The Control of Ribonucleic Acid Synthesis in Bacteria

STEADY-STATE CONTENT OF MESSENGER RIBONUCLEIC ACID IN *ESCHERICHIA COLI*
M.R.E. 600

By W. J. H. GRAY AND J. E. M. MIDGLEY

Department of Biochemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, U.K.

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1. The technique of DNA–RNA hybridization was used to follow changes in the amount and average lifetime of unstable messenger RNA in *Escherichia coli* M.R.E. 600 over a wide range of different growth conditions. The method of analysis was based on the kinetics of incorporation of exogenous labelled nucleic acid bases into the RNA of steadily growing cultures, as described by Bolton & McCarthy (1962). 2. The ratio of the average lifetime of messenger RNA to the mean generation time of *E. coli* cultures was constant over the temperature range 25–45°C in a given medium, but the constant varied with the nature of the growth medium. For cultures growing in sodium lactate–salts or glucose–salts media the ratio was 0.046 ± 0.005 and in enriched broth it was 0.087 ± 0.009 . Measurements of the amounts of transfer RNA, ribosomal RNA and messenger RNA were also made. The results confirmed earlier reports that the ratio of the amount of messenger RNA to the amount of ribosomes in the cells is virtually constant. On the other hand, the ratio of the amount of transfer RNA to the amount of ribosomal RNA decreased with increasing growth rate at a given temperature. 3. In cultures at temperatures higher than necessary for optimum rates of growth the average lifetime of messenger RNA lengthened in harmony with the increased time required for cell division. It seems that suboptimum growth rates at higher temperatures cannot be explained simply as a combination of increased rates of synthesis and breakdown of messenger RNA with a grossly decreased efficiency of translation. The absolute rate of messenger RNA synthesis was lowered, and its amount in the cells was typical of all other cultures grown at lower temperatures in the same medium. 4. The rate of entry of exogenous labelled uracil into unstable messenger RNA and stable ribosomal RNA was constant in all media at all temperatures in the approximate ratio 1:2. In media supporting a lower rate of growth, e.g. lactate–salts or glucose–salts media, the messenger RNA fraction constituted $2.2 \pm 0.3\%$ of the total cellular RNA. In enriched broth $3.6 \pm 0.3\%$ of the total RNA was messenger.

Although many workers have measured the steady-state concentrations of mRNA in bacterial cells by using different experimental approaches (e.g. Bolton & McCarthy, 1962; Forchhammer, Kjeldgaard & Moldave, 1965; Salser, Janin & Levinthal, 1968), most have restricted their studies to specific measurements in a given medium at one temperature. Of those studies that were intended as a systematic examination of mRNA concentrations in various environmental conditions, some have used the antibiotic actinomycin D as a means of determining the amounts and lifetime of mRNA in bacteria. The basic assumption made in these studies was that the fraction of RNA decaying to acid-soluble nucleotides after antibiotic inhibition of RNA synthesis was identifiable with mRNA only (Levinthal, Keynan & Higa, 1962; Levinthal, Fan,

Higa & Zimmermann, 1963; Fan, Higa & Levinthal, 1964; Leive, 1965). Another method (Forchhammer *et al.* 1965; Forchhammer & Kjeldgaard, 1967, 1968) used a cell-free system to measure the catalytic activity of mRNA in promoting polypeptide synthesis *in vitro*.

The accuracy of the first type of measurement is made suspect by the probability that, on inhibition of RNA synthesis by actinomycin D, precursors of mature ribosomes are also broken down (Acs, Reich & Valenju, 1963; Kennell, 1964; Fry & Artman, 1969). As the amount of these is greater than that of mRNA (McCarthy, Britten & Roberts, 1962; Mangiarotti, Apirion, Schlessinger & Silengo, 1968), this extra degradation must lead to a considerable overestimation of the true mRNA fraction (Midgley, 1969). Also, it is not clear that the rates

of mRNA degradation and turnover in these special conditions can be correlated with the rates ruling in steadily growing cultures (Fry & Artman, 1969).

