Early Changes in the Messenger Ribonucleic Acid Concentration of Amino Acid-Starved Cells of *Escherichia coli* are not Dependent on the State of the *rel* Gene

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Measurements of the concentration of mRNA in *rel*⁺ and *rel*[−] strains of *Escherichia coli* shortly after the imposition of amino acid deprivation indicate that there is a temporary fall in the amount of this fraction relative to the total cellular RNA.

In the presence of trimethoprim, bacterial cultures supplemented with adenine and guanosine rapidly respond to the *rel* gene regulatory function for RNA synthesis. In *rel*⁺ strains of *Escherichia coli* the rate of RNA accumulation is lowered some 20-fold only 2–3 min after addition of this substance, whereas in *rel*[−] strains RNA accumulation is not affected (Midgley & Smith, 1973; Smith & Midgley, 1973*a,b*). It has been shown that a deficiency in the supply of glycine and methionine is primarily responsible for this behaviour (Then & Angehrn, 1972; Smith & Midgley, 1973*a,b*). The rapid response of the cells to trimethoprim allows a study of the earliest effects of amino acid deprivation, without the practical difficulties raised by the more laborious filtration or centrifugation techniques commonly used to deprive auxotrophs of required amino acids.

It has been suggested that, at the onset of amino acid deprivation of *rel*⁺ strains of *Escherichia coli* and other organisms, the gross rate of stable RNA-chain initiation is considerably diminished, but that of the messenger fraction is only mildly affected (Gallant *et al.*, 1970; Lazzarini & Dahlberg, 1971; Gallant & Margason, 1972). This suggestion has been made on indirect grounds, arising from discrepancies between the changes in the observed rate of stable RNA accumulation in steadily growing and amino acid-starved *rel*⁺ cells, and changes in the relative rate of synthesis of the stable RNA fraction and mRNA (Lazzarini & Dahlberg, 1971; Gallant & Margason, 1972). It has been proposed that increased concentrations of ppGpp that arise in *rel*⁺ cells during amino acid starvation (Cashel, 1969; Cashel & Gallant, 1969) may selectively inhibit the synthesis of some species of mRNA (Gallant & Margason, 1972).

A necessary result of any diminution in the gross rate of mRNA synthesis in amino acid-starved *rel*⁺ cells is that the absolute amount of this fraction, relative to total RNA, should be lowered, if turnover

rates for the mRNA are unaltered (Kennell & Simmons, 1972). This communication describes the direct measurement of the mRNA concentration of randomly labelled cultures of *E. coli* CP78 (*rel*⁺) and CP79 (*rel*[−]) immediately before and after the onset of a defined amino acid deficiency, caused by trimethoprim. The cells were grown in glucose-salts medium supplemented with 50 µg each of L-arginine, L-histidine, L-leucine and L-threonine/ml and 10 µg of thiamin hydrochloride/ml (Gray & Midgley, 1972). At a culture turbidity $E_{650} = 0.1$, sufficient [5-³H]uracil (The Radiochemical Centre, Amersham, Bucks., U.K.) was added to allow its continuous incorporation into nucleic acids over a further growth period, in which the turbidity reached 0.5. A sample (50 ml) was then taken into crushed ice. Trimethoprim lactate (50 µg/ml) (Wellcome Research Laboratories, Dartford, Kent, U.K.) was added to the remainder, together with adenine and guanosine (30 µg/ml each). At various times, further samples were poured on to crushed ice, and the ³H-labelled RNA was purified (Pigott & Midgley, 1968). A standard ¹⁴C-labelled RNA sample was prepared from a culture of *E. coli* CP78 (*rel*⁺) grown in glucose medium with enough [2-¹⁴C]uracil (The Radiochemical Centre) to label randomly all the RNA fractions to the same specific radioactivity. Samples of the ¹⁴C-labelled standard and each of the ³H-labelled RNA species were mixed, so that the approximate ³H/¹⁴C ratio of c.p.m. was 3:1 (as measured in the Beckman LS200 liquid-scintillation counter). The RNA mixtures were then hybridized with denatured *E. coli* DNA immobilized on cellulose nitrate membrane filters [DNA:RNA ratio (w/w) 5:1 (Midgley & Gray, 1971)]. The ³H/¹⁴C ratio in the hybrid was compared with that of the input, and, after corrections for quench and for contributions to the hybrids by rRNA, the proportion of mRNA in the ³H-labelled specimens was estimated (Midgley & Gray, 1971; Midgley & Smith, 1974).

The results (Fig. 1) show that, in both the *rel*⁺ and the *rel*[−] strain, the onset of amino acid deprivation gave rise to an immediate fall in the

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Fig. 1. Changes in the amount of mRNA in *E. coli* during amino acid deprivation brought about by trimethoprim

(a) Strain CP78 (rel^+); (b) strain CP79 (rel^-). For details see the text. The significance limits on each point are determined from the standard deviations of observed c.p.m. in 3H and ^{14}C radioactivity in each hybrid. A value of 2.2% in mRNA as the fraction of total RNA in steadily growing cultures was assumed (Midgley & Smith, 1974).

cellular content of mRNA. In the rel^+ strain CP78 the mRNA concentration fell by a maximum of 30–40%, whereas in the rel^- strain CP79 the maximum fall was a little larger, being of the order of 50% (see also Midgley & Smith, 1974). In both cases, however, further deprivation of the cultures led to a recovery of the mRNA concentration to a value more comparable with that found in steady growth. In the rel^- strain, prolonged amino acid withdrawal allowed mRNA to accumulate to concentrations almost three times those typical of steady growth (Turnock & Wild, 1965; Friesen, 1966; Gray & Midgley, 1972). In the rel^+ strain, the amount of mRNA in amino acid-starved cells never rises significantly above steady-growth concentrations (Gray & Midgley, 1972; Midgley & Smith, 1974).

It is clear therefore that the early changes in the

concentration of mRNA in amino acid-starved cultures of *E. coli* are independent of the state of the *rel* gene, though later accumulation of mRNA (or fragments of these molecules) is unique to the rel^- strain. A convincing explanation of the behaviour of the rel^- strain in this respect has already been given (Gray & Midgley, 1972). However, changes in the concentration of mRNA in these conditions must be independent of the accumulation of ppGpp (Cashel, 1969; Cashel & Gallant, 1969). Suggestions that the mild inhibition of mRNA synthesis is due to the inhibitory effect of ppGpp on the synthesis of a limited fraction of mRNA molecules (Gallant & Margason, 1972) do not seem to be correct. Rather it appears that normal repression of some mRNA species (e.g. those responsible for the synthesis of amino acids unaffected by trimethoprim) can account for a temporary loss of mRNA in *E. coli* at the outset of amino acid deprivation.

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