Expression of RNA Polymerase and Ribosome Component Genes in *Escherichia coli* Mutants Having Conditionally Defective RNA Polymerases

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The expression of the genes coding for the β and β' subunits of RNA polymerase, ribosomal RNA, ribosomal proteins, and β-galactosidase was investigated in strains carrying conditionally lethal mutations affecting either RNA polymerase core assembly or RNA polymerase enzyme activity. The mutant strain XH56 produces a temperature-sensitive β' subunit and at 42°C is defective in RNA chain initiation; consequently, little or no transcription occurs at the restrictive temperature. A partial restriction, produced by shifting the strain to 39°C, resulted in a rapid fivefold increase in the transcription of the rpoB and C genes and in the synthesis of the β- and β'-subunit proteins for which they code. The RNA polymerase assembly-defective strains A2R7 and Ts4 exhibited a 1.5- to 2-fold increase in the transcription of the rpoB and C genes and in the synthesis of β- and β'-subunit proteins after prolonged restriction. These results demonstrate (i) that regulation of the synthesis of the β- and β'-RNA polymerase subunits is under these conditions primarily transcriptional rather than translational, and (ii) that a stimulation of rpoB and C gene expression results from a restriction on RNA synthesis caused by either RNA polymerase inactivation or inhibition of its assembly. During restriction of the mutant strains, the transcription of the ribosome component genes exhibited patterns which were similar to transcription of the rpoB and C genes, supporting the evidence that genes coding for RNA polymerase are cotranscribed with ribosomal protein genes; transcription of the lacZ gene was observed to decrease concomitant with the stimulation of the rpoB and C genes.

The *Escherichia coli* RNA polymerase (nucleotidetriphosphate:RNA nucleotidyltransferase [EC 2.7.7.6]) is responsible for virtually all cellular RNA synthesis. The RNA polymerase is a complex enzyme consisting of four different subunits in the stoichiometry of α2ββ'σ (3, 6, 40). The rpoA gene, coding for the α subunit, is situated in a ribosomal protein gene cluster near 72 min on the *E. coli* chromosome (24, 38; all map positions are designated according to reference 1) and is cotranscribed with several ribosomal protein genes (24). The rpoB and C genes, coding for the β and β' subunits, respectively, are in the same transcription unit (17, 27, 29) and are situated adjacent to a ribosomal protein gene cluster near 88 min (24, 38). Recent experiments indicate that the rpoB and C genes form a common transcription unit with two of the adjacent ribosomal protein genes; the organization is promoter, rplJ, rplL, rpoB, and rpoC (39). The rpoD gene, coding for the σ subunit, has recently been located near the dnaG gene at 66 min (18, 20, 33); no ribosomal protein genes have yet been located in this region of the chromosome.

The core enzyme components α, β, and β' are synthesized in relatively constant molar ratios during steady-state growth and nutritional shift conditions, whereas the σ subunit appears to be synthesized at essentially the same rate under different growth conditions (2, 9, 16, 22, 23, 31). Because the core enzyme subunits are normally as stable as bulk *E. coli* protein (23), the rates of subunit synthesis must therefore control the intracellular levels of these proteins. The regulation of the synthesis of the RNA polymerase subunits and its relationship to ribosomal protein synthesis, however, are poorly characterized.

The isolation of strains containing conditionally lethal mutations in genes coding for RNA polymerase subunits has facilitated investigations of the regulation of subunit synthesis (7, 26, 32). By using such strains, it was possible to
uncouple $\beta$- and $\beta'$-subunit synthesis from $\alpha$-subunit synthesis, suggesting that the synthesis of these subunits is regulated at least in part by different mechanisms (37). Studies with a mutant strain containing a thermosensitive RNA polymerase unable to catalyze nucleoside triphosphate incorporation into RNA under restrictive conditions indicated that the regulation of $\beta$- and $\beta'$-subunit synthesis is transcriptional and is somehow related to a decrease in the ability of the enzyme to initiate general transcription. Furthermore, this regulation is related to the transcription of ribosomal protein genes near 72 and 86 min on the chromosome (10, 12).