The second method is capable of giving accurate measurements, so long as reliance can be placed on the belief that stimulation of polypeptide synthesis *in vitro* is a property of mRNA alone. However, there is still some uncertainty as to whether newly formed ribosomal RNA, or fragments thereof, may also stimulate the systems *in vitro* (Otaka, Osawa & Sibatani, 1964; Nakada, 1965; Holland, Buck & McCarthy, 1966; Muto, 1968; Fry & Artman, 1969). Further, difficulties may be encountered in quantifying the values obtained, which may be comparable only between different RNA samples from the same organism.

Absolute measurements of mRNA concentrations in bacterial cells can be made by the technique of DNA-RNA hybridization (Pigott & Midgley, 1968). The basic assumption made is that, in a specimen of RNA, that fraction hybridizing efficiently at DNA/RNA ratios less than 5:1 (w/w) is essentially mRNA (Pigott & Midgley, 1968). We have used this technique to assess quantitatively the relationships between the lifetime of mRNA, bacterial growth rate, the nature of the growth medium and temperature. Preliminary accounts of this work have been given (Gray & Midgley, 1968, 1969).

MATERIALS AND METHODS

Organism. *Escherichia coli* M.R.E. 600 was used. This strain is deficient in the extramembranous enzyme ribonuclease I (EC2.7.7.17).

Growth of bacterial cultures. The organism was grown under forced aeration, in the following media: (1) enriched broth; this contained (per litre) 8 g of Oxoid Brain-Heart Infusion (Oxoid Ltd., London E.C.4, U.K.) and 5 g of NaCl; (2) glucose-salts medium; this contained (per litre) 2 g of NH_4Cl , 3 g of KH_2PO_4 , 6 g of anhydrous Na_2HPO_4 , 3 g of NaCl, 0.12 g of Na_2SO_4 , 0.05 g of anhydrous MgCl_2 and 2 g of D-glucose; (3) lactic acid-salts medium; this contained the same salts as medium (2) but the glucose was replaced by 8 ml of lactic acid as sole source of carbon for growth; the medium was adjusted to pH 7.2 with M-NaOH.

The harvesting of cultures, the incorporation of radioactive substances and the preparation of cell-free extracts, DNA and RNA were carried out as described by Pigott & Midgley (1968).

Cell counting. The numbers of bacterial cells in unit volume of a given culture were determined by counting formaldehyde-killed cells on a haemocytometer slide with Thoma ruling (A. Gallenkamp and Co. Ltd., London E.C.2, U.K.), under a Zeiss RA38 microscope at 40 $\times$ magnification. Enough counts were made to ensure an accuracy of $\pm 5\%$ in the final determination of cell number/ml.

DNA-RNA hybridization. *E. coli* DNA was denatured and immobilized on cellulose nitrate membrane filters as

described by Gillespie & Spiegelman (1965). Hybridization of DNA with RNA and the assay of filter-bound materials were performed as described by Pigott & Midgley (1968).

Determination of RNA. RNA was determined either by the colorimetric method of Schneider (1957) or by its extinction in solution at 260 nm. It was assumed that the extinction coefficient of RNA was $E_{1\%}^{1\text{cm}}$ 200.

Determination of DNA. Amounts of DNA in samples of bacterial cells were determined by the diphenylamine method of Burton (1956). DNA immobilized on cellulose nitrate membrane filters was determined by the method of Pigott & Midgley (1968).

