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Abortive termination of *bioBFCD* RNA synthesized *in vitro* from the *bioABFCD* operon of *Escherichia coli* K-12

(biotin DNA restriction fragments/*in vitro* RNA synthesis/inosine triphosphate/S100 extract)

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ABSTRACT **The nature of divergent transcription from the *bioABFCD* gene cluster of *Escherichia coli* K-12 has been studied *in vitro* by using DNA restriction fragments as templates. The *in vitro* RNA transcript initiated at the promoter p_A was found to be similar to the *in vivo bioA* transcript, whereas the transcription initiated at p_B produced a small piece of RNA less than 200 nucleotides long. Substituting ITP for GTP or using an S100 cell-free extract as a source of antiterminator permitted transcription of the *bioBFCD* gene cluster initiating at the promoter p_B to be read through. The possible site for *in vitro* transcription termination in the *bioB* region is discussed.**

The biotin genes of *Escherichia coli* K-12 are clustered in two transcriptional units and are divergently transcribed (1, 2). One of the transcripts proceeds anticlockwise into the *bioA* gene, and the other clockwise into the *bioBFCD* genes; opposite DNA strands of the *E. coli* chromosome are transcribed (1). Both transcripts are coordinately repressed by *d*-biotin (1). However, *bioA* and *bioBFCD* genes are differentially transcribed when bacteria are grown in the presence of the *d*-biotin analogues *d*-homobiotin and *d*-dehydrobiotin. This explains the noncoordinate nature of expression of the *bioA* and *bioBFCD* genes *in vivo* (3). Differential repression of enzymatic activity from the *bioA* and *bioBFCD* segments in a coupled transcription–translation system has been demonstrated in the presence of *d*-biotin (4), which supports a model of independent control of *bioA* and *bioBFCD* transcription as suggested by Vrancic and Guha (3). A model of transcriptional overlap controlled by a single operator situated at the overlap region was proposed on the basis of genetic studies (5). A similar model, suggesting that the *bioA* and *bioB* promoters lie *vis-a-vis* with the common operator in between and partially overlapping the promoters, was proposed on the basis of nucleotide sequences of the *bio* regulator region (6, 7).

In the wake of such complexity, the precise control mechanisms of *bioA* and *bioBFCD* transcription still remain in obscurity. We have undertaken the study of *in vitro* transcription using purified restriction fragments containing the *bio* genes in order to characterize the nature of transcriptional control in the *bio* operon. In this communication we report an abortive synthesis of approximately 200 nucleotide long RNA species, initiated at the promoter p_B of the *bioBFCD* segment of the *bio* operon. Transcription of a full length of RNA from the *bioBFCD* segment has been demonstrated by employing an essentially ribosome-free S100 extract or ITP (substituted for GTP) in the *in vitro* transcription system.

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MATERIALS AND METHODS

Restriction fragments of *E. coli* K-12 *bio* operon were derived from plasmids pNG11 and pNG6. Plasmid pNG11 is selected from a clone of the recombinant of mini-ColE1, pMB8, and the 9-kilobase pair (kb) *bio* DNA fragment reported previously (8), which carries all the *bio* genes of *E. coli* K-12 (Fig. 1). pNG6 is a recombinant of *Eco*RI-cleaved pMB8 and 3.66-kb *bio* DNA fragments. The *bio* DNA fragment of 3.66 kb was derived from the *Eco*RI-cleaved 7.4-kb DNA fragment from λ*bio*3h-1 phage DNA (9), which carries the entire *bioA*($p_A o p_B$) segment of the *bio* locus. The deletion end point at the right end is within the *bioB* cistron but close to the promoter, p_B. The 3.66-kb fragment contains a negligible amount [less than 30 base pairs (bp)] of unresolved *E. coli* DNA. A diagram of the 3.66-kb fragment is shown in Fig. 1. Restriction mapping of the entire *bio* operon of *E. coli* K-12 will be published elsewhere.