In this investigation, the use of temperature-sensitive mutants defective in RNA polymerase assembly or activity has allowed the correlation of $\beta$- and $\beta'$-subunit protein synthesis with transcriptional activity at the rpoB and C genes. This supports the evidence that the regulation of $\beta$- and $\beta'$-subunit synthesis is primarily at the level of transcription. The results indicate that a general decrease in overall transcription rather than accumulation of assembly intermediates is probably responsible for the stimulation of $\beta$ and $\beta'$ synthesis. During restriction of the mutant strains, the transcriptional patterns of ribosome component genes were perturbed in a manner similar to transcription of the rpoB and C genes, consistent with the idea that genes coding for RNA polymerase subunits and ribosome components are cotranscribed (39). In contrast, transcription of the gene specifying $\beta$-galactosidase was severely depressed under conditions where transcription of the $\beta$- and $\beta'$-subunit genes was stimulated.

**MATERIALS AND METHODS**

Bacterial strains and growth conditions. The isogenic wild-type and mutant strains, obtained from J. Miller and J. Kirschbaum, were derived by P1 transduction of the respective mutations to E. coli strain X239 (F$^{\ast}$ his metB thr bfe purD argH2 rpsL lac) (26). The wild-type strain X240 (F$^{\ast}$ his metB thi rpsL lac) was obtained by using a P1 lysate from a wild-type donor and selecting the arg$^{\ast}$ pur$^{\ast}$ transductants. The mutant strains Ts4 and XH56, in addition, have the respective temperature-sensitive mutations rpoC4(Ts) and rpoC56(Ts) in the gene coding for the $\beta'$ subunit of RNA polymerase. The mutant strain A2R7 contains a double mutation, having both the temperature-sensitive and spontaneous rifampcin resistance mutations rpoB7 rpoB2(Ts) in the gene coding for the $\beta'$ subunit of RNA polymerase. Cultures were grown in M9 minimal salts medium supplemented with 0.2% glucose or 0.2% glycerol (and 10$^{-3}$ M isopropyl $\beta$-D-thiogalactoside) as the carbon and energy source and L-methionine (50 $\mu$g/ml), L-histidine (50 $\mu$g/ml), and thiamine (0.5 $\mu$g/ml) at the permissive temperature of 30°C (26). Cellular mass accumulation was determined by measuring the absorbance at 460 nm ($A_{600}$). Cellular RNA accumulation was monitored as the $A_{260}$ of the acid-insoluble, alkali-labile material (5). Before experimentation, cultures were grown for at least 10 exponential cell doublings. At an $A_{600}$ of 0.25 to 0.35, the cultures were shifted to the nonpermissive temperature of 42°C unless otherwise indicated.

Protein labeling and quantitation of $\beta$ and $\beta'$ subunits of RNA polymerase. Cellular proteins were radioactively labeled at 30°C and at various times after a temperature shift to 42°C by the addition of [14C]leucine (specific activity, 348 mCi/mmol; 1.0 $\mu$Ci/ml) to 5-ml sample volumes. Incorporation was terminated after 3 min by the addition of excess (50 $\mu$g/ml) nonradioactive L-leucine. Samples were further incubated for 2.5 min to allow for completion of nascent polypeptide chains. The cells were harvested, and lysates were prepared for sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Cellular proteins were separated by using 1.5-mm-thick SDS slab gels (7.5% polyacrylamide) with 3% stacking gels. Gels were run at 25 mV/cm for 2.5 h after which the $\beta$ and $\beta'$ subunits had migrated 6.5 to 7.0 cm into the gels and were separated by about 2.5 mm. The gels were dried, and autoradiograms were obtained with Kodak XR-2 X-ray film. Quantitation of radioactivity in the $\beta$ and $\beta'$ region of the gels was obtained by scanning the autoradiograms with a Quick Scan Jr. scanner (Helena Institute, Beaumont, Tex.) equipped with an integrator. The areas of the $\beta$ and $\beta'$ peaks were determined, corrected for different amounts of total radioactivity applied to the gels, and compared. In all instances, with the exception of strain XH56 at 42°C (see below), the amounts of total radioactivity applied to a gel differed by less than ±10%.

