A Consequence of the *rel* Gene during a Glucose to Lactate Downshift in *Escherichia coli*

THE RATES OF RIBONUCLEIC ACID SYNTHESIS

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SUMMARY

*Escherichia coli* strains with relaxed (CP79) and stringent (CP78) RNA control were examined in regard to their RNA synthesis and accumulation during balanced growth in two media supporting different growth rates, and during downshift transitions between them. Although the RNA content was greater for CP79 at both growth rates, the total rate of RNA synthesis was found to be proportional to the growth rate in both strains.

Both strains traversed a downshift transition in about equal periods of time, but as a consequence of the relaxed mutation the reduction of the rate of RNA synthesis in CP79 was much less; the total rate of RNA synthesis was reduced during the downshift to 5% of the preshift rate in CP78 but to only 50% of the preshift rate in CP79.

Therefore, cessation of RNA accumulation during the downshift in CP79 implies that considerable RNA degradation was required. Thus, in addition to its function during amino acid starvation, the *rel* gene increases the efficiency of the transition from fast to slow growth rates.

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*Escherichia coli* is able to maintain a constant relation between the concentration of ribosomes and its expressed protein synthesizing capacity. Thus, at all but very slow growth rates, the number of ribosomes per cell and amount of mRNA per cell increase linearly with the growth rate. (See Kjeldgaard (1) and Maaløe (2) for recent reviews.)

The possibility that the rate of RNA synthesis might be constant and that the rate of RNA accumulation might be adjusted to the growth rate by degradative reaction has been proposed (3, 4). Although indirect evidence against this model has been adduced (5-7) it has never been eliminated. The present data exclude such a model and establish that the rate by RNA synthesis varies with the growth rate.

When faced with the necessity of adjusting to a slow growth rate from a fast one (downshift), *E. coli*, as well as other bacteria, is able to attenuate the rate of RNA accumulation for a time, until the RNA content is reduced to that characteristic of cells grown at the slower rate. Certain characteristics of this downshift have led several authors to suggest that the mechanism involved may be related to or the same as that which operates during amino acid starvation (1, 2, 8, 9).

The cellular response to conditions of amino acid starvation is greatly influenced by the *rel* gene (see Reference 10 for a review of the properties of the *rel* gene). Thus, in mutants carrying the relaxed mutation, RNA synthesis and accumulation continue under conditions of amino acid starvation, whereas in the wild type stringent counterpart, RNA accumulation ceases and RNA synthesis is moderately reduced (11-13). The present communication is concerned with the importance of the *rel* gene in the regulation of RNA synthesis during balanced growth and during downshift transitions. The results of this study demonstrate that both CP78 (rel+1) and CP79 (rel-1) are able to adjust their RNA content and rate of RNA synthesis to the values characteristic for slow growth. However, during a downshift transition the rates of RNA synthesis in the two strains are markedly different. Thus, it is concluded that the *rel* gene product (the identity of which is at present unknown), functions not only during amino acid starvation but also during downshift transitions and greatly enhances the efficiency with which a downshift in growth rate is executed.

EXPERIMENTAL PROCEDURE

Materials—[3H]Uracil (9.2 mCi per μmole) and Liquifluor were purchased from New England Nuclear. H232PO4 was obtained from Tracerlab. PEI cellulose sheets (cellulose polyethyleneimine MN-polygram 300 cel PEI) were the product of Brinkmann. Glass fiber filters (type A, 1 inch diameter) were purchased from Gelman Instrument Company and nitrocellulose filters (25 mm diameter, 0.45 μm pore size) from Millipore. Orcinol was obtained from Fisher.

*Bacteria and Media*—*E. coli* CP78 (thr-, arg-, his-, leu-, rel-) and CP79 (thr-, arg-, leu-, his-, rel-) were used. Tris-minimal medium was used.