***In Vitro* Transcription, Hybridization Assay, and Polyacrylamide Gel Electrophoresis.** *In vitro* transcription experiments were carried out as described by Miller and Burgess (10) with some modifications. In the transcription experiments, DNA fragments were added (final concentration 30 μg/ml) to a 200-μl reaction mixture containing: 33 mM Tris acetate, pH 7.9; 0.4 mM dithiothreitol; 10 mM $MgCl_2$; 200 mM KCl; three unlabeled nucleoside triphosphates, 0.094 mM each ATP and GTP, 0.063 mM CTP, and 0.03 mM unlabeled UTP; 2.05 pmol of [^{14}C]UTP (specific activity 486 mCi/mmol, New England Nuclear; 1 Ci = 3.7×10^{10} becquerels); 20% (vol/vol) glycerol; and 50 units of *E. coli* RNA polymerase (P-L Biochemicals or Boehringer Mannheim) per ml. The amount of [^{14}C]UTP to be used in the reaction was vacuum dried at 50°C for 5 min to remove ethanol and water. The rest of the components except the $MgCl_2$ were added to it, and the reaction mixture was preincubated for 10 min at 37°C. $MgCl_2$ at a final concentration of 10 mM was added to start the reaction, and the reaction was allowed to proceed for 20–60 min at 37°C. Rifampicin was then added to a concentration of 20 μg/ml and the mixture was incubated for 5 min more to allow initiated RNA chains to be completed. The reaction mixture was transferred to an ice-water bath, 20 μl of RNase-free DNase I (Worthington, electrophoretically purified; 1 mg/ml in autoclaved distilled water) was added to the reaction. Digestion was carried out for 15 min at 30°C, and then the activity of DNase I was destroyed by keeping the sample in a boiling water bath for 2 min. The above basic method was used in all *in vitro* transcription experiments. Vari-

Abbreviations: bp, base pair(s); kb, kilobase pair(s); *l* and *r* strands, leftward- and rightward-transcribing strands of λ*bio* transducing phage DNA, respectively; NaCl/Cit, 0.15 M NaCl/0.015 M trisodium citrate, pH 7.2.