Nucleic acid preparation and hybridization. Pulse-labeled RNA was prepared by exposing 10- to 20-ml samples of the cultures to [5-3H]uracil (specific activity, 26 Ci/mmol; 10 $\mu$Ci/ml) for 1 min. The cells were rapidly cooled to 0°C in the presence of 10$^{-3}$ M sodium azide, harvested, and lysed in SDS, and cellular RNA was extracted by the phenol method (14). The RNA preparations were appropriate diluted to a concentration of about 1 $A_{260}$ unit per ml (equivalent to 50 $\mu$g of RNA per ml) and used for hybridization as described previously (12).

The DNAs used for hybridizations were from phage $\lambda$, from the specialized transducing phages $\lambda$trkr, $\lambda$drpc, $\lambda$nu5, and $\lambda$nu5c, and from the ColE1-derived composite plasmids pJC701, pJC703, and pJC720 (8, 12, 13, 14; the composite plasmids previously have been referred to as pcc 701, pcc 703 and pcc 720, respectively). The $\lambda$ and $\lambda$trk DNAs were employed to measure nonspecific hybridization. The $\lambda$trk DNA contains the $aroE$ and trkA genes located near 72 min on the E. coli chromosome. The $\lambda$drpc1 DNA, in addition to $aroE$ and trkA, contains a cluster of 15 ribosomal protein genes from this same region of the chromosome ($rpsD, E, F, H, K, M, Q, rplE, F, N, rpsM, D$) and the gene coding for the $\alpha$ subunit of RNA polymerase (rpoA). The difference in radioactivity hybridized with $\lambda$trkr and $\lambda$drpc1 DNAs, therefore, represents specifically that mRNA which is homologous to the DNA of the ribosomal protein gene cluster and the rpoA gene. The $\lambda$nu5 DNA contains an rRNA.
transcription unit and hybridizes almost exclusively stable rRNA (8, 11). The λlac5 DNA contains the lacZ gene and hybridizes mRNA transcribed from the lactose operon DNA. Hybridizations of radioactivity to λlac5 DNA were corrected for nonspecific binding of radioactivity by subtracting the radioactivity associated with λ DNA.

The composite plasmid pJC720 carries the genes coding for the β and β′ subunits of RNA polymerase (rpoB and C, respectively), located near 88 min on the E. coli chromosome. The pJC703 DNA contains, in addition to rpoB and C, the adjacent ribosomal protein gene cluster (rplA, J, K, L). Radioactivity hybridizing to pJC720 DNA represents mRNA specifically homologous to the DNA of rpoB and C. The difference in radioactivity binding to pJC703 and pJC720 DNAs therefore represents that mRNA which is homologous to the adjacent ribosomal protein gene cluster (12). All values were corrected for nonspecific hybridization to λ DNA.

The pJC701 DNA contains the rpoB gene. When used in competition experiments with pJC720 DNA, the pJC701 DNA competes for the DNA sequences which are homologous to rpoB but not to rpoC. Thus, the reduction in hybridization of RNA to pJC720 DNA in the presence of excess pJC701 DNA is rpoB mRNA; the residual hybridization to pJC720 DNA is rpoC mRNA. Further details are described elsewhere (12).

In the temperature-sensitive strain XH56, when labeled with [3H]uracil at 39°C, the amount of radioactivity incorporated into RNA is small because of the restriction on RNA synthesis. Consequently, the specific activity of the rRNA is too low to employ the standard RNA hybridization to excess λib5 DNA. To overcome this problem, a double-label technique was employed in which the hybridization of the [3H]-pulse-labeled RNA could be compared with a standard [32P]RNA preparation. The standard [32P]RNA was prepared from a culture of homogeneously labeled E. coli strain NF314 as previously described (11, 15). Under standard hybridization conditions of DNA excess, 40% of the [32P] radioactivity hybridized to 20 μg of λib5 DNA. To assay for RNA in the [3H]-pulse-labeled RNA preparations, samples were mixed with portions of the [32P] RNA and hybridized. The assay contained radioactive RNA, one λ DNA filter, and two λib5 DNA filters (5 μg of DNA per filter). Incubation was for 18 h at 67°C. The isotope ratio of [3H] to [32P] in the input RNA and in the RNA hybridized to the λib5 DNA filters was determined, and the [3H] hybridization was corrected according to the efficiency of hybridization of the [32P] radioactivity. All measurements were in triplicate, and in all cases a minimum of 10² cpm of each isotope was hybridized to each λib5 DNA filter.