The abbreviations used are: thi, thiamine; rel1, stringent RNA control; rel*, relaxed RNA control; ppGpp, guanosine tetraphosphate.
medium has been previously described (5). All experiments were done at 27° in a reciprocally shaking water bath. Growth was measured by following the absorbance at 600 nm with a Zeiss PMQII spectrophotometer. In the downshift experiments, 0.025% glucose and 0.25% lactate were present. The break in absorbance (A) denoting the period of transition from glucose to lactate metabolism occurred at 0.25 to 0.30, corresponding to a cell density of about 2.0 × 10^8 cells per ml.

Measurement of Cellular RNA—At appropriate times, 5 ml of culture were withdrawn, mixed with 0.5 ml of 50% trichloroacetic acid, and chilled. These suspensions were collected on glass fiber filters, washed with 5% trichloroacetic acid, and placed in test tubes with 2.0 ml of 5% trichloroacetic acid. The cells were hydrolyzed by heating to 90° for 20 min. After sedimenting the debris by centrifugation, 0.5 ml of the supernatant fluid was removed and the RNA content was assayed by the orcinol method (14).

Rates of RNA Synthesis—When cultures had reached the approximate A_600, [3H]uracil (final concentration, 20 μCi per ml; specific activity, 0.2 mCi per μmole) was added, and 5-ml samples were removed at 10-sec intervals and mixed with an equal volume of ice-cold 10% trichloroacetic acid. These suspensions were sedimented by centrifugation, and the cells were washed three times with 25% trichloroacetic acid containing uracil, 0.1 mg per ml. The cells were hydrolyzed in 1.0 ml of 0.3 M KOH for 16 hours at 37°, and the radioactivity of the hydrolysates was measured (15). The method, which makes use of a 32P, 3H double labeling technique as well as the measurement of the specific activity of the intracellular CTP and UTP pools, has been previously described (5). In experiments with CP78, in which the specific activity of the UTP pool was very low, acid-soluble extracts were concentrated with charcoal before chromatographic isolation as described previously (11).

Concentration of ppGpp and Nucleoside Triphosphate Pools—At least one full doubling before the downshift, H_2PO_4 was added to give a final concentration of 20 to 25 μCi per ml. Aliquots (100 μl) were removed at intervals and mixed with 50 μl of ice-cold 4 M formic acid. These suspensions were sedimented by centrifugation and 10 μl of the supernatant fluids applied to PEI sheets for two-dimensional chromatography. The chromatographic isolation of the four major nucleoside triphosphates was identical with that previously described (5). In addition, some experiments were done with 2.2 x 10^4 μCi/Pi in the second dimension for the isolation of ppGpp. These compounds were located by autoradiography, and the portions of the PEI sheets containing them were cut out and counted in the toluene-Liquifluor scintillator. The specific activity of the culture medium was determined by counting an aliquot and measuring the phosphate concentration (16).

RESULTS

Rates of RNA Synthesis during Balanced Growth—The general methods for the measurement of the rates of RNA synthesis during balanced growth by means of uracil labeling and the measured specific activities of the UTP pools have been described.

TABLE I
Rates of RNA synthesis in CP78 and CP79 during balanced growth on glucose and lactate media

| Carbon source | CP78 | CP79 |
|---------------|------|------|
| Glucose | Lactate | Glucose | Lactate |
| μm (hr⁻¹) | 0.455 | 0.300 | 0.492 | 0.300 |
| RNA (μg/A_600) | 65 | 49 | 90 | 67 |
| RNA accumulation (μmole/sec/A_600) | 3.3 | 1.1 | 4.9 | 1.5 |
| Observed synthesis (μmole/sec/A_600) | 11 | 5 | 12 | 6 |
| mRNA synthesis (μmole/sec/A_600) | 7.7 | 3.9 | 7.1 | 4.5 |
| Stable RNA synthesis (%) | 30 | 22 | 41 | 25 |

