The Control of Ribonucleic Acid Synthesis in Bacteria

FLUCTUATIONS IN MESSENGER RIBONUCLEIC ACID SYNTHESIS IN CULTURES RECOVERING FROM AMINO ACID STARVATION

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(Received 19 July 1973)

Changes in the cell content and rate of synthesis of mRNA were studied in auxotrophs of *Escherichia coli* recovering from a period of amino acid deprivation. Parallel studies were carried out on bacterial strains inhibited with trimethoprim, when glycine and methionine were added to relieve an amino acid deficiency. In the latter case, protein synthesis was still severely inhibited through a lack of *N*-formylmethionyl-tRNA^{fMet} for chain initiation, so that fewer ribosomes were attached to mRNA chains. (1) In RC^{str} strains recovering from amino acid starvation, there was a transient oversynthesis of mRNA, but the amounts returned to normal after about a 15-min period of recovery. RC^{rel} strains did not show this effect; any extra mRNA accumulated during the previous starvation period was rapidly lost, but no oversynthesis occurred during the resumption of growth. (2) In trimethoprim-inhibited cultures supplemented with glycine and methionine, mRNA was produced at the same rate, relative to stable RNA species, as during normal growth. The evidence implied that decreased rates of ribosome attachment had no effect on the functional or chemical lifetime of the mRNA fraction. This suggests that mRNA stability does not depend on the frequency of translation by ribosomes. (3) Changes in the mRNA contents of trimethoprim-inhibited RC^{str} and RC^{rel} cultures were noted soon after supplementation with glycine and methionine. These closely followed those observed in cultures recovering from simple amino acid withdrawal.

When bacteria are inhibited with trimethoprim the tetrahydrofolate pool is depleted (Burchall & Hitchings, 1965) and several precursors of macromolecules rapidly become in short supply (Shih *et al.*, 1966). Synthesis of RNA and DNA can be resumed in *Escherichia coli* only when suitable nucleotide bases are added (Shih *et al.*, 1966; Moivic & Pizer, 1971; Dale & Greenberg, 1972; Then & Angehrn, 1972; Harvey, 1973; Smith & Midgley, 1973*a*). However, if amino acids are not supplied, the cells become deprived of glycine and methionine. In RC^{str} but not RC^{rel} strains, the expression of the RC function then decreases the rate of stable RNA accumulation (Shih *et al.*, 1966; Dale & Greenberg, 1972; Then & Angehrn, 1972; Smith & Midgley, 1973*a*) with the accumulation of ppGpp (guanosine tetraphosphate) in the nucleotide pools (Smith & Midgley, 1973*b*). Protein synthesis does not resume at a normal rate, even if all 20 amino acids are added as supplements (Dale & Greenberg, 1972; Then & Angehrn, 1972; Harvey, 1973; Midgley & Smith, 1973; Smith & Midgley, 1973*a*). With RC^{rel} strains, protein synthesis is similarly restricted, though the addition of glycine and methionine corrects inaccurate translation arising from amino acid lack (Hall & Gallant, 1971; Midgley & Smith, 1973). The permanent decrease

in the rate of protein synthesis in trimethoprim-inhibited cultures supplemented with amino acids is probably due to a lack of *N*-formylation of methionyl-tRNA^{fMet} for chain-initiation processes (Eisenstadt & Lengyel, 1966; Lengyel, 1967; Kondo *et al.*, 1968). However, Harvey (1973) has shown that in cells growing in certain richly supplemented media, though not in glucose-salts, protein chain initiation by aminoacylated tRNA^{fMet} can occur without the initial formylation of tRNA-bound methionine that is usual in prokaryotic systems (Adams & Capecchi, 1966; Sundararajan & Thach, 1966).

Lower rates of protein synthesis in trimethoprim-inhibited bacteria could also be due, in part, to decreases in the amount of mRNA made available for translation. Imamoto (1973) postulated a close relationship between the rate of ribosome translocation of mRNA chains and the rate of transcription of the *trp* mRNA system. A logical extension of this idea might be that the permitted rate of ribosome attachment to mRNA chains is a rate-limiting step for mRNA synthesis. Supporting this possibility, greatly decreased amounts of poly-ribosomes have been observed in trimethoprim-inhibited bacteria (Phillips & Franklin, 1969; Harvey, 1973; J. E. M. Midgley, unpublished work).