Fractionation of tRNA and rRNA. *E. coli* cultures were grown at 37°C in the media described above. Enough [^{14}C] or [^3H]-uracil was added to allow continuous incorporation over at least six cell divisions, so as to randomly label the nucleic acids. Portions (0.5 ml) of the cultures were then precipitated in ice-cold 5% (w/v) trichloroacetic acid and the acid-precipitable radioactivity (c.p.m.)/ml of cultures was measured by filtration through Oxoid (Oxo) membrane filters and by counting the radioactivity of the dry filters in a Beckman L200 scintillation counter, with correction to constant quench. Further portions (5 ml) were killed with a few drops of formaldehyde and cell counts were made. Thus the radioactivity (c.p.m.)/organism present in nucleic acid was determined. The remainder of the culture was harvested, crushed and extracted by the phenol-cresol technique of Pigott & Midgley (1968) to purify the nucleic acids. Portions of the resulting solutions were dissolved in 0.5 M-KOH and were then incubated at 37°C for 18 h. This hydrolysed the RNA, leaving the DNA virtually intact. The hydrolysates were then made up to 10% (w/v) trichloroacetic acid and the alkali-resistant acid-precipitable radioactivity (c.p.m.) was measured. The difference between this and the total acid-precipitable radioactivity (c.p.m.) accounted for the labelled RNA in the mixture. It was initially assumed that the proportions of labelled DNA and RNA in the phenol-extracted nucleic acids were the same as those in the intact cells. This assumption was verified by similar treatment of intact-cell suspensions, when the same fraction of acid-precipitable radioactivity was resistant to exhaustive digestion by alkali.

The total nucleic acids were fractionated in two ways. (1) The nucleic acids were subjected to gel filtration through columns (2 cm $\times$ 25 cm) of Sephadex G-200 (medium grade) (Pharmacia, Uppsala, Sweden) in 0.01 M-sodium acetate-acetic acid buffer, pH 4.6. rRNA, mRNA and DNA were excluded from the gel by their molecular size, whereas 5S rRNA and tRNA were not (e.g. Midgley, 1965). The radioactivity (c.p.m.) in the labelled nucleic acids acting as material of very high molecular weight (rRNA + mRNA + DNA) was now measured by trichloroacetic acid precipitation of portions (1 ml) of the appropriate pooled fractions. Further portions were incubated with 0.5 M-KOH to allow corrections to be made for the presence of the DNA. The radioactivity (c.p.m.) present as rRNA + mRNA could now be compared with that in the fractions containing tRNA + 5S rRNA. A further correction was necessary because the nucleotide base composition of tRNA is different from that of rRNA. The pyrimidine nucleotide content of tRNA was taken as 47 mol/100 mol and that of rRNA as 42 mol/100 mol

(Midgley, 1962; Stanley & Bock, 1965). As the specific radioactivity of the UMP and CMP residues of RNA randomly labelled by $[2\text{-}^{14}\text{C}]$ - or $[5\text{-}^3\text{H}]$ -uracil is the same, the radioactivity (c.p.m.) measured in the fractions containing rRNA was multiplied by the factor 1.12 to account for compositional differences. Finally, the contamination of the tRNA peak with 5S rRNA was corrected for. It is known that the molecular weight of 5S rRNA is about 4×10^4 (Brownlee & Sanger, 1967) and that there is only one molecule of 5S rRNA in a 70S ribosome (Rosset, Monier & Julien, 1964). As the molecular weight of the remaining rRNA in a 70S ribosome is 1.65×10^6 (Stanley & Bock, 1965), about 2.5% of the rRNA is in the form of the 5S component. It should also be noted that mRNA is a minor contaminant of the rRNA fraction. Initially no allowances were made for this, and the values in the tables are given for rRNA+mRNA. (2) The nucleic acids were fractionated by ion-exchange column chromatography on DEAE-cellulose. A 100g portion of Bio-Rad Cellex D (exchange capacity 0.87 mequiv./g) (Calbiochem, Los Angeles, Calif., U.S.A.) was suspended in 1 litre of 0.01 M-tris-0.01 M-MgCl₂ buffer, adjusted to pH 7.2 with HCl. After the suspension had been left to settle for 2 h the fines were removed by decantation. This process was repeated twice. The DEAE-cellulose was then filtered at the pump and washed with 1 litre of 0.1 M-NaOH and was again filtered. It was resuspended in 1 litre of M-HCl and filtered, and was then suspended in water and filtered until the washings were virtually acid-free. The material was finally suspended in 1 litre of tris-MgCl₂-HCl buffer, and was stored in a sealed dark bottle at 5°C. Columns (1 cm $\times$ 10 cm) were prepared. The nucleic acids were applied in the above buffer. They were then fractionated by stepwise elution with 0.6 M-NaCl in the same buffer. DNA, tRNA and probably 5S rRNA were eluted, whereas the remaining mRNA and rRNA were tightly bound, being eluted only with difficulty by 1.2 M-NaCl (Midgley, 1962; Stanley & Bock, 1965). The nucleic acids eluted by 0.6 M-NaCl were pooled, and the contribution of DNA was determined by incubation with KOH as described above. The radioactivity (c.p.m.) was also corrected for as before for 5S rRNA contamination and for differences in specific radioactivity of tRNA and rRNA. Tests of the efficiency of elution of tRNA from the columns by 0.6 M-NaCl, with pure tRNA, showed that more than 98% was released. This method of separation of different RNA species has the advantage of being based on the differing secondary structure of the various species (Stanley & Bock, 1965). Further, the degradation of high-molecular-weight RNA to sizes comparable with that of tRNA does not alter its chromatographic characteristics (Midgley, 1962). Thus, unlike the first method, ion-exchange column chromatography on DEAE-cellulose gives results that are independent of slight degradation in the high-molecular-weight fraction of rRNA. However, the non-enzymic method of preparation of RNA has been shown to produce undegraded rRNA (Midgley, 1965), mRNA and tRNA (Volkin & Riggsby, 1969).

