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Biochemistry

Regulation of transcription factor ρ and the α subunit of RNA polymerase in *Escherichia coli* B/r

(suA gene product/polarity/two-dimensional gel electrophoresis/ribosomal protein S6/RNA nucleotidyltransferase)

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ABSTRACT **Transcriptional termination factor ρ, the α subunit of RNA polymerase (RNA nucleotidyltransferase, nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6), and ribosomal protein S6 were resolved from whole-cell extracts of *E. coli* B/r by a high-resolution, two-dimensional polyacrylamide gel electrophoretic technique, and were identified through coelectrophoresis with the purified proteins. The regulation of ρ, α, and S6 was studied, in steady-state cultures of *E. coli* B/r growing at rates ranging from 0.6 to 2.1 generations per hr, through the use of this gel technique and a double radioisotope labeling procedure. The regulatory patterns of ρ and α are distinct from, but similar to, one another. Neither ρ nor α shows the sharply increasing levels with increasing growth rate shown by the ribosomal proteins as exemplified by S6. The difference between the levels of ρ and α, on the one hand, and S6, on the other, is most pronounced during rapid growth. The regulatory pattern of α is interesting, given the recent suggestion that the gene coding for α is cotranscribed with genes coding for ribosomal proteins.**

The termination factor ρ was first isolated by Roberts (1), and was characterized as being a tetrameric protein of identical 50,000-dalton subunits. Mutants in the *suA* locus, at 74 min on the genetic map of *Escherichia coli* K12, possess an altered ρ (2) and show much less transcriptional polarity than otherwise isogenic wild-types for *suA* (3, 4). *In vitro*, ρ has been shown to cause transcriptional termination on a number of DNA templates at specific sites that seem to correspond to the sites of transcriptional termination *in vivo* (1, 5, 6).

Very little has been learned about the regulation of ρ, although variation in its levels could play an important role in the regulation of gene expression. It has been seen *in vitro* that the apparent efficiency of ρ-mediated transcriptional termination is dependent upon the relative amounts of ρ and RNA polymerase present (5, 7). Furthermore, there is evidence that the efficiency with which ρ acts is also dependent upon the nucleic acid sequence being recognized, since the ρ concentration required for half-maximal termination varies from one site to another (6). Estimates of the ρ content of *E. coli* range from 1 to 15 ρ tetramers per 100 RNA polymerase (RNA nucleotidyltransferase, nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) molecules (8, 9). Since ρ can be classified as a transcriptional protein, it seemed logical to compare its regulation to that of the RNA polymerase subunits, particularly in the light of the apparent sensitivity of ρ efficiency to the ρ/RNA polymerase ratio. The development of a high-resolution, two-dimensional polyacrylamide gel electrophoresis technique (10) has enabled us to study the regulation of ρ and α levels in *E. coli* B/r. The regulation of ribosomal protein S6 was also studied, for comparison.

Abbreviations: ρ_B, ρ-factor from *Escherichia coli* B; ρ_{K12}, ρ-factor from *E. coli* K12; α, α subunit of RNA polymerase; S6, ribosomal protein S6.

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MATERIALS AND METHODS

Strains. NC3 is an *hsr*$^-$ derivative of *E. coli* B/r and is the parent strain from which NC119 and NC120 were constructed. NC119 carries *leuA*^{ts}, *lac*L1R4, and *trpE*9851, while NC120, in addition, carries *suA* (11). Thus, NC119 has ρ_B, while NC120 has ρ_{K12}. All strains were constructed in this laboratory by Dr. B. L. Wanner.

Marker Proteins. RNA polymerase and ρ, isolated from *E. coli* K12, were the gifts of Dr. F. Engbaek. ρ from *E. coli* B was donated by Dr. J. Richardson, and ribosomal proteins from *E. coli* K12 were given to us by Dr. L. Lindahl.

Growth and Labeling of Cells. All cultures were grown in "MOPS"-based media (12), with 0.027 mM K_2SO_4 (one-tenth the normally used concentration). This concentration of sulfate will support growth, with no deviation in the growth rate, up to an $OD_{420\,nm}$ of 1.1. All sterilizations were performed by filtration. The cultures were grown at 37°, with L-tryptophan and L-leucine added when required. The cultures were grown exponentially for 8–10 generations in a given medium before labeling in that medium.