Alternatively, if mRNA is produced at normal rates, a large decrease in the rate of ribosome attachment may affect the molecular stability of the mRNA chains. If fewer ribosomes are present on each mRNA strand, then the relatively unprotected molecules will be more open to attack by nucleases (see, e.g., Morse & Guertin, 1971).

In the present paper, we have examined possible interrelationships between the rate of supply of formylated or nonformylated methionyl-tRNA^{fMet} (Harvey, 1973) for protein chain initiation (and therefore ribosome-mRNA complex formation; Hille *et al.*, 1969), the rate of mRNA synthesis and its stability in the cells. Within the limits of a probable fivefold decrease in the rate of protein chain initiation (Smith & Midgley, 1973a) there was no discernible effect on the synthesis and stability of mRNA in *E. coli*. We have also observed an accumulation of mRNA in RC^{str}, but not RC^{rel}, strains shortly after the addition of glycine and methionine to trimethoprim-inhibited cultures. On comparing these effects with those in auxotrophs recovering from a period of amino acid deprivation, it appears that such changes are typical of starved cells soon after the resumption of growth and do not result from the more widespread effects of trimethoprim.

Materials and Methods

Methods

The strains of bacteria used were *E. coli* CP78 (F⁻, Thr⁻, Leu⁻, His⁻, Arg⁻, Mal⁻, Xyl⁻, Ara⁻, Gal⁻, Sm^s, RC^{str}) and CP79 (isogenic with CP78 except for RC^{rel}). The bacteria were grown with forced aeration in the media described by Gray & Midgley (1972). Cultures inhibited with trimethoprim were invariably given adenine and guanosine (30 µg/ml of each) 10 min before the addition of the drug. Methods for the harvesting of cultures, radioactive labelling and extraction of RNA are given by Smith & Midgley (1973a). The method of DNA-RNA hybridization was essentially that of Pigott & Midgley (1968), by using the cellulose nitrate membrane filter technique of Gillespie & Spiegelman (1965). Measurements of the mRNA content of randomly labelled bacterial RNA were made by the technique of 'coincident hybridization' (Midgley & Gray, 1971). The relative content of labelled mRNA in rapidly labelled specimens was measured by a modification of this technique. A ¹⁴C-labelled specimen of rapidly labelled RNA was obtained by allowing a culture of *E. coli* growing in glucose-salts medium to incorporate [2-¹⁴C]uracil (sp. radioactivity 54 mCi/mmol; concentration 0.2 µCi/ml) for 1 min. A suitable amount of the RNA was mixed with any other specimen of rapidly labelled

RNA obtained by adding [5-³H]uracil (sp. radioactivity 0.1–1 Ci/mmol; concentration 0.5–1 µCi/ml). The proportion of ³H-labelled RNA that hybridized at the overall DNA/RNA ratio of 5:1 used (Pigott & Midgley, 1968) was measured by comparing the ¹⁴C/³H ratio in the hybrid with the ratio in the input material. A Beckman LS200 three-channel liquid-scintillation counter was used to count the radioactivity of specimens. Measurements of the ¹⁴C contributions to count rates in each of the three channels were made by the 'swamp' technique. After counting the samples normally as filter-bound material in a toluene-based scintillant (Gray & Midgley, 1970), the filters were removed and dried. Enough ¹⁴C-labelled uracil was placed on each filter to swamp out the ³H contributions to the count rate. The filters were again dried and replaced in the scintillant. The vials were again counted, and the ratio of c.p.m. in the three channels was used to calculate the ¹⁴C/³H c.p.m. ratio (corrected to constant quench) in the original samples. Small corrections were made for non-specific binding of RNA to the filters and for rRNA hybridized at low efficiency to its specific DNA sites (Midgley & Gray, 1971). Filters that fortuitously bound large amounts of RNA non-specifically could easily be detected. In this way, changes of the order of 10% in the relative amount of mRNA in rapidly labelled and randomly labelled cultures could be measured.

Antibiotics

Rifampicin was a gift from Lepetit Pharmaceuticals Ltd., Maidenhead, Berks., U.K. Trimethoprim lactate was a gift from Wellcome Research Laboratories, Dartford, Kent, U.K.