Determination of the cell ribosome content. From knowledge of the radioactivity (c.p.m.)/organism present as RNA, the fraction of the radioactivity in tRNA and rRNA, their specific radioactivities and the molecular weight of the rRNA in a 70S ribosome (1.69×10^6) the number of ribosomes/cell can be calculated. The nucleic acids were

incubated with 10 μ g of deoxyribonuclease (EC 3.1.4.5) (ribonuclease-free; Worthington Biochemical Corp., Freehold, N.J., U.S.A.)/ml for 30 min at room temperature. The remaining RNA was again extracted with phenol-cresol mixture and was subjected to gel filtration on Sephadex G-200 as described above. The specific radioactivity of the high-molecular-weight fraction was obtained (16S+23S rRNA). By multiplying this value by the factor 1.12, the corresponding value was given for tRNA (see above for derivation of this factor).

Radiochemicals. $[5\text{-}^3\text{H}]$ Uracil (sp. radioactivity 1000 mCi/mmol) and $[2\text{-}^{14}\text{C}]$ uracil (sp. radioactivity 54.5 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

RESULTS

Bacterial growth rate in the presence of exogenous uracil. In cultures of *E. coli* M.R.E. 600 considerable changes occurred in the rates of cell division when exogenous uracil was added to the medium. This phenomenon was most pronounced when the base was added to cultures growing in media supporting only a low rate of growth, e.g. lactate-salts or glucose-salts medium. The effect was also more prominent at temperatures less than 35°C. The mean generation time was decreased by as much as 25% by the addition of uracil. Table 1 shows the growth rates typical of cultures used in this study when uracil was present or absent. The concentration of exogenous base had no further effect on the degree of acceleration of growth. Fig. 1 shows the details of a typical growth curve of a culture growing in glucose-salts medium at 30°C. The acceleration in growth caused by uracil was virtually instantaneous.

Since the kinetic analysis of RNA labelling requires the stabilization of growth rates during an experiment, it is essential to add unlabelled carrier some minutes before labelling is commenced. It is likely that some reaction in the biosynthesis of pyrimidine nucleotides *de novo*, earlier than the point of entry of exogenous uracil, is rate-limiting for nucleic acid synthesis in this strain. Such accelerations were not noted in *E. coli* K 12 cultures.

This phenomenon was not appreciated in the preliminary studies by Gray & Midgley (1969). However, if their estimate of mRNA is raised by the factor 1.1-1.2 to account for growth rate changes, their results agree with those presented in this paper.