For the steady-state labeling of NC3, carrier-free $^{35}SO_4$ or L-[4,5-^{3}H]leucine and L-[4,5-^{3}H]isoleucine were added to specific activities of 20–25 Ci/mmol. This yielded 540 μCi/ml of $^{35}SO_4$, or 150 μCi/ml of [^{3}H]leucine and 75 μCi/ml of [^{3}H]isoleucine in the labeling media. The cultures were grown, in the presence of label, from an $OD_{420\,nm}$ of 0.1 to one of 0.4, at which point they were harvested. NC119 and NC120 were labeled from 0.1 to 0.8 in $OD_{420\,nm}$ with carrier-free $^{35}SO_4$ (250 μCi/ml of culture). The growth rates obtained for NC3 are shown in Table 1. All radioisotopes were purchased from New England Nuclear.

Harvesting, Extraction, and Electrophoresis. The cells were collected by centrifugation, washed once with 10 mM Tris·HCl (pH 7.8), 5 mM $MgCl_2$, and stored at −80°. When ^{3}H-labeled and ^{35}S-labeled cells were to be electrophoresed together, appropriate amounts of the two were mixed, brought to a volume of 80 μl (with the Tris-$MgCl_2$ solution described above), sonically disrupted, treated with RNase A and DNase A (2 μl of a solution containing 2 mg/ml of each enzyme), and electrophoresed as described (10). Since the isoelectric point of ρ is rather alkaline (~9.0), 2% Ampholines (LKB) of pH range 3.5–10 were used. Under the conditions described, 60–70% of the total labeled material is recoverable from the gels. At least 20% of the unrecoverable material is either consistently precipitated, or is outside the pH range of the isoelectric focusing gels used. The staining, cutting, and measurement of radioactivity of the gels were performed as described (13).

Table 1. Steady-state growth rates of NC3 in media used

Medium	Generations/ hr (μ)	k (hr^{-1})
0.2% Acetate + Leu + Ile	0.67	0.46
0.2% Glycerol + Leu + Ile	1.13	0.78
0.2% Glucose + Leu + Ile	1.50	1.04
0.2% Glucose + FS	2.14	1.48

Growth conditions are described in *Materials and Methods*. k is the growth rate constant. FS stands for full supplement, and has been described elsewhere (11). In this work, the FS was made without methionine or cysteine, but contained the other 18 common amino acids, vitamins, adenine, guanine, cytosine, and uracil. No effects on the growth rate were observed during labeling (at least in glucose and glucose + FS media, in which the growth rate of control cultures was monitored). If any growth rate effects by the label occurred in the other media, it is unlikely that they affected the data since (*a*) reversing the isotopes in the labeling protocol did not affect the results, and (*b*) for all four growth media, when both labeled cultures were grown in the same medium, the isotope ratio in a given protein spot did not change from one growth medium to another.

Identification of Proteins. To identify α, ρ_B, and ρ_{K12}, approximately 0.1 μg of the purified protein was mixed with a nonstainable amount of total cell extract from ^{35}S-labeled *E. coli* NC3. After electrophoresis, the stained gel was compared to the autoradiogram of that gel.

RESULTS

Further identification of the proteins

The proteins being studied were identified as described above. The identification of α was confirmed through analysis of extracts from UV-killed *E. coli* infected with λfus_3 (13), which carries the gene coding for α. The spot identified as representing α was one of the few spots present on gels of those extracts. Further identification of S6 will be reported later (Reeh and Pedersen, manuscript in preparation). In the case of ρ, the identification could be confirmed by comparing *E. coli* NC119 with NC120 (Fig. 1). It has been shown (9) that the molecular weight of ρ_{K12} is 2000–3000 greater than that of ρ_B. The molecular weights calculated from the gels shown in Fig. 1 are 48,800 for ρ_B and 51,300 for ρ_{K12}. Note that the ρ_{K12} isoelectric point is apparently more acid than that of ρ_B (by about 0.8 pH unit).