Radiochemicals

[2-¹⁴C]Uracil, (sp. radioactivity 54 mCi/mmol), [5-³H]uracil (sp. radioactivity 1 Ci/mmol) and L-[U-¹⁴C]tyrosine (sp. radioactivity 405 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Results

Changes in the relative rates of synthesis of mRNA and stable RNA in cultures recovering from amino acid starvation

Gray (1970) showed that in RC^{str}, but not RC^{rel}, amino acid auxotrophs of *E. coli* recovering from a period of amino acid deprivation, the rate of synthesis of mRNA relative to that of stable RNA was at first higher than in steadily growing organisms (Sarkar & Moldave, 1968; Lazzarini & Winslow, 1970; Lazzarini & Dahlberg, 1971; Stamato & Pettijohn,

1971; Gray & Midgley, 1972). As normal rates of stable RNA synthesis were resumed the relative flux of precursors into mRNA was not typical of the value found in normal growth for some minutes after the addition of the required supplements. Gray's (1970) findings suggested that there may be over-synthesis of mRNA in the early stages of recovery, or a temporary decrease in the rate of degradation of the mRNA fraction.

Gray's (1970) experiments used the usual technique of simple hybridization of RNA labelled by only one radioisotope. However, to determine accurately any changes in the relative fluxes of radioactive precursors entering mRNA and stable RNA in recovering cells, the technique of 'coincident hybridization' (see the Methods and Materials section) was used.

Cultures of RC^{str} or RC^{rel} strains were starved of amino acids for 30 min at 37°C. Required amino acids (50 µg/ml) were then added. Samples of the recovering cultures were taken at intervals and labelled for 1 min with [5- 3H]uracil (sp. radioactivity 1 Ci/mmol; concentration 1 µCi/ml). RNA extracted from the samples was co-hybridized with rapidly ^{14}C -labelled RNA obtained from a culture steadily growing in supplemented glucose-salts medium. The relative flux of radioactivity entering the mRNA fraction in the 3H -labelled cultures was calculated by comparing the $^3H/^{14}C$ ratio in the hybridized RNA with the ratio in the input (see the Methods and Materials section for details). The results are shown in Fig. 1.

Similar experiments were carried out, in which RC^{str} or RC^{rel} cultures were first inhibited with

Fig. 1. Percentage of hybridizable mRNA in the rapidly labelled RNA of RC^{str} and RC^{rel} cultures recovering from amino acid starvation

Samples of recovering cultures were labelled with [5- 3H]uracil for 1 min (for details see the text) and the labelled RNA was co-hybridized with a rapidly ^{14}C -labelled RNA standard (see the Methods and Materials section). (a) Strain CP78 (RC^{str}). (b) Strain CP79 (RC^{rel}). ○, Recovering cultures; ●, growing control.

Fig. 2. Percentage of hybridizable mRNA in the rapidly labelled RNA of RC^{str} and RC^{rel} cultures inhibited with trimethoprim, and then given glycine and methionine

The sampling and labelling of cultures was as described in Fig. 1, and the hybridization of mRNA was measured by co-hybridization with a standard rapidly ^{14}C -labelled RNA. (a) Strain CP78 (RC^{str}), (b) Strain CP79 (RC^{rel}). ○, Cultures given glycine and methionine at time zero; ●, growing control.

Fig. 3. Kinetics of RNA labelling in cultures of RC^{str} *E. coli* first starved of amino acids and then given the amino acids simultaneously with the addition of labelled uracil

(a) Strain CP78 starved of amino acids by simple withdrawal. (b) Strain CP78 deprived of glycine and methionine through trimethoprim inhibition.

trimethoprim (50 μ g/ml) in the presence of exogenous adenine and guanosine. In these conditions the cells became deprived of glycine and methionine (Then & Angehrn, 1972; Smith & Midgley, 1973a). After 30 min of incubation with the drug, glycine and methionine (50 μ g/ml of each) were added to inhibited cultures when stable RNA synthesis (but not protein synthesis) was immediately resumed at a high rate (Smith & Midgley, 1973a). Rapidly 3H -labelled RNA was obtained at suitable times, as described above, and was co-hybridized with standard rapidly ^{14}C -labelled RNA (Fig. 2).