Determination of the rRNA, tRNA and 70S ribosome content of *E. coli*. A detailed study has been made of the relationships between the rates of synthesis of tRNA and rRNA in bacterial cells in different growth media (Kjeldgaard & Kurland, 1963; Maaløe & Kjeldgaard, 1966). Briefly, it was shown that, with increasing rates of growth at a given temperature, the ratio of the cellular contents

Table 1. *Rates of growth of E. coli M.R.E. 600 cultures in various media*

Growth medium	Temp (°C)	Cell division time (min)	
		Before addition of uracil	After addition of uracil
Broth	25	95	73
	30	47	44
	35	33	33
	40	28	28
	45	53	50
Glucose-salts	25	223	167
	30	122	87
	35	84	57
	40	63	53
	45	110	94
Lactate-salts	25	300	280
	30	199	163
	35	144	114
	40	106	92
	45	284	142

Fig. 1. Growth curve for a culture of *E. coli* M.R.E. 600 in glucose-salts medium at 30°C in the presence or the absence of exogenous uracil.

of tRNA to those of rRNA decreased. Non-coordinate control was thus implicated in the bio-synthesis of these RNA fractions. Although this finding was challenged by Rosset, Julien & Monier (1966), it was later substantiated by Forchhammer & Kjeldgaard (1968). These latter authors also found that the ratio of the amount of mRNA to the amount of rRNA was virtually constant in cells grown in various environments.

Similar measurements for tRNA and rRNA (for details see the Materials and Methods section) are shown in Table 2. In Table 3 measurements of the

number of DNA genome equivalents/cell, the number of tRNA molecules/cell and the number of 70 S ribosomes/cell are given for *E. coli* M.R.E. 600 grown in three media. Fig. 2 indicates the relationship between bacterial growth rate and nucleic acid content. The results for the DNA content of bacterial cultures were in agreement with those of Cooper & Helmstetter (1968).

In general the results confirmed the findings of Maaløe & Kjeldgaard (1966). The ribosome and tRNA contents of the cells rise with increasing growth rate of cultures, as does the amount of DNA. The rRNA content, expressed per DNA genome equivalent, remains constant, and the proportion of RNA as tRNA decreases with increasing growth rate.

mRNA content and lifetime in E. coli. Steadily growing cultures of *E. coli* in each of three media (see the Materials and Methods section), were allowed to incorporate [5-³H]uracil into their nucleic acids. Samples (50 ml) of the cultures were removed at intervals and plunged into crushed ice, and the RNA was isolated by phenol-cresol extraction of broken cells, in a suitable buffer. The RNA was then purified further in preparation for DNA-RNA hybridization (Pigott & Midgley, 1968). Hybridization was then carried out by using the membrane-filter technique of Gillespie & Spiegelman (1965). The labelled RNA that was hybridized was corrected for non-specific binding and for a small rRNA component (Pigott & Midgley, 1968). Typical kinetic plots of the time-course of incorporation of [5-³H]uracil into the various RNA fractions are shown in Fig. 3. This shows that, in all growth media, the early kinetics of incorporation of labelled precursors into RNA give the same ratio of the

Table 2. Nucleic acid content of *E. coli* M.R.E. 600 cells in various media at 37°C

Growth medium	Method of estimation	% of RNA species in total RNA	
		rRNA+mRNA	tRNA
Broth	Gel filtration	(i) 79.5	20.5
		(ii) 76.9	23.1
		(iii) 77.8	22.2
	DEAE-cellulose	(i) 80.4	19.6
		(ii) 78.5	21.5
		(iii) 79.2	20.8
	Mean value	78.8	21.2
Glucose-salts	Gel filtration	(i) 66.4	33.6
		(ii) 67.1	32.9
		(iii) 65.0	35.0
	DEAE-cellulose	(i) 65.2	34.8
		(ii) 64.2	35.8
		(iii) 64.5	35.5
	Mean value	65.4	34.6
Lactate-salts	Gel filtration	(i) 49.1	50.9
		(ii) 49.5	50.5
		(iii) 48.0	52.0
	DEAE-cellulose	(i) 47.7	52.3
		(ii) 47.0	53.0
		(iii) 48.0	52.0
	Mean value	48.3	51.7

Table 3. Nucleic acid content of *E. coli* M.R.E. 600 cells at 37°C in various media

It was assumed that the molecular weight of 1 genome equivalent in *E. coli* DNA is 2.8×10^9 (Kit, 1963).