RESULTS

RNA synthesis in the RNA polymerase mutant strains. Strains X240, XH56, Ts4, and A2R7 are isogenic strains of E. coli differing only in the genetic constitution of the rpoB and C genes. Strain X240 is wild type, whereas strains XH56 and Ts4 carry the thermosensitive mutations rpoC56(Ts) and rpoC4(Ts), respectively, and strain A2R7 carries the thermosensitive and rifampicin-resistant mutations rpoB7 rpoB2-(Ts). At the restrictive temperature of 42°C, strain XH56 is defective in RNA synthesis (7, 10, 19), whereas strains Ts4 and A2R7 are defective in different steps of core RNA polymerase assembly (21, 35, 36). The accumulation of cellular mass and RNA in these strains after a temperature shift from 30 to 42°C is illustrated in Fig. 1. Within 3 to 5 min of a shift to 42°C, cellular RNA and, somewhat later, cellular mass ceased to accumulate in the activity-defective strain XH56. In contrast, in the RNA polymerase assembly-defective strains Ts4 and A2R7, the accumulation of cellular mass and RNA increased essentially arithmetically after the temperature shift. This suggests that RNA polymerase assembled before the temperature shift remains active but that little or no additional core RNA polymerase is assembled after the temperature shift.

Synthesis of β and β′ subunits of RNA polymerase at restrictive temperatures. The measurement of β and β′ synthesis was undertaken to determine the effects of a temperature shift on the synthesis of the β and β′ subunits of RNA polymerase. Samples from cultures of each strain were radioactively labeled at 30°C and at times after a shift to 39 or 42°C. Lysates were prepared, and cellular proteins were separated by SDS-polyacrylamide gel electrophoresis. The amounts of β and β′ subunits relative to total radioactivity applied to the gels were determined (Table 1).

The wild-type strain X240 exhibited a slight decrease in the relative synthesis rate of the β and β′ subunits after a shift to 42°C. In the assembly-defective mutant strain Ts4, the relative synthesis rate of the β and β′ subunits was elevated about twofold after a prolonged period of incubation at the restrictive temperature. In the assembly-defective strain A2R7, a transient decrease was followed by a slight increase in the relative synthesis rates of the β and β′ proteins. These observations are consistent with previously reported measurements (26, 37) and indicate that a restriction in core RNA polymerase assembly over an extended period of time can cause an increase in the relative synthesis rates of the β- and β′-subunit proteins.

When strain XH56, defective in RNA polymerase activity, was shifted to 42°C, the inhibition of total protein synthesis was virtually complete within 10 min, and by 30 min no radioactivity was detectable on the autoradiogram in the vicinity of the β- and β′-subunit proteins. (Staining with Coomassie brilliant blue demonstrated that the β- and β′-subunit proteins were present in the extracts but, because they were not radio-
Fig. 1. Accumulation of cellular mass (A) and RNA (B) after a temperature shift to 42°C. Strains X240 (▼), XH56 (▲), TS4 (■), and A2R7 (□) were grown at the permissive temperature of 30°C. At zero time (t) the cultures were shifted to the nonpermissive temperature of 42°C. Cellular mass was monitored as A_{660}. Cellular RNA was determined as the A_{260} of the acid-insoluble, alkali-labile fraction of the cell mass. The broken lines extrapolate the preshift rates into the postshift region.

**Actively labeled RNA** was prepared before (rather than after) the temperature shift. Shifting strain XH56 to 39°C rather than 42°C only partially inhibits the activity of RNA polymerase (10). In such a shift it was observed that the relative synthesis rate of the β and β'-subunits increased almost fivefold within 20 min and then decreased slightly. Thus, it was apparent from these results that partially inhibiting RNA synthesis by restricting RNA chain initiation profoundly affected the synthesis of the β- and β'-RNA polymerase subunit proteins.