In both cases, the relative flux of radioactivity into the mRNA fraction of RC^{str} strains was high at the onset of recovery, and declined steadily to values typical of normal growth over the next 10 min. Conversely, the flux into the mRNA of RC^{rel} cultures at no time was altered from the usual values found in steadily growing cells. Since it took a finite time for the relative flux into mRNA to settle to normal values in the recovering RC^{str} strain, it might be considered that, over the first 10 min, the rate of synthesis of stable RNA had not reached a steady maximum value.

If the kinetics of labelling of the total nucleic acids was followed after the addition of amino acids to starved RC^{str} cultures, there was a delay of about 2 min before the rate of RNA labelling became more like that in normally growing cultures (Fig. 3). In this early period, the initial rate of RNA labelling

was very low, since the cells were relatively inaccessible to exogenous nucleotide precursors (Edlin & Neuhaud, 1967). Thus even though there was a relatively high flux of material into the messenger RNA fraction, this should not affect the later kinetics of labelling of mRNA and stable RNA at times when the overall labelling rates were much greater. The fact that steady, high rates of RNA labelling recommenced soon after amino acid addition and continued through a period in which the relative flux of precursors into mRNA was decreasing, suggests that there was oversynthesis of mRNA in the early stages of recovery (Gray, 1970). In RC^{rel} strain CP79 there was no evidence of such overproduction, as the relative rates of mRNA and stable RNA labelling did not alter (Figs. 1b and 2b).

Fluctuations in the mRNA content of RC^{str} and RC^{rel} cultures during recovery

The results in Figs. 1 and 2 show that the relatively high proportion of precursors entering the mRNA fraction in recovering RC^{str} cultures persisted for some 5–10 min into the recovery period. To test that this actually led to temporary increases in the mRNA content of recovering cells, randomly labelled cultures were used. Cultures of *E. coli* CP78 (RC^{str}) and CP79 (RC^{rel}) were grown

[Fig. 4. mRNA content of RC^{str} and RC^{rel} cultures recovering from amino acid starvation

Randomly 3H -labelled RNA from recovering cultures was co-hybridized with randomly ^{14}C -labelled standard RNA from a growing culture (see the Methods and Materials section). The percentage of mRNA in the ^{14}C -labelled standard was set at 2.2% (by wt.) of the total RNA (Gray & Midgley, 1970). Any inaccuracy in this value does not affect the accuracy of other values if expressed as relative changes in mRNA contents. (a) Strain CP78 (RC^{str}). (b) Strain CP79 (RC^{rel}). $\circ$, Recovering cultures; $\bullet$, growing control.

Fig. 5. mRNA content of RC^{str} and RC^{rel} cultures inhibited with trimethoprim and given glycine and methionine

The labelling of cultures and the method of hybridization of labelled RNA were as described in Fig. 4. (a) Strain CP78 (RC^{str}). (b) Strain CP79 (RC^{rel}), inhibited with trimethoprim for 5 min. $\circ$, Cultures given glycine and methionine at time zero; $\bullet$, growing control.

over many generations in the presence of $[5-^3H]$ -uracil, with a constant rate of uptake of the isotopically labelled precursor. The cultures were then suspended for 30 min in glucose-salts medium containing $[^3H]$ uracil of the same specific radioactivity. Samples were taken for extraction of randomly 3H -labelled RNA. The required amino acids were then added and further samples were taken. Similar experiments were carried out in which the cultures were randomly labelled throughout initial growth and, later, a period of trimethoprim inhibition. The 3H -labelled RNA species were then hybridized together with a standard randomly ^{14}C -labelled RNA, in which the mRNA content was assumed to be 2.2% by weight (Gray & Midgley, 1970). By comparing the $^3H/^{14}C$ ratio of the

hybrids with that of the input, the amount of mRNA in the randomly 3H -labelled cultures could be calculated. The results are shown in Figs. 4 and 5.

