicin/l. Rapid sampling was carried out over a period of 40 min and the trichloroacetic acid-precipitable radioactivity resistant to incubation for 30 min at 100°C was measured (Fig. 8). In agreement with Lancini & Sartori (1968), it took about 15 min after rifampicin inhibition before incorporation of exogenous leucine into protein ceased. However, the results shown in Fig. 6 indicated that hybridizable mRNA molecules formed after the addition of the antibiotic persisted for a further considerable time (at least 40 min after inhibition). This shows that the cessation of protein synthesis in rifampicin-inhibited cells may not be solely the result of the complete degradation of mRNA. It is possible that the initiation of ribosomes on the mRNA may be prevented by proximal degradation of the initiator sites, without the degradation proceeding far enough to complete the hydrolysis of the mRNA fraction. Conversely, however, this finding makes it difficult to say with certainty that the events leading to a decline in protein biosynthesis in inhibited cells can be compared with the situation in steadily growing organisms. If, as has been shown, some part of the mRNA made in the presence of rifampicin remains stable in these conditions, some degree of translative capacity may be retained beyond the point normally to be expected if all the mRNA was unstable.

The results in Fig. 8 were plotted in a different way, to give some information about the decline in translative capacity in rifampicin-inhibited cells. In Fig. 9, a plot was made of (14 C radioactivity finally incorporated into protein after 20 min inhibition – 14 C radioactivity in protein at any earlier time) against the time of rifampicin inhibition. This plot measures the decline in protein-synthesizing capacity with time in the inhibited cells. A semi-log plot gave a straight line, indicating a half-life for this function of about 4 min. If this can be equated with the decay of the mRNA fraction in steadily growing cultures, as measured by average lifetime (Gray & Midgley, 1970), the value is

Fig. 7. Time-course of labelling RNA and DNA in rifampicin-inhibited cultures grown at 37°C in various media, in the presence or the absence of 0.2 g of chloramphenicol/l. Rifampicin (0.1 g/l) was added at times indicated by the arrow. For conditions of inhibition by chloramphenicol, see the text. The approximate doubling times of the growing cultures at 37°C were: broth, 33 min; glucose-salts, 61 min; and lactate-salts, 118 min. (a) Broth; (b) glucose-salts; (c) lactate-salts; (d) broth+chloramphenicol; (e) glucose-salts+chloramphenicol; (f) lactate-salts+chloramphenicol. ○, RNA labelling; ●, DNA labelling.

Table 1. Rate of chain elongation of *E. coli* 23S rRNA in various media at 37°C as calculated from the results in Fig. 7

Nucleotide addition rates were calculated by assuming that 23S rRNA is a polynucleotide of 3100 residues (mol.wt. 1.0×10^6) (Kurland, 1960). Doubling times of cultures were: broth, 33 min; glucose-salts, 61 min; lactate-salts, 118 min.

Growth medium	Time for completion of 23S rRNA in inhibited cultures (min)	RNA chain-elongation rate (nucleotides added/s at 37°C)
Broth	2.0 ± 0.2	26 ± 2
Broth+chloramphenicol	2.5 ± 0.3	21 ± 3
Glucose-salts	2.5 ± 0.3	21 ± 3
Glucose-salts+chloramphenicol	2.5 ± 0.4	21 ± 3
Lactate-salts	3.0 ± 0.4	17 ± 3
Lactate-salts+chloramphenicol	3.0 ± 0.4	17 ± 2

Fig. 8. Time-course of incorporation of L-[1- 14 C]leucine into protein in rifampicin-inhibited cultures. Rifampicin (0.1 g/l) was added at the time indicated by the arrow. For further details see the text.

Fig. 9. Half-life of the biosynthetic capacity for protein in rifampicin-inhibited cultures of *E. coli*. For further details, see the text.

somewhat larger than that expected for growing cells in glucose-salts media, dividing every 60 min.

DISCUSSION

The results presented in the present paper have fully confirmed that, at least for the 23S rRNA of *E. coli*, the rate of polymerization of new RNA chains on the specific cistrons is constant at a given temperature, and is therefore essentially unaffected by the nature of the growth medium, the bacterial growth rate or by the presence of inhibitory concentrations of chloramphenicol (Midgley & Gray, 1971). In this, our findings are in basic agreement with those of other workers (Bremer & Yuan, 1968; Manor *et al.* 1969; Rose, Mosteller & Yanofsky, 1970). Thus, from studies of the synthesis of

rRNA and mRNA in normally growing and chloramphenicol-inhibited cultures of *E. coli* (Midgley & Gray, 1971) it is apparent that the control of RNA synthesis is directed at the rate of initiation of new RNA chains rather than on the rate of polymerization of chains already in the course of completion.