**Transcription of rpoB and C and ribosomal protein genes in RNA polymerase assembly-defective mutants.** The patterns of β- and β'-subunit synthesis obtained for strains Ts4 and A2R7 during restriction presumably result from alterations either in the transcription of the rpoB and C genes or in the translation of the rpoB and C mRNA. If regulation of β- and β'-subunit synthesis is primarily at the level of transcription, the patterns of β- and β'-subunit synthesis would be expected to correlate with the amount of transcription occurring from the rpoB and C genes. To examine this, samples from cultures of strains X240, Ts4, and A2R7 were pulse-labeled with [5-3H]uracil at 30°C and at various times after a shift to 42°C. Radioactive RNA was prepared and used for hybridizations to an excess amount of pJC720 DNA containing the rpoB and C genes.

Transcription from the rpoB and C genes in the wild-type strain X240 remained essentially constant or increased slightly after the temperature shift (Fig. 2A). In the mutant strain Ts4, transcription from the rpoB and C genes increased steadily during the incubation period at 42°C, reaching a level about twofold above the 30°C control level after 120 min (Fig. 2B). The amount of transcription from the rpoB and C genes at the permissive temperature of 30°C was elevated in the mutant strain Ts4 as compared with the wild-type strain X240; the relative amounts of β- and β'-subunit proteins were also elevated in strain Ts4 compared with the wild-type strain at the permissive temperature. This suggests that even at 30°C assembly of core RNA polymerase in strain Ts4 may be partially defective.

Transcription of the rpoB and C genes in the rpoB mutant strain A2R7 exhibited an altered pattern in the first 30 min after a shift to the nonpermissive temperature (Fig. 2C). After 15 min, the level of rpoB and C transcription was decreased slightly relative to the 30°C control level; at times after 30 min the amount of transcription gradually increased to a final level about 1.5-fold greater than that of the control.
TABLE 1. Relative rates of β- and β′-subunit synthesis after a shift to restrictive conditions*  

<table>
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<tr>
<th>Temperature shift</th>
<th>Time after temperature shift (min)</th>
<th>Relative rate of β- and β′-subunit synthesis in strain:</th>
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<tr>
<td></td>
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<td></td>
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* Cultures of X240, Ts4, A2R7 and XH56 were grown at 30°C, the permissive temperature. At zero time, the cultures were shifted to the indicated temperature. At zero time (30°C control) and at 30 and 120 min after the shift to the elevated temperature, 5-ml samples were pulse-labeled with [14C]leucine for 3 min. Nonradioactive leucine was added in excess, and incubation was continued for 2.5 min to allow for completion of nascent polypeptide chains. The cells were harvested and lysates were prepared.

Cellular proteins were separated by electrophoresis on 7.5% SDS-polyacrylamide slab gels. The amount of radioactivity corresponding to β and β′ peaks was determined by integration and corrected for the different amounts of radioactivity applied to the gels (from 1 × 10^5 to 2 × 10^6 cpm). The relative rates of synthesis are standardized so that the rate in the 30°C control for each strain represents 1.0. The absolute amount of labeled β- and β′-subunit protein in strain Ts4 was estimated to be about 2.7-fold greater than that in the parental X240 strain at the permissive growth temperature of 30°C. The relative rates of subunit synthesis in the 30°C controls, compared with a value of 1.0 for the wild-type strain X240, were 2.0, 0.9, and 1.1, respectively, for Ts4, A2R7, and XH56.

Although the apparent decrease at 15 min may be artifactual, the same pattern has been observed in independent experiments. In addition, previous investigators have also observed a decrease in the synthesis of β- and β′- subunit proteins during this period (37).

Because of the proximity of the RNA polymerase genes and ribosomal protein genes and because of the physiological relationships between transcription elements and translation elements, the transcription of ribosomal protein genes located at 72 and 88 min was examined in the assembly-defective mutants during incubation at the nonpermissive temperature. This was achieved by hybridizing the pulse-labeled RNAs to Adspl and pJC703 DNAs. In the wild-type strain X240 and the mutant strain A2R7, the ribosomal protein genes near 72 and 88 min were essentially unaffected by the shift to 42°C. In strain Ts4, a slight increase in the level of ribosomal protein transcription was observed after 120 min at the restrictive temperature.