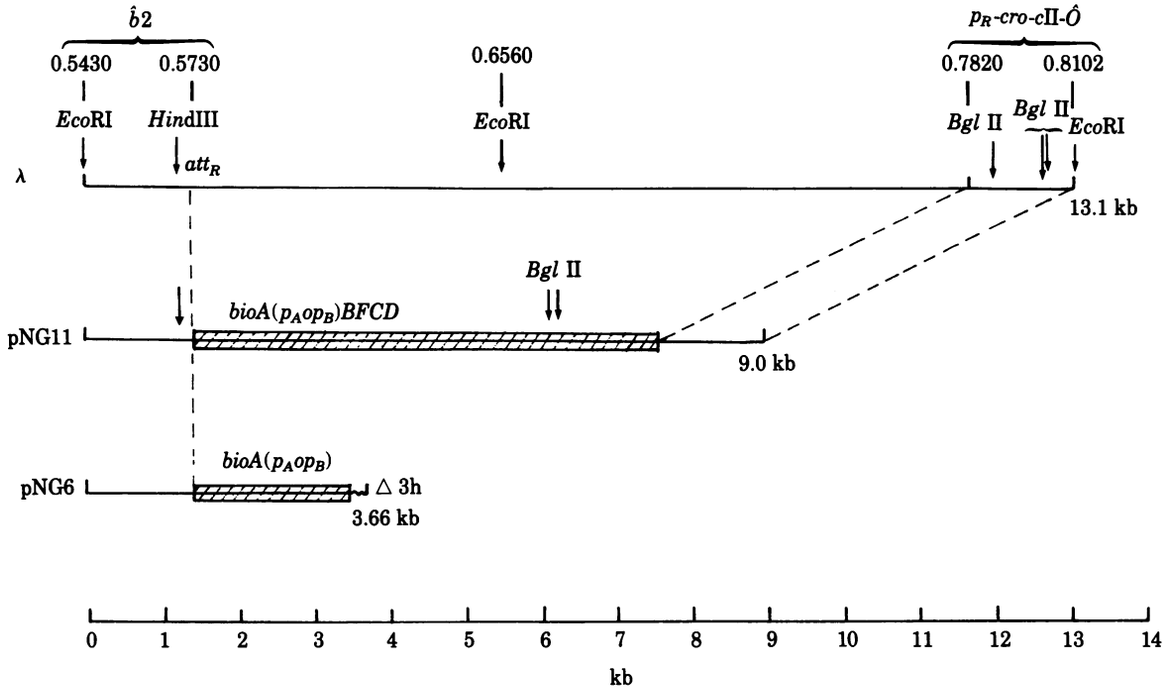

FIG. 1. Diagram of the *Eco*RI fragments from plasmids pNG11 and pNG6 containing the *bio* genes of *E. coli* K-12. The DNA segments containing the biotin genes are represented by the hatched bars. The insertions of *E. coli* and λ DNA are represented by the wavy and continuous lines, respectively. The pMB8 vector DNA is not shown. The 9-kb *bio* DNA fragment was originally derived from λ*bio*t124-10 phage DNA, which carries the entire set of *bioABFCD* genes (8). The 3.66-kb *bioA* DNA fragment has been derived from λ*bio*3h-1 phage DNA, which carries the *bio* locus with a deletion of *bioBFCD* genes (see *Materials and Methods* and footnote *; unpublished work). The *Eco*RI, *Hin*dIII, and *Bgl* II cleavage sites beginning at 0.5430 and extending to 0.8102 λ length and the important λ genes within that region (16) are shown in the upper drawing and aligned with the respective segments of the *bio* substitutions. The caret (^) indicates that parts of the *b*2 region and *O* gene are present in the λ *Eco*RI pNG11 and pNG6 fragments. The *bio* genes begin about 1,440 bp away from the left end point of the fragments. One of the *Bgl* II cleavage sites in the *bio* locus is 6,150 bp away from the left end point of the 9.0-kb *bio* DNA fragment. The second *Bgl* II site is approximately 135 bp to the right of the first one.

ations of the basic method are described under the appropriate tables.

RNA samples were directly used for hybridization assay, as described by Vrancic and Guha (3) with modifications. To each vial containing filters impregnated with 5 μg each of separated λ*bio*t124-10 or λ*c*72 phage DNA strands (conditions of DNA excess), varied amounts of [^{14}C]RNA (6,000–21,000 cpm), dimethyl sulfoxide at final concentration of 25% (vol/vol), and phenol-saturated NaCl/Cit of sufficient concentration to give each hybridization mixture a final volume of 1.0 ml of 2× NaCl/Cit (1× NaCl/Cit = 0.15 M NaCl/0.015 M trisodium citrate, pH 7.2) were added. The filters with hybridization mixture were incubated for 20 hr at 37°C. The [^{14}C]RNA·DNA hybrids were washed, treated with a solution of pancreatic and T1 RNase (made up of 25 μg of pancreatic RNase and 25 units of T1 RNase per ml of 2× NaCl/Cit) for 30 min at 37°C. After the enzyme treatment, the filters were washed on both sides with 2× NaCl/Cit and dried, and the radioactivity was determined in a Beckman liquid scintillation spectrometer (LS7500).

For polyacrylamide gel electrophoresis and fluorography, 250 μg of *E. coli* tRNA as carrier and 0.1 vol of 3 M sodium acetate were added to the reaction mixture. RNA was precipitated by adding 3 vol of ethanol at −20°C. RNA samples were resuspended in sterile 33 mM Tris·HCl, pH 7.9, mixed with 7 M urea/bromophenol blue dye solution or 14 mM methylmercuric hydroxide/dye solution, and electrophoresed on denaturating gels containing 4 mM methylmercuric hydroxide (11, 12).