In Fig. 4(a) the mRNA content of the RC^{str} strain virtually doubled in the first 10 min of recovery from amino acid starvation, but then declined to normal values. In contrast, the increased mRNA content typical of amino acid-starved RC^{rel} cultures (Friesen, 1966; Gray & Midgley, 1972) rapidly declined to normal values after the addition of amino acids (Turnock & Wild, 1965), without any evidence of a temporary oversynthesis in the recovery period (Fig. 4b). Qualitatively, the same phenomena were noted in trimethoprim-inhibited cultures given glycine and methionine (Figs. 5a and 5b). In this case, the mRNA content of the

recovering RC^{str} cells was temporarily almost fourfold greater than in normally growing cultures but again rapidly declined to normal values. For the RC^{rel} cultures we deliberately chose a short period of trimethoprim inhibition, where the mRNA content of the cell was initially much decreased (R. J. Smith, unpublished work). We argued that if a similar gross oversynthesis of mRNA occurred in this strain, it should be made apparent if mRNA accumulation was avoided during amino acid deprivation. Fig. 5(b) shows that no oversynthesis of mRNA occurred during the recovery of RC^{rel} cultures from amino acid deprivation, and the mRNA content eventually became only slightly higher than that found in normally growing cultures.

It is probable that, in these conditions where stable RNA can be made at normal rates in the trimethoprim-inhibited cells, protein synthesis (and therefore polyribosome formation) is inhibited through lack of *N*-formylmethionyl-tRNA^{fMet} (Eisenstadt & Lengyel, 1966; Lengyel, 1967; Harvey, 1973; Midgley & Smith, 1973), but not through any further

Fig. 6. Decay of protein-synthesizing capacity in RC^{str} strains of *E. coli* during recovery from amino acid starvation, as measured by residual labelling after rifampicin inhibition

The potential for residual protein synthesis at any time after the addition of rifampicin and $[U-^{14}C]$ tyrosine was expressed as the difference between the radioactivity incorporated into protein at that time and the final maximum radioactivity incorporated after 30 min of incubation (Gray & Midgley, 1971). ○, Culture first starved of amino acids, then given amino acids for 5 min and then given simultaneously rifampicin (400 μ g/ml) and $[U-^{14}C]$ tyrosine (for details see the text). ●, Culture as above, but given amino acids for 60 min before addition of rifampicin and labelled tyrosine. ◐, Steadily growing culture given rifampicin and labelled tyrosine. Strain CP79 (RC^{rel}) gave results that were essentially the same as in the RC^{str} experiments shown here.

lack of amino acids. We thus have a situation in both RC^{str} and RC^{rel} cultures in which the rate of protein chain initiation is very greatly decreased, and yet the relative rates of synthesis of mRNA and stable RNA, and the steady-state content of the mRNA fraction itself, are the same as in normally growing cultures. This indicates that the chemical lifetime of the mRNA fraction overall must also be unchanged, though it is possible that temporary changes in the rate of mRNA degradation may explain in part the transient accumulation of mRNA in the recovering RC^{str} strain.

Functional lifetime of mRNA in recovering RC^{str} and RC^{rel} cultures

(a) *Cultures resuming growth after amino acid starvation.* Strains CP78 (RC^{str}) and CP79 (RC^{rel}) were starved of required amino acids for 30 min. The stationary cultures were then supplemented, and 5 min afterwards the functional lifetime of the mRNA was measured by using rifampicin to stop further RNA synthesis (Gray & Midgley, 1971). A sample of the culture was given simultaneously L- $[U-^{14}C]$ -tyrosine (sp. radioactivity 20 mCi/mmol) and 400 μ g of rifampicin/ml. Residual labelling of protein by translation of the decaying mRNA pool was followed by rapid sampling and precipitation by trichloroacetic acid. The functional half-life of mRNA was plotted from the results, as described by Gray & Midgley (1971) (Fig. 6). In this figure, this parameter was also measured after a further 60 min of growth, when the mRNA contents in the cells had returned to normal values. In both stringent and relaxed strains, the half-life of functional messenger was about 2.5 min at 37°C and there was no evidence of any increase in mRNA half-life in the RC^{str} strain shortly after the resumption of growth. However, lack of evidence for an altered functional lifetime for mRNA under these conditions does not rule out the possibility of an altered chemical lifetime. It may be that changes in the latter are more important in controlling mRNA contents in the early stages of recovery after amino acid withdrawal.