Our values for the rates of chain elongation of 23S rRNA at 37°C are in agreement with those of Mangiarotti *et al.* (1968), but are consistently less than the chain-polymerization rates of 40–55 nucleotides/s quoted by others (Bremer & Yuan, 1968; Manor *et al.* 1969; Winslow & Lazzarini, 1969a,b; Rose *et al.* 1970). However, since by very different methods of approach (Midgley & Gray, 1971) we have estimated the rate of chain growth of 23S rRNA in *E. coli* to be in the region of 22 nucleotides added/s at 37°C, we consider that there is no reason to suspect that our estimations are excessively low. Any complications that might be caused by the choice of organism may be discounted, since similar experiments with *E. coli* CP78 and CP79 and with *Bacillus subtilis* at the same temperature give the same results (W. J. H. Gray & J. E. M. Midgley, unpublished work; Avery & Midgley, 1971).

The results do not permit, even in the most rigorous analysis, estimations of RNA polymerization rates more than 10–20% in excess of those quoted here. From the evidence presented in Fig. 6 of the present paper, at no time during the inhibition by rifampicin does the proportion of radioactivity in the mRNA fraction exceed one-third of that in total RNA. The possibility that the hybridization technique underestimates the mRNA contribution to the labelling patterns may be discounted, since the net loss of labelled RNA due to mRNA decay, after 4–5 min labelling, plus the hybridizable RNA that remains stable after this period is equal to the proportion of label entering the mRNA pool at early times in the labelling process. Further, most of the mRNA molecules produced in *E. coli* are probably of sizes smaller than the 23S rRNA, and so, in any case, should be completed more rapidly in rifampicin-inhibited cultures. The difficulties that might arise from our experimental designs, in that the labelled precursor was added 2 min before the addition of rifampicin, can be shown to be negligible, as experiments in which the labelled precursor and antibiotic were added together gave identical labelling patterns for the RNA (J. E. M. Midgley, unpublished work). Finally, as it is unlikely that in *E. coli* the stable rRNA molecules are produced from much larger precursors by degradative processing (Adesnik & Levinthal, 1969), errors of more than 10% in our measurements caused by underestimating the size of the precursor rRNA are not permissible.

A possible rationalization of the disparities in the estimations of polymerization rates for specific mRNA molecules (Rose *et al.* 1970) and for rRNA (Midgley & Gray, 1971) is that, for some reason, RNA chain growth is connected with the ultimate function of the molecular products. It may be that the movement of DNA-dependent RNA polymerase molecules along the cistrons depends on the presence of ribosomes on the mRNA strands behind the point of transcription. If in the synthesis of rRNA molecules, there is no concomitant ribosomal attachment and translocation of the newly formed strands (Mangiarotti & Schlessinger, 1967), the absence of ribosomes may affect the rate of growth of the RNA chains. Alternatively, the fact that active ribosomal attachment, translocation and translation is occurring on mRNA strands in the process of completion (Morse *et al.* 1969) may force transcription to take place at a higher rate. In this regard, it is notable that the rate of amino acid incorporation into protein and the transcription rate of mRNA in *E. coli* indicate similar rates of movement of ribosomes in translation and of DNA-dependent RNA polymerase in transcription (Lacroute & Stent, 1968; Rose *et al.* 1970).

The evidence that the rate of chain polymerization of stable rRNA may be different from that of translatable mRNA is of great importance in any consideration of the overall control mechanisms for RNA synthesis. It does not devalue the importance of initiation as the main regulatory mechanism for RNA synthesis in both the rRNA and mRNA fractions (Midgley & Gray, 1971), but may superimpose a further control on this special group of cistrons responsible for rRNA synthesis. Possibly, in conditions where the supply of amino acids becomes limiting, the pressure exerted by translating ribosomes eases on the transcribing polymerases, leading to a diminished rate of transcription (Winslow & Lazzarini, 1969*a,b*). However, from our results, it is possible that other factors may be more important in the control of rRNA synthesis, such as nucleotide supply.

W. J. H. G. thanks the Medical Research Council for providing a research studentship. J. E. M. M. is a Senior Research Associate in the Medical Research Council Research Group in the Structure and Biosynthesis of Macromolecules, University of Newcastle upon Tyne. We thank Mrs P. Hislop for her careful technical assistance.

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