RNA polymerase gene expression in a mutant defective in initiation of RNA chains. A mutant defective in RNA polymerase activity exhibited properties substantially different from mutants defective in RNA polymerase assembly. When shifted to 42°C, strain XH56, carrying the thermosensitive mutation rpoC56(Ts), almost immediately ceased accumulation of RNA (Fig. 1). This cessation appears to result in vivo from an inhibition of RNA chain initiation rather than RNA chain elongation (10). After the initial few minutes at 42°C, essentially no RNA was synthesized, and, consequently, the effect of such a restriction on the general pattern of transcription of the chromosome cannot be determined directly. However, at temperatures several degrees below the absolutely restrictive temperature, initiation of RNA transcripts is only partially inhibited, and RNA continues to accumulate, although at a somewhat reduced rate.

Strain XH56 was grown at 30°C in glucose or glycerol minimal medium and at zero time was shifted to 39°C. At various times, portions of the cultures were labeled with [3H]uracil for 1 min or [14C]leucine for 3 min. In the respective samples RNA was prepared for hybridization or protein extracts were prepared for SDS gel electrophoresis. The imposition of a partial restriction in glucose minimal medium resulted in a four- to fivefold increase in the relative synthesis rate of β and β′ proteins during the initial 30 min at 39°C (Table 1). During the same time interval the relative level of transcription from the rpoB and C genes was elevated about fivefold (Fig. 3) (10). By using a competition hybridization assay it was possible to show that transcription of the rpoB and rpoC genes increased coordinately (Fig. 4); this result was expected because these two genes are in the same transcription unit (17, 27, 29, 39). A similar restriction carried out in glycerol minimal medium resulted in a five- to sixfold increase in the level of transcription of the rpoB and C genes (Fig. 3).

The transcription of genes specifying the RNA and protein components of the ribosome was also examined during the partial restriction of strain XH56 (Fig. 3). In both the glucose- and glycerol-grown cells, the transcription of ribosome component genes was perturbed in a manner similar to the transcription of the RNA polymerase genes. After the temperature shift to 39°C, the amount of transcription from rRNA and ribosomal protein genes increased to levels...
about 1.2- to 1.5-fold greater than those of the 30°C controls and then decreased, after 60 min, to approximately the preshift values. In the wild-type strain X240, a temperature shift to 42°C had virtually no effect on the transcription of ribosomal protein genes (Fig. 2A) or rRNA genes (data not shown).

Finally, the transcription of the lacZ gene, specifying the inducible enzyme β-galactosidase, was examined in glycerol-grown cells in the presence of the inducer isopropyl β-D-thiogalactoside. It was apparent that transcription of the lactose operon was extremely sensitive to the restriction. At 5 min the level of transcription was only about 10% of the prerestriction level and may have represented runoff of RNA polymerase which had initiated transcription of the lactose operon during the first few minutes of the restriction. After 20 min at 39°C, when the relative transcription of the rpoB and C genes had increased about sixfold, transcription from the lactose operon was essentially undetectable.

**DISCUSSION**

**Regulation of β- and β'-subunit synthesis.** The temperature-sensitive mutant strain XH56 is unable to initiate transcription at nonpermissive temperatures, but at intermediate temperatures initiation of transcription is only partially restricted (10). When this strain was shifted to a partially restrictive temperature, the relative rate of transcription of the rpoB and C genes exhibited a dramatic increase; this was accompanied by a concomitant increase in the synthesis of the β- and β'-subunit proteins (Fig. 3 and Table 1). Similar results were obtained when another rpoC mutant strain, also having temperature-sensitive RNA polymerase activity, was shifted to restrictive temperature (25). The results are also in agreement with those obtained when a wild-type strain of *E. coli* was treated with the transcription initiation inhibitor rifampin (3a). These observations indicate that an inhibition of transcription initiation selectively stimulates the relative rate of rpoB and C transcription; the resultant increase in rpoB and C mRNA production leads to a corresponding increase in the relative rate of β- and β'-subunit protein synthesis.