(b) *Cultures resuming stable RNA accumulation after trimethoprim inhibition.* When glycine and methionine are added to RC^{str} or RC^{rel} cultures inhibited with trimethoprim, all the amino acids required for normal protein synthesis are then present (Dale & Greenberg, 1972; Smith & Midgley, 1973a,b; Then & Angehrn, 1972; Midgley & Smith, 1973), but initiation of protein chains is inhibited (Eisenstadt & Lengyel, 1966). Thus the addition of the two amino acids to trimethoprim-inhibited cultures is analogous to the relief of amino acid deprivation in auxotrophs, in that the accumulation of ppGpp is suppressed in RC^{str} strains (Smith & Midgley, 1973b) and stable RNA is synthesized at a

Fig. 7. Decay of protein-synthesizing capacity in *RC^{str}* and *RC^{rel}* strains of *E. coli* after addition of glycine and methionine to trimethoprim-inhibited cells, as measured by residual labelling after rifampicin

The method of expressing the results was as described for Fig. 6. ○, *RC^{str}* culture first inhibited with trimethoprim, then given glycine and methionine for 5 min and then given simultaneously rifampicin (400 µg/ml) and [¹⁴C]tyrosine (see the text for details). ●, As above but *RC^{rel}* strain used. ●, As above (*RC^{str}* strain) but given glycine and methionine for 60 min before addition of rifampicin and labelled tyrosine.

high rate (Smith & Midgley, 1973a; Midgley & Smith, 1973).

Experiments equivalent to those described above for recovering *RC^{str}* and *RC^{rel}* auxotrophs were carried out on trimethoprim-inhibited cultures, after the addition of glycine and methionine. Measurements of the functional half-life of mRNA in these cells are shown in Fig. 7. Samples taken both early and late after amino acid addition showed the same half-life of about 2.5 min for the total mRNA pool. On comparing these results with those for normally recovering cells (Fig. 6), it is clear that the large decrease in the rate of protein chain initiation and polyribosome formation (Phillips & Franklin, 1969; Harvey, 1973) had no discernible effect on the functional lifetime of the mRNA as a whole.

Discussion

Several models, on the basis of either studies of general instability or the turnover of individual mRNA species, have been put forward to explain how mRNA lifetimes are regulated in bacteria. The existence of an exonuclease specific for mRNA

degradation was first proposed by Kuwano *et al.* (1970, 1971) and Mangiarotti *et al.* (1971), but this activity is now believed to be an artifact (Bothwell & Apirion, 1971; Holmes & Singer, 1971). Ribonuclease II has also been implicated in mRNA turnover (Lennette *et al.*, 1971). However, regardless of which nuclease activities are important in the degradation of mRNA, it is probable that individual mRNA species have different stabilities, implying that specific regions of the chains have different affinities for the enzyme (Blundell *et al.*, 1972; Kennell & Simmons, 1972; Kennell & Bicknell, 1973). A recent model of mRNA turnover in *E. coli* proposed that mRNA is degraded in a 5' → 3' direction overall, that is, following the polar events of transcription and translation (Forchhammer *et al.*, 1972).

The movement of ribosomes over the mRNA during translation has been implicated as a factor in the control of its turnover rate (Lennette *et al.*, 1971). Whether in fact ribosomes play an effective part in controlling the speed or direction of mRNA breakdown is not yet clear (cf. Lennette *et al.*, 1971; Kennell & Simmons, 1972; Kennell & Bicknell, 1973). A study of trimethoprim-inhibited cells allows a further examination of the relationship between ribosomal translocation of mRNA and its functional and chemical lifetime. Since translation rates are probably not affected in trimethoprim-inhibited cultures given glycine and methionine (Smith & Midgley, 1973a,b), the principal effect of trimethoprim is to greatly decrease the rate of attachment of ribosomes to mRNA, and thus to make more freely available any putative nuclease-sensitive sites near the 5' terminus of the chains (Forchhammer *et al.*, 1972). Unless ribosomes play no effective part in regulating mRNA turnover, and the amount of nuclease activity in the cells is already rate-limiting with respect to sensitive sites, any situation that drastically decreases ribosomal attachment to mRNA should have an effect on its stability.