Preparation of S100 Cell Free Extract. S100 extract (free of nucleic acid and ribosomes) was prepared from *E. coli* PR7 (*rns*$^-$ *pnp-7 bio*$^+$) as described by Ishii *et al.* (13) with modifications. A culture of *E. coli* PR7 was grown to about 5 × 10^8 cells per ml at 37°C in 1 liter of yeast extract/salt medium as described (14). The culture was rapidly chilled in ice and the cells were harvested by centrifugation. The cell pellets were kept frozen overnight at −70°C. The frozen cells were thawed, and then homogenized in 20 ml of ice-cold buffer I (10 mM Tris acetate, pH 8.2/14 mM magnesium acetate/60 mM KCl/1 mM dithiothreitol). The cell suspension was treated with 1 ml of lysozyme (5 mg/ml in 0.25 M Tris acetate, pH 8.0) at 0°C for 15 min to yield spheroplasts. The cells were then lysed by sonication and the lysate was freed from cell debris by centrifugation at low speed. The supernatant was then centrifuged for 3 hr at 37,000 rpm in a Beckman model L2 65B ultracentrifuge (SW 50.1 rotor). The clear supernatant (S100 extract) was collected and treated with streptomycin sulfate to a final concentration of 4%. The slurry was centrifuged for 30 min at 18,000 rpm in a Beckman L2 65B ultracentrifuge (SW 40.1 rotor) to remove the precipitated nucleic acids. Solid ammonium sulfate (0.54 g/ml of the supernatant) was added to the supernatant. The resulting precipitate was collected by centrifugation, dissolved in a minimal volume of 33 mM Tris acetate, pH 7.9, and dialyzed against the same buffer. The S100 extract was then further concentrated by dialyzing against 50% (vol/vol) glycerol in 33 mM Tris acetate, pH 7.9. All the above steps were carried out at 0–4°C unless mentioned otherwise. Protein concentration for S100 extract was determined by the Lowry technique (15).

RESULTS AND DISCUSSION

Transcription from the *bio* DNA Templates. The 9-kb DNA fragment obtained by *Eco*RI cleavage of the λ*bio*t124-10 phage

Table 1. Filter hybridization of the *in vitro* synthesized [^{14}C]RNA, specific for the *bio* locus of *E. coli* K-12, with an excess of separated strands of λ*bio*t124-10 phage DNA

Source of [^{14}C]RNA		[^{14}C]RNA hybridized, % of total				*bio*-specific [^{14}C]RNA hybridized, % of total			
		l strand		*r* strand					
Template DNA	*bio* locus	λ*bio*t124-10	λ*c*72	λ*bio*t124-10	λ*c*72	*l* strand (*bioA*-specific)	*r* strand (*bioBFCD*-specific)	*l* + *r*	*l*:*r*
bio DNA (9 kb) from the plasmid pNG11	*bioABFCD* (Fig. 1)	15.2	5.6	10.5	6.4	9.60	4.10	13.70	70:30
bioA DNA (3.66 kb) from the plasmid pNG6	*bioA*($p_A o p_B$) (Fig. 1)	17.1	5.7	5.2	0.4	11.4	4.8	16.2	70:30

Approximately 19,000–21,000 cpm of [^{14}C]RNA was hybridized with 5 μg each of *l* and *r* strands of separated λ*bio*t124-10 and λ*c*72 phage DNAs (in excess). The numbers in the *l* + *r* column are the sums of the numbers in the preceding two columns.