The temperature-sensitive RNA polymerase assembly mutant strains T64 and A2R7 are defective in different steps in the assembly of core RNA polymerase (21, 35, 36). At the nonpermissive temperature, only RNA polymerase molecules which have been assembled before the
temperature shift is active in transcription. As cellular mass accumulation and cell division occur, the intracellular concentration of this active and preassembled RNA polymerase progressively decreases. Eventually, a concentration incapable of sustaining further growth is reached because these strains cannot form colonies when incubated at the nonpermissive temperature of 42°C. As seen in Fig. 1, cellular mass and RNA continued to accumulate when strains Ts4 and A2R7 were shifted to 42°C, although the accumulations became arithmetic rather than exponential. This indicates that the RNA polymerase assembled before the shift retains most, if not all, of its activity. In strain Ts4, the restriction of RNA polymerase assembly led to a gradual increase in the relative rate of rpoB and C transcription to double the preshift rate, with a concomitant increase in the synthesis of β- and β'-subunit proteins (Fig. 2 and Table 1) (37). In strain A2R7, the restriction of RNA polymerase assembly resulted in a transient decrease in rpoB and C transcription, but the relative rate of transcription of these genes eventually increased to 1.5 times the preshift rate; the synthesis of β- and β'-subunit proteins responded in a qualitatively similar fashion. Thus, restricting the assembly of RNA polymerase also stimulates the rate of rpoB and C transcription and relative synthesis rate of β- and β'-subunit proteins.

In summary, these results indicate that the regulation of β- and β'-subunit synthesis under these conditions is primarily transcriptional. Furthermore, it is evident that a rapid partial inhibition of transcription leads to a rapid rela-
tive increase in rpoB and C transcription, whereas a gradual inhibition of transcription leads to a gradual and less dramatic increase in rpoB and C transcription. This is most easily explained by proposing a mechanism which couples rpoB and C transcription, in an inverse manner, to the total transcription capacity of the cell. Such a proposal is quite different from previous models which invoke subunits or assembly intermediates as autogenous regulatory effectors (see reference 35 for a review).

Expression of other genes during the restriction. The transcription of the rpoB and C genes has been shown to be coordinate with, but fivefold less frequent than, the transcription of the adjacent ribosomal protein gene cluster during steady-state growth (12). Recent genetic experiments indicate that the rpoB and C genes form a single transcription unit together with two of the adjacent ribosomal protein genes; the organization is promoter, rplJ, rplL, rpoB, and rpoC (39). The reduced frequency in transcription between the ribosomal protein and RNA polymerase genes is presumably the result of an attenuator which terminates about 80% of the transcripts during exponential phase growth. It is further apparent that, under certain conditions, the expression of the rpoB and C genes may be dissociated from that of the adjacent ribosomal protein genes. For example, the transcription of the rpoB and C genes and the synthesis of the β- and β'-subunit proteins appears to be insensitive to stringent control during a partial amino acid deprivation, whereas transcription of ribosomal protein genes and the synthesis of ribosomal proteins is substantially reduced (4, 11, 13, 30). Under these conditions, termination at the attenuator seems to be relaxed. Similarly, in vitro experiments with a coupled transcription-translation system suggest that guanosine tetraphosphate reduces the production of ribosomal proteins but not the β- and β'-subunit proteins (28). A second condition which dissociates the expression of the rpoB and C genes from the ribosomal protein genes has been achieved by using the temperature-sensitive RNA polymerase mutant strain XH56. A partial restriction of this strain stimulated transcription of the rpoB and C genes about fivefold, whereas transcription of the adjacent ribosomal protein genes was stimulated only about twofold (see above; 10). Similarly, the expression of the rpoB and C genes was dissociated from that of the adjacent ribosomal protein genes in studies with the transcription initiation inhibitor rifampin. When a wild-type strain of E. coli was subjected to low concentrations of rifampin, the transcription of the rpoB and C genes was stimulated about threefold, whereas transcription of the adjacent ribosomal protein genes was stimulated by only about 50% (3a). In all instances, however, the frequency of transcription of the ribosomal protein genes remained greater than the frequency of transcription of the rpoB and C genes. These observations are consistent with the proposal of an attenuator, with unique physiological properties, being located between the rplL gene and the rpoB and C genes.

The transcription of the lactose operon was found to drop to essentially undetectable levels in conjunction with the stimulation of rpoB and C transcription in strain XH56 (Fig. 3). This response is probably not due to exclusion of the inducer isopropyl β-D-thiogalactoside or to the catabolite repression system and could reflect either a competition for active RNA polymerase based upon promoter strength or functional heterogeneity in the pool of RNA polymerase (3a). In either case, it is clear that the selective increase in transcription of rpoB and C and ribosomal genes occurs even when transcription of the lac operon, which has a very strong promoter, is severely depressed.

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