Growth medium	No. of doublings/h	DNA genomes/cell	Ribosomes/cell	Ribosomes/genome	tRNA molecules/cell	tRNA molecules/genome
Broth	1.74	3.4	2.2×10^4	6.6×10^3	4.04×10^5	1.19×10^5
Glucose-salts	0.97	2.2	5.8×10^3	2.7×10^3	2.20×10^5	1.02×10^5
Lactate-salts	0.34	1.6	2.3×10^3	1.4×10^3	1.49×10^5	0.93×10^5

Fig. 2. Nucleic acid content of *E. coli* cells from cultures grown in various media at 35°C. The nucleic acid content and cell division rate of *E. coli* cultures growing in sodium lactate-salts medium was set at unity. Δ , rRNA; $\bullet$, tRNA; $\circ$, DNA.

Fig. 3. Kinetics of incorporation of [5-³H]uracil into the RNA of *E. coli* cultures growing in various media. (a) Long-term kinetics of incorporation of exogenous uracil into total RNA and mRNA of a culture in lactate-salts medium at 40°C. (b)–(d) Early kinetics of incorporation of label into total RNA and mRNA in cultures growing in (b) enriched broth at 30°C, (c) glucose-salts medium at 25°C and (d) lactate-salts medium at 30°C. ○, Total RNA; ●, mRNA (in *a* corrected for exponential expansion of the pool).

radioactivity (c.p.m.) in hybridized material to that in non-hybridized material. It should be remembered that the incorporation of labelled precursors into tRNA is appreciably delayed (Midgley & McCarthy, 1962; Midgley, 1963), so that the early kinetics of labelling involve mRNA and rRNA only. DNA labelling is similarly delayed (Midgley & McCarthy, 1962). Thus in *E. coli* cells grown in different media a variable fraction of tRNA in total RNA will have a minimal effect on the very early patterns of labelling, although, of course, it will exert an effect proportional to its amounts in the cells at later times.

The mRNA content of the total cellular RNA was then calculated. For simplicity it was initially assumed that the model of Britten (1963) held for the nucleotide flows into RNA and that the diluting effect of the intracellular nucleotide pool is negligible. Thus it was assumed that the specific radioactivity of the newly formed RNA is the same as that of the exogenous precursor. If S is the specific radioactivity of the precursor, T_0 is the amount of RNA initially present and T is the amount at any later time t , then, if the cultures are growing exponentially:

$$T - T_0 = T_0(e^{kt} - 1) \quad (1)$$

where k is the growth constant. Thus the radioactivity (c.p.m.) in the newly formed RNA will be given by:

$$S \cdot T_0(e^{kt} - 1) \quad (2)$$

Considering mRNA (hybridizable RNA) at times when the mRNA pool is saturated with labelled material, pre-existing unlabelled RNA will have broken down and will have been replaced by labelled RNA. Thus the radioactivity (c.p.m.) in the mRNA fraction will be given by:

$$S \cdot M_0 \cdot e^{kt}$$

where M_0 is the amount of mRNA at the start of labelling. If the radioactivity (c.p.m.) in the mRNA fraction after saturation of its pool is corrected for exponential expansion, we can set the radioactivity (c.p.m.) in hybridized mRNA equal to $S \cdot M_0$.

Graphically, when radioactivity (c.p.m.) in total hybridized RNA is plotted against t , we can extrapolate the corrected saturation plot for hybridized mRNA to the line for the incorporation into total RNA. Now:

$$S \cdot M_0 = S \cdot T_0(e^{kt} - 1) \quad (3)$$

If the exponential term is expanded, then to a first approximation;

$$M_0 = T_0 \cdot kt \quad (4)$$

Table 4. *Amount and lifetime of mRNA in E. coli RNA in various environments*

The assumption implicit in these calculations was that the relative amounts of tRNA and (rRNA+mRNA) in the cells were virtually unaffected by temperature but were affected by the nature of the growth medium employed.