DNA carries the entire *bio* gene cluster, *bioABFCD* of *E. coli* K-12, as previously reported (8). The 9-kb *bio* DNA fragment and an *Eco*RI-cleaved λ*bio*3h-1 phage DNA fragment 7.4 kb in length (9), from which the *bioBFCD* genes in the *bio* locus from the region proximal to the *bioB* promoter (p_B) are deleted, were cloned in mini-ColE1 plasmid pMB8. A DNA fragment 3.66 kb in length derived from one of the recombinant plasmids, pNG6, was not altered in the *bioA*($p_A o p_B$) segment (Fig. 1), but the non-*bio* segment of the *E. coli* DNA that is included in the λ*bio*3h-1 phage (9) was deleted from the plasmid DNA during cloning (unpublished). The 9-kb and the 3.66-kb DNA fragments described above were employed in the present *in vitro* transcription studies and will henceforth be mentioned simply as the *bio* and *bioA* DNA fragments, respectively (Fig. 1).

The *in vitro* transcripts from the *bio* and *bioA* DNA fragments (Fig. 1) were characterized by hybridization with the separated *l* and *r* strands (the leftward- and rightward-transcribing strands, respectively) of λ*bio*t124-10 phage DNA, which carries the entire *bio* locus (Table 1), and by polyacrylamide gel electrophoresis (Fig. 2). Seventy percent of the [^{14}C]RNA synthesized from the 9-kb *bio* DNA template hybridized with the *l* strand (*bioA* specific) and 30% hybridized with the *r* strand (*bioBFCD* segment specific) of λ*bio*t124-10 phage DNA (Table 1), which is contrary to what is obtained when *in vivo* transcripts isolated from derepressed *E. coli* cells are hybridized (1). If the efficiency of the p_A (left) and p_B (right) promoters are to be considered equal, the ratio of the *l* (*bioA* RNA) and *r* (*bioBFCD* RNA) transcripts should be approximately 30:70, in compliance with the ratio of the sizes of the *bioA* and *bioBFCD* segments (1, *). The hybridization results with *in vitro* RNA showed a ratio of reverse order (*l* to *r* = 70:30). A similar ratio was obtained when the 3.66-kb *bioA* DNA fragment (Fig. 1) was used as the template DNA for transcription (Table 1).

Electrophoretic analysis of the RNA products from the *in vitro* transcription system containing purified *E. coli* RNA polymerase and the *bio* and *bioA* DNA templates but without ρ factor shows a major band of *bioA* RNA corresponding to approximately 1,200 nucleotides (Fig. 2, bands 1 in lanes a and b). The autoradiogram shows no band corresponding to the 3,200-nucleotide-long *bioBFCD* RNA. One major band of λ*b*2 transcript of approximately 1,000 nucleotides belonging to the λ*b*2 region† from both the *bio* and *bioA* templates (Fig. 2, bands 2 in lanes a and b) and three other major and minor bands corresponding to sizes between 500 and 900 nucleotides, probably initiating at the λp_R sites, were observed. These transcripts were present because the 9-kb *Eco*RI fragment contains the λ DNA portion on both the right and left sides of the *bioABFCD* genes (Fig. 1). The most interesting observation was the presence of a very fast moving [^{14}C]RNA band obtainable from either the *bio* or the *bioA* DNA template (Fig. 2, band 6 in lane a and band 3 in lane b); either it is the piece of RNA initiating at p_B (*bioBFCD* promoter) and terminating at some region proximal to it or it may belong to the λ*b*2 region initiating at a λ*b*2 promoter that is included in the *Eco*RI fragments of the *bio* DNA. There are two pieces of evidence supporting the view that the small piece of RNA is initiated at p_B. The first evidence was obtained from the result of hybridization (Table 1), which indicated that approximately 30% of the *in vitro* RNA hybridized specifically with the *r* strand of λ*bio*t124-10 phage DNA when the same DNA strand of λ*c*72 was used as control. The second piece of evidence was obtained from the analysis of *in vitro* RNA transcripts from the 3.66-kb *bioA* DNA fragment and a 4.7-kb *Hin*dIII/*Bgl* II DNA subfragment containing the *bio* operator–promoter region, derived from the 9-kb *bio* DNA (Figs. 1 and 3). The *bioA* transcript from both the DNA templates and the λ*b*2 RNA from the *Eco*RI *bioA* DNA template remained on the top of the 5% gel (Fig. 3, bands 1 in lanes a and b). A small piece of RNA consisting of approximately 200 nucleotides (Fig. 3, bands 2 in lanes a and b) was obtained with either the 3.66-kb *bioA* or 4.7-kb *Hin*dIII/*Bgl* II subfragment used as the template. The 120-nucleotide-long λ*b*2 RNA transcript (Fig. 3, band 3 in lane a) from the *Hin*dIII/*Bgl* II subfragment of *bio* DNA (see Fig. 1) was banded below the 200-nucleotide-long RNA (Fig. 3, bands 2 in lanes a and b). The small piece of RNA initiating at p_B and terminating at some region within 200 bp after initiation accounted for 30% of *in vitro bio* RNA hybridizing with the *r* strand of λ*bio*t124-10 phage DNA (Table 1).