Fig. 1. The determination of the rate of RNA synthesis for Escherichia coli CP78 in lactate medium. A culture of CP78 was supplemented with all growth requirements and 0.25% lactate. Two hours before [3H]uracil labeling, the culture was divided, and H_2PO_4 was added (final concentration, 20 μCi per ml) to one subculture. [3H]Uracil was then added to both cultures (final concentration, 20 μCi per ml; 9.2 mCi per μmole, specific activity), and both cultures were sampled simultaneously. A, the time course of uracil uptake into base-hydrolyzed, trichloroacetic acid-precipitable material. B, the time course of [3H]uracil labeling of the parallel 32P culture. This culture was sampled after the addition of [3H]uracil and formic acid extracts were prepared. These extracts were subjected to fractionation of the nucleoside triphosphate components with two-dimensional thin layer chromatography. UTP and CTP were located and cut out, and the radioactivity was determined. This distribution of label between UTP and CTP was used to determine the radioactivity in RNA due to UMP alone. Thus, the values in A were reduced by the fraction of label in B due to CTP at corresponding time points. The dashed line in B shows the specific activity of newly synthesized RNA which was used to convert the data of A to molar amounts of UTP accumulated at each time point. These final data are shown in C.
in detail elsewhere (5). Fig. 1 illustrates this method, showing the results obtained with CP78 growing exponentially in lactate medium. The rate of RNA synthesis for CP79 under the corresponding conditions was determined according to the same method. The rate of uracil uptake is slightly faster for CP79 than for CP78, and the UTP pool specific activity for CP79 is slightly less than for CP78. Thus, the total rate of RNA synthesis is higher for CP79 than for CP78, in spite of their similar growth rates. These data are summarized in Table 1.

Table I also shows the RNA content under various conditions of growth. The rates of RNA accumulation were calculated with these values and the composition of total cellular RNA given by Midgley (17). The difference between these rates of RNA accumulation and the measured rates of RNA synthesis must be the rates of synthesis of unstable RNA. If it is assumed that all rRNA and tRNA synthesized is essentially stable, then the unstable fraction of total RNA is mRNA.

Rate of Synthesis of RNA during the Downshift—Fig. 2 demonstrates that for both CP78 and CP79 the period of transition between glucose and lactate is marked by an absolute cessation of RNA accumulation for a period of about 3 hr. During this period, as well as before and after it, the instantaneous rates of RNA synthesis were measured. In these experiments, cells were cultured in a downshift medium, which contained 0.25% lactate and 0.025% glucose, so that at a cell density of 2 x 10^6 cells per ml, glucose would be exhausted and lactate would serve as the sole source of carbon and energy. At an A_{600} value of about 0.1, the culture was split, and H_3P0_4 (20 μCi per ml) was added to one of the subcultures. At various times during the course of the downshift, the two cultures were labeled simultaneously with [3H]uracil (final concentration, 20 μCi per ml; specific activity, 9.2 mCi per μmole). The ^3P culture was used to determine specific activity of the UTP pool, and the other culture was used to determine the [3H]uracil labeling of total cellular RNA.

The results of the rate measurements are described in Table II and in Fig. 3. These data show that in CP78 there is a drastic inhibition of the rate of RNA synthesis, 20-fold being the maximum measured. However, the actual value for the maximal reduction is probably not significant, owing to the rapidly changing rate. In contrast, the rate of synthesis in CP79 appears to fall smoothly to reach the new value of about 50% of the glucose rate, without going through a minimum as is the case with CP78. It is clear that very much more RNA is actually synthesized in CP79 during this transition period than is synthesized in CP78, but RNA accumulates in neither organism.

Nucleoside Triphosphate and ppGpp Pool Changes—When rel^+ cells are deprived of a required amino acid, the intracellular concentration of GTP falls precipitously (18). This decrease is accompanied by the similarly abrupt appearance of an unusual guanosine tetraphosphate (19–21). Recently, this compound as well as the changes in the purine triphosphates were measured during the glucose to lactate shift in the present study. Fig. 4 shows that the intracellular concentration of ATP and GTP fall to reach new values in CP78. This reduction is abrupt for GTP in CP78, but more gradual in CP79. For CP79, ATP falls only transiently, then returns to the preshift level.