Variation of the proteins with growth rate

By using a double radioisotope labeling technique (see Fig. 2), the regulation of ρ, α, and S6 was studied. The $^3H/^{35}S$ ratio in a given spot on the gel depends upon (*a*) the amino acid composition of the protein and (*b*) the relative amounts of the protein in cells grown on glucose and on the various other carbon sources. Thus, the purity of the spots can be (and was) ascertained by determining that the $^3H/^{35}S$ ratio within a spot is constant. In those gels on which the ^3H- and the ^{35}S-labeled cells were grown on the same carbon source, the $^3H/^{35}S$ ratio of the ρ spot agrees well with the ratio predicted from the amino acid composition of ρ (14).

It can be seen that the regulatory patterns of ρ and α are similar, with α increasing with the growth rate between the acetate and glucose steady-states, but remaining fairly constant with a further increase in growth rate. ρ, on the other hand, remains relatively constant over the range of growth rates studied. In contrast to these patterns is that of S6, whose intracellular level increases in a linear manner with the growth rate.

Relative amounts of ρ and α

The relative amounts of ρ and α were determined from gels of an extract of *E. coli* NC3 that had been uniformly labeled with D-[^{14}C]glucose (by Dr. P. L. Bloch). The spots representing these proteins were cut out quantitatively and were treated as described (13), with the exception that the H_2O_2 was made basic (with NH_4OH, to 2% vol:vol of the concentrated solution) (15) to prevent loss of $^{14}CO_2$. Using these procedures, 3140 cpm were in the ρ spot, and 6379 cpm were in the α spot. By correcting for differences in the molecular weights of these proteins (48,800 for ρ_B and 39,000 for α) the mole ratio of ρ/α was calculated to be 0.39:1.00. The significance of this ratio will be discussed below.

DISCUSSION

Comparison with earlier studies

The regulation of ribosomal protein S6 has been studied in *E. coli* B/r (16). In that study, it was found that S6 levels varied 2-fold over a range of growth rates of 0.65–1.9 doublings per hr. Here, we have reported a 2.6-fold variation in S6 levels over a range of growth rates of 0.67–2.14 doublings per hr. These results agree quite well and indicate the veracity of the techniques used in this work. Since the Dennis study (16) involved

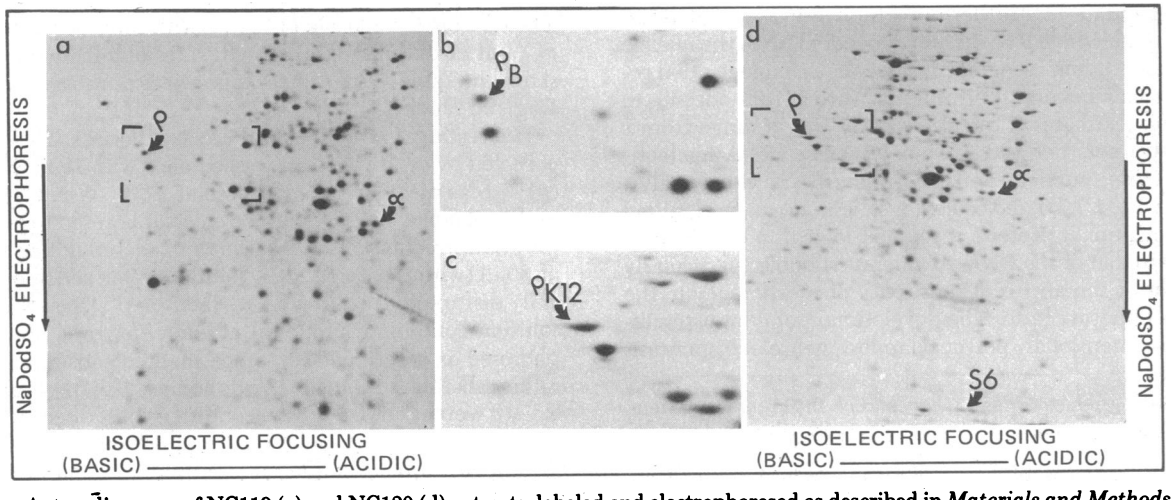

FIG. 1. Autoradiograms of NC119 (a) and NC120 (d) extracts, labeled and electrophoresed as described in *Materials and Methods*. ρ, α, and S6 are shown. The indicated areas of the NC119 (b) and NC120 (c) autoradiograms have been enlarged to clarify the changed spot pattern. NaDodSO$_4$, sodium dodecyl sulfate.