Transcription with ITP in Place of GTP. *In vitro* transcription using ITP instead of GTP results in complete readthrough on the wild-type template DNA, ignoring some terminator sites (18–20). We have also used ITP in our purified *in vitro* transcription system. Our results indicated that IMP-containing *bio*RNA hybridized with the *r* strand (*bioBFCD* segment) of the λ*bio*t124-10 phage DNA with the *l* to *r* strand ratio of 38:62 (Table 2), whereas with the *bioBFCD*-deleted λ*bio*3h-1 DNA strands (9, *), the *l* to *r* ratio was obtained as 70:30 (not shown in the table). The increased amount of hybridization of RNA with the *bioBFCD* segment of the λ*bio*t124-10 *r* DNA strand is consistent with the readthrough of p_B-initiated RNA transcription in the presence of ITP, ignoring the promoter-proximal termination.

***In vitro* Synthesis of the *bioBFCD* RNA in Presence of Ribosome-Free S100 Extract.** In order to demonstrate an antitermination function present in *E. coli* cells that antagonizes ter-

* Szybalski, E. H. & Szybalski, W. (1977) 77th Annual Meeting of the American Society of Microbiologists, New Orleans, LA, May 11, 1977, Abstr. 325.

† Rosenvold, E. C., Blattner, F. R. & Szybalski, W. (1979) 79th Annual Meeting of the American Society of Microbiologists, Honolulu, HI, May 10, 1979, Abstr. 178.