The basal concentration of ppGpp appears to be slightly higher in the glucose medium for CP78 as compared with CP79. These data show considerable scatter, but the observation agrees with similar measurements by Lazzarini, Cashel, and Gallant (22). The concentration of this compound as well as the changes in the purine triphosphates were measured during the glucose to lactate shift. The peak concentration of ppGpp rises very abruptly in both strains, and appears to be concomitant with the cessation of RNA accumulation and the break in the growth curve. The peak concen-

![Fig. 2. Growth and accumulation of RNA in Escherichia coli CP78 and CP79 during a glucose to lactate downshift. Cells were cultured in a shift medium containing 0.25% lactate and 0.025% glucose, so that the glucose would be exhausted in mid-log phase and metabolism of lactate would begin. At intervals the cultures were sampled and their A_{600} was measured. Simultaneously, the RNA concentration was assayed according to the orcinol method described under "Experimental Procedure."](image-url)

**Table II**

**Determination of amount of UMP incorporation into RNA after 30-sec pulse of [3H]uracil**

Data are shown from experiments during a glucose to lactate downshift at 40 min before (A), 30 min after (B), 60 min after (C), and at 165 min after (D) the break in the A_{600} curve shown in Fig. 2.

|        | A              | B          | C          | D          |
|--------|----------------|------------|------------|------------|
|        | CP78           | CP79       | CP78       | CP79       | CP78      | CP79      | CP78      | CP79      |
| UTP specific activity (dpm/μmole × 10^-3) | 4.4          | 4.2        | 1.6        | 1.3        | 3.3       | 1.8       | 4.4       | 2.3        |
| RNA specific activity (dpm/μmole × 10^-3) | 2.8          | 2.9        | 0.8        | 0.9        | 1.8       | 1.2       | 2.5       | 1.7        |
| RNA labeling (dpm/A_{600} × 10^-3) | 8.76         | 9.34       | 1.00       | 2.50       | 0.32      | 2.16      | 5.20      | 2.98       |
| UMP (μmole/A_{600}) | 0.13          | 0.32       | 126        | 278        | 38        | 100       | 200       | 175        |
The rates of RNA synthesis for *Escherichia coli* CP78 and CP79 during a glucose to lactate downshift. Cultures were grown in the shift medium, and the instantaneous rates of RNA synthesis were measured at the four times indicated at A, B, C, and D. The methods used were the same as described in the legend to Fig. 1. The bottom panels show the time course of incorporation of UMP residues into RNA, and the upper panels summarize the initial rates measured from these curves at each time point.

Fig. 4. The changes in the purine nucleoside triphosphate pools and ppGpp during a glucose to lactate downshift for *Escherichia coli* CP78 and CP79. Cultures were grown in the shift medium and 2 hours before the break in Asoo, H3PO4 was added (final concentration, 20 to 25 μCi per ml). Samples (100 μl) were mixed with 50 μl of 1 M formic acid at the times indicated, and the intracellular nucleoside triphosphate pools and ppGpp were isolated according to the chromatographic procedure described under "Experimental Procedure." The radioactivity of each compound was measured and converted to molar amounts by means of the phosphate specific activity of the medium at the time of sampling.

Ammun acid starvation, where it is absent altogether (19, 20). Further, the disappearance of the compound is more rapid in CP78 than in CP79, suggesting either that it is produced in the latter for a longer period, or that it is consumed in the former at a faster rate.
The rate of RNA accumulation in *E. coli* is exponentially related to the growth rate (23). The variation of the rate of RNA synthesis with the growth rate has been assumed (2) but never proven. Moreover, alternate models have been proposed (3, 4), which involve degradation of ribosomal RNA at slow growth rates to achieve reduced rates of RNA accumulation. Indeed, *E. coli* has been shown to degrade ribosomal and transfer RNA in certain circumstances (3, 4, 24).

Recently, methods for the measurement of the instantaneous rates of RNA synthesis have been described (5). These methods have been used in the present experiments to measure the rates of RNA synthesis in two media which permit different growth rates. The results indicate that *E. coli* is able to adjust its rate of RNA synthesis as well as its rate of RNA accumulation to its growth rate. This is true for cells with both relaxed and stringent RNA control.