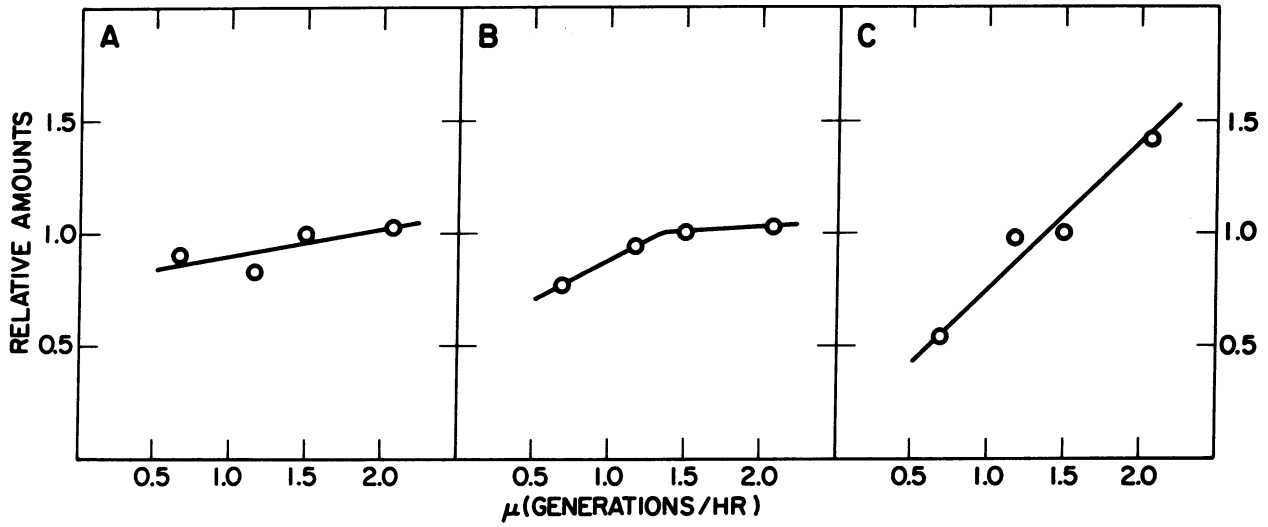

FIG. 2. The regulatory patterns of ρ (A), α (B), and S6 (C) versus the growth rate. The regulatory pattern of a protein was determined through comparison of the $^3H/^{35}S$ ratios in its spot and in the total extract when glucose-grown, ^3H-labeled cells were mixed with cells grown in a different medium and labeled with ^{35}S, as described in *Materials and Methods*. Reversing the isotopes in this protocol did not change the pattern obtained. These values have been normalized such that the value obtained when both the ^3H- and ^{35}S-labeled cells are glucose-grown is 1.0. Typically, about 100 μCi of ^{35}S or 20 mCi of ^3H were incorporated per ml of culture, approximately 5 μCi of ^{35}S and 1 mCi of ^3H were loaded onto a gel, and, of this, the radioactivity recovered in the ρ, α, and S6 spots was around 0.1, 0.2, and 0.2%, respectively, of the radioactivity loaded.

analysis of fractionated ribosomal proteins, while this work involved analysis of unfractionated cell extracts and used no chase period after the labeling period, the close agreement of the results indicates that there is no change in the efficiency of S6 incorporation into ribosomes over the growth range common to both studies.

The regulation of α has also been studied previously (17), in *E. coli* K12. A fairly linear 4-fold increase in the level of α, over almost a 9-fold range in the growth rate, was seen. The growth rates obtained in that study are lower (ranging from 0.22 to 1.87 generations per hr) than those obtained in this one (which is due, in part, to strain differences), and it can be seen that between growth rates of 0.67 and 1.50 generations per hr our measurements of α also show a fairly rapid increase with the growth rate. We also observe a leveling off of the rate of increases in α levels with increases in the growth rate.

We have studied the variation of ρ with the growth rate. The amounts of ρ found in this study are approximately 10- to 100-fold higher than preliminary predictions had indicated (8, 9).

Transcriptional and translational proteins have rarely been studied in the same cell extracts. The ability to do so here, with no preliminary fractionation of the extracts required, reflects the power that the techniques used have brought to the study of cellular physiology.