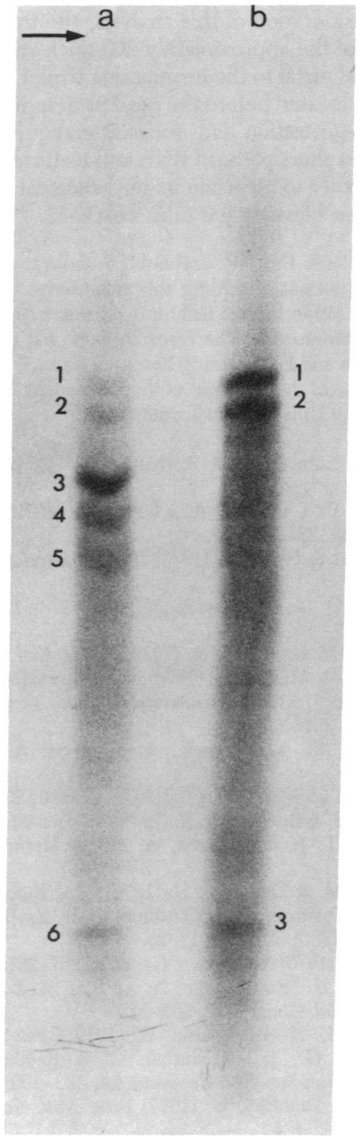

FIG. 2. Autoradiogram of polyacrylamide/agarose gels of [^{14}C]-RNAs synthesized *in vitro* by *E. coli* RNA polymerase holoenzyme. The 9-kb *bio* DNA and the 3.66-kb *bioA* DNA fragments (Fig. 1) were transcribed under standard reaction conditions. ^{14}C-Labeled RNA transcripts were separated electrophoretically on a composite gel containing 2.5% acrylamide and 0.7% agarose. Electrophoresis with Tris/borate/EDTA buffer, pH 8.3, was carried out at 30 V for about 8 hr at room temperature until the bromophenol blue dye marker reached the bottom of the gel. After electrophoresis, gels were processed as described by Fairbanks *et al.* (17) treated with New England Nuclear EN^{3}HANCE (for fluorography), dried, and exposed to Kodak XR x-ray film at −70°C. Lanes a and b show RNA transcripts synthesized from the 9-kb *bio* DNA fragment and the 3.66-kb *bioA* DNA fragment, respectively. Arrow indicates origin of electrophoresis.

mination and accommodates synthesis of complete polycistronic RNA chains once initiated at p_B, we employed S100 extract (free of nucleic acid and ribosomes) from *E. coli* PR7 in the *in vitro* transcription system. We measured differential *bioA* and *bioBFCD* RNA synthesis by hybridizing the transcription products with the separated *l* and *r* strands of λ*bio*t124-10 phage DNA. The [^{14}C]RNA transcripts synthesized from the *bio* DNA template in this transcription system showed an *l* to *r* ratio of 47:53 when hybridized with the separated λ*bio*t124-10 phage DNA strands (Table 3). This higher ratio in favor of hybridization with the *r* strand (*bioBFCD* genes specific) of λ*bio*t124-10

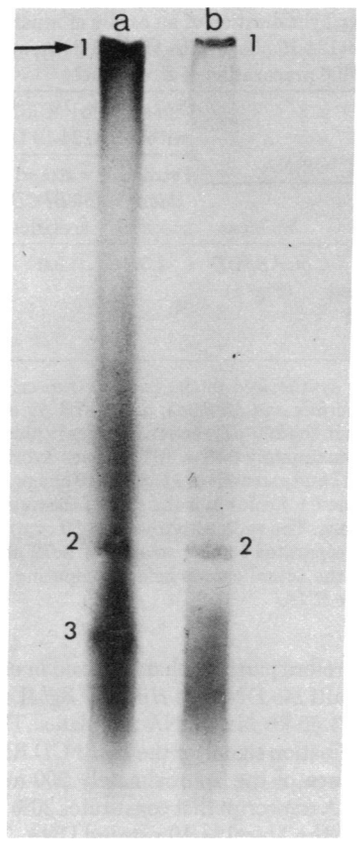

FIG. 3. Autoradiogram of polyacrylamide/agarose gels of [^{14}C]RNA products from purified *in vitro* transcription systems employing 3.66-kb *bioA* DNA and 4.77-kb *Hin*dIII/*Bgl* II *bio* DNA templates. RNA transcribed from the *Hin*dIII/*Bgl* II *bio* DNA fragment (Fig. 1) (lane a), and the *bioA* DNA fragment (lane b) were resolved on 5% polyacrylamide/0.65% agarose (composite) Tris/borate/EDTA gels containing 4 mM methylmercuric hydroxide. Electrophoresis and processing for fluorography were carried out as described for Fig. 2. Arrow indicates the origin of electrophoresis.

phage DNA may indicate that the ribosome-free S100 extract possesses some antitermination function resulting in the elongation of the *bioBFCD* transcript beyond the *in vitro* termination site.