The instability of RNA in CP79 during the shift, implied by its faster rate of synthesis relative to that of CP78, suggests either that the RNA which is being made is mRNA and normally unstable, or that rRNA is rendered unstable. Hybridization-competition experiments (13) have demonstrated that the RNA synthesized is predominantly mRNA, and that there is selective inhibition of rRNA synthesis during the downshift. However, as previously noted (13), the failure of RNA to accumulate during the downshift although a small amount of rRNA is synthesized implies that degradation of rRNA also takes place.

In the calculations summarized in Table I, the rates of tRNA and rRNA synthesis were assumed to be the same as the rate of RNA accumulation by the cell. If that assumption is correct, and tRNA and rRNA are not overproduced and subsequently degraded, proportionality should exist between the rate of mRNA synthesis measured in this way and the growth rate (micra), and between the rate of rRNA synthesis and the square of the growth rate, squared micra (2). This relation predicts that as micra decreases, the proportion of RNA synthesis devoted to rRNA and tRNA should decrease. This prediction is borne out, at least qualitatively, by the calculations shown in Table I, in which the percentage of stable RNA synthesis is shown to be less in lactate medium for both strains than in glucose medium. Hence, a model in which RNA accumulation at different growth rates is regulated by the degradation of excess RNA (25) seems to be ruled out by the present data.

If the rate of elongation of nascent RNA chains is more or less constant at all growth rates (5-7, 26), these observations allow the prediction that the cell maintains a number of nascent RNA chains at any instant which is adjusted to suit its growth rate. Thus, it appears that although *E. coli* has mechanisms for the degradation of ribosomal RNA under conditions of unbalanced growth, the most efficient mechanism for the regulation of the rate of synthesis during balanced growth involves maintenance of a number of growing chains which is proportional to the growth rate. This type of regulation minimizes the wastage of newly synthesized RNA.

The downshift transition, like amino acid starvation, represents a period of unbalanced growth. During this time, no RNA accumulates, and the cell, by continued protein and DNA accumulation, adjusts its composition to that characteristic of cells growing at the slower rate. If indeed there is a relationship between regulation during amino acid starvation and during a downshift, then manifestation of the rel gene should be detectable during the downshift, as well as during amino acid starvation.

Accordingly, the rates of RNA synthesis have been measured in CP78 and CP79 during a downshift from glucose to lactate media. Despite the fact that RNA accumulation ceases for 24 hours during the downshift (Fig. 2) substantial rates of RNA synthesis are demonstrable in both strains. As amino acid starvation, the relaxed strain synthesizes considerably more RNA than its stringent counterpart (Fig. 3). These findings indicate that the relaxed strain meets the challenge of the downshift much less efficiently than does the stringent. These results support the conclusion that the amino acid control and growth rate control are interrelated since a single mutation affects both processes.

The guanosine tetraphosphate has been reported to appear during amino acid starvation of rel+ but not rel− strains (19-21), and during downshift transitions in both rel+ and rel− strains, but the actual quantitative relationships are strain-dependent (22). In the present experiments this compound appeared in both strains, in slightly higher concentration in CP78, and with a more rapid disappearance. Its appearance at all in the relaxed CP79 served to distinguish the downshift from amino acid starvation (20).

A model in which ppGpp might be an inhibitor of RNA synthesis has been suggested (20), and is consistent with the present data. However, the appearance of this compound in CP79 mitigates against the possibility that ppGpp is a specific inhibitor of RNA polymerase which appears under conditions of unbalanced growth and the production of which is eliminated by the rel− mutation. The experiments with CP79 demonstrate that ppGpp correlates not with the inhibition of RNA synthesis, but with the inhibition of RNA accumulation. That is, in all circumstances studied so far, ppGpp appears only under conditions of restricted RNA accumulation. Therefore, if ppGpp is involved in regulation of RNA synthesis, it seems likely that it participates in the selective inhibition of synthesis of stable forms of RNA rather than in the promotion of degradation of these species.

Equally consistent with these data is the proposal that ppGpp is the product of a reaction which results in the degradation of forms of RNA which would be stable under conditions of balanced growth. This cause or effect dilemma remains to be resolved.

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