ρ/RNA polymerase ratio

The ρ/RNA polymerase ratios were calculated from the ρ and α measurements made in this study. The β and β' subunits, which are more commonly used as indices of the cellular RNA polymerase levels, were not used here because the spots representing those proteins were not pure (as evidenced by different isotope ratios in different parts of the spots). Since several proteins in that molecular weight range can be resolved on the two-dimensional gels, the contamination is not necessarily due to χ (17), which is known to be present in *E. coli* NC3 (18).

The ρ_4/α_2 ratios are shown in Fig. 3. Obviously, these ratios reflect the ρ/RNA polymerase ratios directly only if (*a*) ρ is active only as a tetramer, (*b*) all α is incorporated into RNA polymerase, and (*c*) there is no functional sequestering of ρ, α, or RNA polymerase. There is good evidence *in vitro* for *a* (1, 5). As for the second qualification (*b*), recent evidence (18) indicates that the mole ratio of α to β' is between 5 and 7, which represents 2.5–3.5 times as much α as could be incorporated into RNA polymerase. If this excess of α is constant at all growth rates (which would not be likely, given the regulatory pattern for α reported here), then the ρ/RNA polymerase ratios in the cell are 2.5- to 3.5-fold higher than the ratios shown in Fig. 3. The third qualification (*c*) does not, at least in the case of RNA polymerase (19), appear to be satisfied, but it is impossible to predict at this time what effects such functional sequestration has on the functional ρ/RNA polymerase ratio *in vivo*.

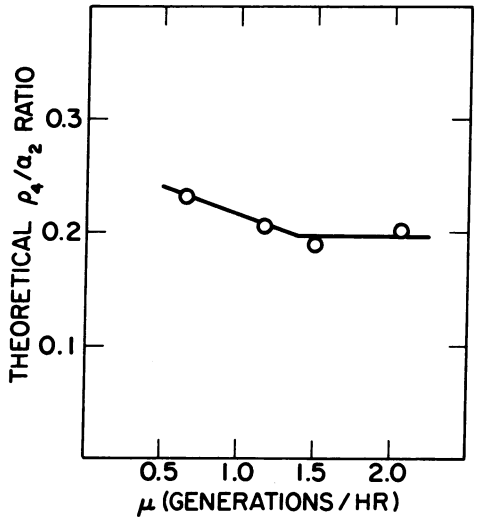

FIG. 3. The theoretical ρ_4/α_2 ratio versus growth rate. These values were obtained by starting with the molar ratio of ρ subunits to α subunits given in the text, correcting for the facts that ρ is active as a tetramer while α exists as a dimer in RNA polymerase, and then extrapolating to the growth rates in the other three media according to the data in Fig. 2. As described in the *text*, the actual ratio of ρ_4/α_2 and the functional ratio of ρ/RNA polymerase are probably somewhat different.

Nevertheless, the measured ratio of ρ to α is relatively constant, at about one ρ tetramer per five α dimers. This ratio increases by about 15% at the slowest growth rate studied. If the data *in vitro* of Goldberg and Hurwitz (5) can be applied to conditions *in vivo*, then the ρ/RNA polymerase ratio is in the most sensitive region of the response curve, such that the increase in this ratio at the slow growth rate will result in around a 10% increase in the efficiency of transcriptional termination. Thus, it is possible that the variation of ρ with respect to α reported here results in a significant variation, with growth rate, of the efficiency of at least some ρ-mediated transcriptional termination.

ρ and α

Although further study is required, it is apparent that neither ρ nor α is responding to changes in the growth rate as one would expect proteins coregulated with ribosomal proteins to do. This is an interesting situation, since it has been found (20) that the gene coding for α appears to be ". . . coordinately expressed and possibly cotranscribed . . ." with those coding for the ribosomal proteins S11, S4, and L17, although this was observed in UV-irradiated bacteria after infection with transducing phages carrying bacterial DNA of that section of the chromosome. Recent evidence (Dennis, Blumenthal, Lemaux, and Neidhardt, manuscript in preparation) indicates that α is not subject to stringent control (as compared to ribosomal proteins), and ρ does not appear to be, either.

One clue as to how ρ is regulated may come out of the observation that the regulatory pattern reported here for ρ bears a good resemblance to that reported elsewhere for σ (17). Another relationship between ρ and σ has to do with the fact that both cycle onto and off of RNA polymerase rapidly (7, 21) in fulfilling their transcriptional roles.

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