Growth medium	Temp. (°C)	Cell doubling time (min)	% of mRNA in total RNA	Average lifetime of mRNA (min)
Broth	25	73	3.3	5.6
	30	44	3.9	4.2
	35	33	3.6	2.9
	40	28	3.5	2.4
	45	50	3.7	4.5
Glucose-salts	25	167	2.3	8.0
	30	87	2.2	4.0
	35	57	2.1	2.5
	40	53	2.5	2.8
	45	94	2.0	3.9
Lactate-salts	25	282	2.2	12.9
	30	163	2.3	7.8
	35	114	2.1	5.0
	40	92	2.4	4.6
	45	142	2.1	6.2

The growth constant $k = (\ln 2)/D$, where D is the doubling time of the cells. Thus the fraction:

$$M_0/T_0 = 0.69 t/D \quad (5)$$

As, in practice, it cannot be assumed that the specific radioactivities of the exogenous precursors and newly formed RNA are identical owing to intracellular dilution by the nucleotide pool, this estimate is somewhat high. The specific radioactivity of the saturated mRNA pool will be higher than that of the total newly formed RNA at early times. However, the proportion of exogenous precursor directly entering the RNA fractions by by-passing the intracellular nucleotide pool is about 50% (Buchwald & Britten, 1963), so that this error is not large. The factor $\ln 2$ was omitted in Midgley's (1969) calculations for *Bacillus subtilis* cultures, and his estimate of the mRNA content of this organism should be decreased to 2.1% of the total RNA.

The average lifetime of the mRNA in each culture was calculated as shown by Midgley (1969), from the observed kinetics of entry of labelled precursors into the hybridized RNA fractions. Table 4 shows the calculated amounts of mRNA in *E. coli* M.R.E. 600 cultures in each of three media at five different temperatures. Fig. 4 shows the relationships between the average lifetime of the mRNA, the mean generation time of cell division and temperature for cultures in each medium.

Table 4 shows clearly that variations in temperature over the range 25–45°C have no effect on the amount of mRNA in cultures growing in a given medium. However, the amount of mRNA in the

cultures growing in enriched broth at high growth rates is greater proportionally in the total RNA than is the case for cultures growing more slowly in glucose-salts or lactate-salts medium. Detailed examination of the kinetics of incorporation of labelled precursors into mRNA and stable rRNA at early times also showed no change in the ratio of the radioactivity (c.p.m.) incorporated with changes in temperature of medium.

When the ratio of weight of mRNA to weight of rRNA is calculated from the results in Tables 2 and 4, the values obtained are 0.046, 0.033 and 0.044 for cultures growing at 35°C in broth, glucose-salts and lactate-salts media respectively. It is clear that the proportions of mRNA and rRNA form an almost constant ratio, regardless of the nature of the growth medium or the growth rate.

DISCUSSION

Bacterial cells respond to long-term changes in their nutritive environment by altering their size and internal composition to suit the new conditions. Of especial importance is the finding that the protein and nucleic acid content of the cells may alter considerably so that cellular concentrations of DNA, 70S ribosomes and tRNA rise when the growth rate is increased by enrichment of the external medium (e.g. Maaløe & Kjeldgaard, 1966). This phenomenon has been linked with a positive control determined directly or indirectly by the supply of amino acids in the cells (Gros & Gros, 1956; Pardee & Prestidge, 1956). Although several theories have been put forward to explain in detail

the interrelationships between amino acid supply and nucleic acid synthesis, none has yet succeeded in satisfying unequivocally all the known facts.

Detailed work has been carried out by Maaløe & Kjeldgaard and their co-workers (e.g. Maaløe & Kjeldgaard, 1966) on the control of biosynthesis of the stable intracellular nucleic acids. Generally the results in the present paper agree with their findings, although, in some ways, *E. coli* M.R.E. 600 is an aberrant strain of the species. For example, its growth rate in a given medium is often lower than those of related strains such as *E. coli* K12 or B (W. J. H. Gray, unpublished work), and it appears to have a growth-limiting lesion in the pathways of biosynthesis of pyrimidine nucleotides. Nevertheless the same basic relationships were discovered between DNA, rRNA and tRNA synthesis as were described by Maaløe & Kjeldgaard (1966) for *Salmonella typhimurium*.