We have shown that neither the functional nor the chemical lifetime of mRNA is altered appreciably in trimethoprim-inhibited cells which are supplemented with all the amino acids necessary for protein synthesis but where there remains a lack of *N*-formylmethionyl-tRNA^{fMet}, necessary for chain initiation. Protection of mRNA by moving ribosomes has therefore little effect on its rate of degradation although if, in an extreme case, ribosome translocation is arrested, mRNA may be stabilized in the immediate locality of the immobilized particles. For example, much of the mRNA formed in chloramphenicol-inhibited *RC^{str}* cultures decays with a normal half-life, but less than half is stable and accumulates in the cells (Friesen, 1966; Midgley & Gray, 1971), owing to the inhibition of peptide bond formation and ribosome movement. In addition, amino acid-starved *RC^{rel}* cultures also accumulate

some mRNA but turn over much of this fraction, again at rates close to normal (J. E. M. Midgley, unpublished work; Gray & Midgley, 1972) probably because ribosomes become immobilized during inaccurate translation (Hall & Gallant, 1971).

Our results also show that mRNA synthesis is not detectably altered compared with the synthesis of stable RNA, when protein chain initiation and polyribosome formation are inhibited (Phillips & Franklin, 1969; Harvey, 1973). Again, this shows that overall rates of transcription and translation are not related (Kennell & Simmons, 1972) in contrast with the original suggestion of Stent (1964, 1966). We find no evidence to support the proposals of Imamoto (1973) for any general coupling in the whole mRNA fraction. This suggests that the rate of mRNA synthesis overall is controlled mainly through the rate of attachment of RNA polymerase to promoter sites on the DNA, and is not influenced by ribosome supply for translation. There are therefore no grounds for proposing any role for *N*-formylmethionyl-tRNA^{fMet} supply and protein chain initiation in the control of the synthesis of mRNA chains.

Finally, we have made the noteworthy discovery that in RC^{str}, but not RC^{rel}, amino acid auxotrophs, the recovery of deprived cultures is accompanied by a temporary overshoot in the synthesis of mRNA, or possibly a temporary decrease in its turnover rate. The most likely explanation of this phenomenon would begin from the observed accumulation of ppGpp in amino acid-starved RC^{str} cells (Cashel, 1969) which decreases the engagement of RNA polymerase on cistrons for stable RNA (rRNA + tRNA) (Travers, 1971, 1973) and allows a higher fraction of the active enzymes to transcribe mRNA cistrons (Sarkar & Moldave, 1968; Lazzarini & Dahlberg, 1971; Stamato & Pettijohn, 1971; Gray & Midgley, 1972; Norris & Koch, 1972). On the re-addition of amino acids, any redistribution of RNA polymerases at the onset of growth is not made immediately. Since, on the addition of amino acids, ppGpp concentrations fall very rapidly (Cashel, 1969; Cashel & Gallant, 1969), this suggests that changes in the concentration of this regulatory nucleotide are not the only arbiters of the relative rates of synthesis of mRNA and stable RNA. It is probable that holdups in translation in RC^{str} strains, arising from amino acid lack, alter, in addition, the molecular state of other factors (e.g., the Ts-Tu complex for protein chain elongation) shown to play an important role in the initiation of stable RNA chains (Travers, 1971, 1973; Travers & Buckland, 1973; Travers *et al.*, 1973). During recovery, the return of the latter factor(s) to a state more typical of steadily growing cells appears to be a time-consuming process. This would allow the temporary overproduction of mRNA by the active

polymerase fraction. Obviously, changes in the rate of ribosomal attachment to and translation of mRNA have no effect on this phenomenon, as it was also noted in RC^{str} cultures inhibited with trimethoprim, when a high rate of stable RNA synthesis was resumed by adding glycine and methionine, but where polyribosome formation was much decreased.

In RC^{rel} strains, such changes in the distribution of enzymes on stable and mRNA cistrons are not expected, since ppGpp does not accumulate during amino acid starvation, and stable RNA synthesis is not curtailed (Borek *et al.*, 1955; Stent & Brenner, 1961; Cashel, 1969; Cashel & Gallant, 1969). The relative rates of synthesis of mRNA and stable RNA do not alter significantly on starvation (Gray & Midgley, 1972). The fact that RC^{rel} strains did not accumulate mRNA when glycine and methionine were added to trimethoprim-inhibited cultures again suggests that the phenomenon is linked to the expression of the RC gene and not to translation *per se*.

R. J. S. thanks the Medical Research Council for a postgraduate studentship. We thank Mrs. M. Bell for excellent technical assistance.

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