Table 2. Filter hybridization of an excess of separated strands of λ*bio*t124-10 phage DNA with *bio* [^{14}C]RNA, synthesized *in vitro* in presence of 5′-ITP in place of 5′-GTP

Source of [^{14}C]RNA		*bio*-specific [^{14}C]RNA hybridized with λ*bio*t124-10 DNA, % of total			
Template DNA	*bio* locus	*l* strand (*bioA*-specific)	*r* strand (*bioBFCD*-specific)	*l* + *r*	*l*:*r*
bio DNA (9 kb) (*Eco*RI fragment of the plasmid pNG11)	*bioABFCD*	0.97	1.6	2.57	38:62

[^{14}C]RNA was synthesized as described in *Materials and Methods*, but substituting ITP at a final concentration of 0.1 mM for GTP. Approximately 5–6 × 10^3 cpm was hybridized with 5 μg each of *l* and *r* strands of separated λ*bio*t124-10 phage DNA (in excess). The number in the *l* + *r* column is the sum of the numbers in the preceding two columns. The radioactivities (210–310 cpm) of controls that hybridized with separated *l* and *r* strands of λ*c*72 phage DNA were subtracted from the actual counts before computing the percentages of *bio*-specific RNA.

Table 3. Filter hybridization of an excess of separated DNA strands of λ*bio*t124-10 phage with *bio* [^{14}C]RNA, synthesized *in vitro* with S100 preparation of *E. coli* PR7

Source of [^{14}C]RNA		*bio*-specific [^{14}C]RNA hybridized with λ*bio*t124-10 DNA, % of total			
Template DNA	*bio* locus	*l* strand (*bioA*-specific)	*r* strand (*bioBFCD*-specific)	*l* + *r*	*l*:*r*
bio DNA (9 kb) (*Eco*RI fragment of the plasmid pNG11)	*bioABFCD* (Fig. 1)	2.3	3.0	5.3	43:57

[^{14}C]RNA was synthesized in the *in vitro* transcription system as described in *Materials and Methods*, using 87.3 μg of protein of the S100 preparation in the 200-μl reaction mixture in place of *E. coli* RNA polymerase. Approximately 6–8 × 10^3 cpm was hybridized with 5 μg each of separated *l* and *r* strands of λ*bio*t124-10 phage DNA (in excess). The number in the *l* + *r* column is the sum of the numbers in the preceding two columns. The radioactivities (70–80 cpm) of controls that hybridized with separated *l* and *r* strands of λ*c*72 phage DNA were subtracted from the actual counts before computing the percentages of the *bio*-specific RNA.

We have therefore partially characterized *in vitro* transcripts from 9.0-kb *Eco*RI *bio* DNA, its *Hin*dIII/*Bgl* II 4.7-kb subfragment, and the 3.66-kb *bioA* DNA templates. The presence of an *in vitro* termination signal for the *bioBFCD* RNA is indicated by the occurrence of the approximately 200-nucleotide-long, p_B-initiated RNA transcript that constitutes 30% of the *bio* RNA hybridized with the λ*bio*t124-10 *r* strand DNA. Transcriptional readthrough has occurred at an early terminator site proximal to the *bioB* promoter in the presence of the GTP analog ITP (Table 2). An antiterminating activity has been found when the *in vitro* transcription experiments were carried out in the presence of ITP or a ribosome-free S100 preparation of *E. coli* PR7 (Table 3).

On analysis of the nucleotide sequences of the *bio* regulatory region (6, 7), one finds a terminating codon UGA in a frame that is 24 codons after the initiating triplet AUG. The *bioB* protein cannot initiate at this site, because the polypeptide will be terminated after only 24 amino acid residues, whereas the suggested molecular weight of the *bioB* protein is approximately 36,000*. We thus predict that the location of the *bioB* protein initiating codon is somewhere past this promoter-proximal UGA codon. In consideration of this analysis, the transcription termination site of the approximately 200-nucleotide p_B-initiated RNA is situated distal to the termination triplet. It is suggested that the site is located before the *bioB* protein initiation codon. Further characterization and nucleotide sequence analysis of the p_B-initiated short piece of RNA and its termination site are necessary in order to ascertain its physiological role in the regulation of biotin biosynthesis in *E. coli* K-12.

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