There is less comprehensive information about the relationships between the synthesis of stable RNA and unstable mRNA in bacteria. Forchhammer & Kjeldgaard (1968), on the basis of an assay of mRNA in a protein-synthesizing system *in vitro*, suggested that the proportion of mRNA in the cells always has a fixed relationship with that of the rRNA; in other words there is always a constant ratio between the weight of mRNA and the weight of 70S ribosomes, in whatever medium the bacteria are growing. The present work has verified this constancy and has suggested that it is probably independent of growth medium and of temperature. It thus appears that, for maximum cell economy, the amount of the ribosomal apparatus for protein synthesis may vary with the nature of the growth medium, but the efficiency with which it is used in growing cells is constant in all media studied. From our results it seems that the mRNA constitutes about 4.0% of mRNA+rRNA. Our results are in generally good agreement with those from earlier studies (McCarthy & Bolton, 1964; Leive, 1965; Mangiarotti & Schlessinger, 1967; Kennell, 1968; Mueller & Bremer, 1968; Pigott & Midgley, 1968; Salser *et al.* 1968). If all mRNA is in the form of polyribosomes (Mangiarotti & Schlessinger, 1967) this means that, on the average, the ribosomes in the polyribosomal aggregates are separated by at least 7×10^4 molecular-weight units of mRNA (i.e. their centres are about 700 Å apart). This distance is about three times the diameter of the 70S ribosome

Fig. 4. Relationships between cell division time, the average lifetime of mRNA and temperature in *E. coli* cultures growing in various media. (a) Enriched broth; (b) glucose-salts medium; (c) lactate-salts medium. ●, Average lifetime of mRNA; ○, cell division time.

(Spirin, 1963). This may represent the nearest permitted approach of these particles in the polyribosomes. Our evidence thus supports the concept that the amount of mRNA in the cell is directed by the absolute number of ribosomes and that the rate of synthesis of new mRNA chains is dependent on the frequency of release of ribosomes from polyribosomes after completion of translation (Stent, 1964, 1967).

Additionally notable is the finding that, at temperatures higher than necessary for the optimum rate of cell growth, the rate of synthesis of mRNA and rRNA fractions falls in harmony with the decreased rate of cell division. Further, the average lifetime of the mRNA rises. The increased lifetime of the mRNA can be explained by proposing that, at these higher temperatures, the supply of amino acids as aminoacyl-tRNA to the sites of protein biosynthesis may be diminished and the rate of ribosomal translation of mRNA may be lowered. Decreased rates of ribosomal translation of mRNA may thus increase the average lifetime of mRNA molecules, as the movement of degrading exonucleases along the mRNA chains may be dependent on ribosome movement (Kuwano, Schlessinger & Apirion, 1970).

The decreased rates of mRNA and rRNA synthesis at higher temperatures are explicable in either of two ways. The overall rate of synthesis of RNA molecules from DNA templates is dependent both on the rate of initiation of new chains by DNA-dependent RNA polymerase and on the rate of polymerization of chains in the process of transcription. A decrease in either of these parameters would give rise to lower rates of RNA synthesis. We have evidence (W. J. H. Gray & J. E. M. Midgley, unpublished work) that, although the rate of polymerization of initiated RNA chains continues to rise as the temperature is increased past the point giving optimum rates of bacterial cell growth, the rate of chain initiation falls off rapidly at higher temperatures, so that the rate of RNA synthesis declines overall. The effects of higher temperatures on bacterial growth rates can be seen to be due in part to a greatly decreased rate of initiation of RNA chains on the DNA cistrons, even though the rate of polymerization of those chains that succeed in being initiated is increased. New steady states of mRNA and rRNA content/cell may be set up at lower growth rates, but there may again be maintenance of the greatest possible efficiency of ribosomal attachment to mRNA and ribosome translation. We thus envisage the control of RNA synthesis in steady states of bacterial growth to be primarily concerned with the initiation of RNA chains by DNA-dependent RNA polymerase, in agreement with the suggestions of Maaløe & Kjeldgaard (1966).

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