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Wild-Type and Mutant In Vitro Products of an Operon for Ribonucleic Acid Polymerase Subunits

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An in vitro protein-synthesizing system can synthesize two ribonuclеic acid (RNA) polymerase subunits of *Escherichia coli*, β and β', when a transducing phage deoxyribonucleic acid (DNA) template containing the *rpoB* region of the bacterial chromosome is added. Recombinant *rpoB* transducing phages were isolated that carry "nonsense" mutations of the class *rpo*-rifampin zero amber (formally referred to as rif^{0}_{am}). DNA was extracted from two of these phages. These DNAs are unable to direct the synthesis of β subunits, whereas β' synthesis is unaffected. Both mutations can be efficiently suppressed in vitro by the addition of suppressor transfer RNA. One of the mutations (*rpoB115*) produces a detectable nonsense (or restart) fragment of the β protein in the absence of suppression. It is concluded that *rpoB115* is an amber mutation within the structural gene for the β subunit of RNA polymerase.

The deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase of *Escherichia coli* has three different kinds of subunit in its active core (β,β',α) (6). Genetic evidence shows that β and β' are the products of a single operon, *rpoBC* (formerly *rif* but renamed to conform with the uniform nomenclature proposed by J. Scaife and R. Hayward), located at 79 min on the genetic map (8). Mutations at this locus that cause resistance to the antibiotic rifampin are known to be β structural gene mutations (10). Recent studies show that the structural gene for α maps far from *rpoB*, near 64 min on the genetic map (11). The isolation of φ80 bacteriophages, which carry a fragment of the bacterial chromosome that includes the *rpoB* locus, provides a suitable template DNA for the study of the expression of this region in vitro. We have shown that a coupled transcription-translation system is capable of synthesizing a product that resembles β in antigenicity and molecular weight when φ80d7*rpoB* DNA is included (2). Although early studies showed no β' synthesis, modification of the composition of the preparation used leads to the efficient synthesis of both β and β'-like products (1).

We have previously described a technique for the selection of potentially lethal mutations that affect the expression of RNA polymerase in vivo (3, 4). The essential feature of this selection is that the dominant rifampin sensitivity phenotype of a merodiploid strain, which carries genes for both rifampin sensitivity and resistance, is eliminated by mutation (*rpo*-rifampin zero mutations, formally called rif^{0}). The resulting clones express their recessive rifampin resistance character and are easily selected by their ability to grow on media containing rifampin. Since the method selects against the rifampin sensitivity character, which is known to be determined by the β gene, it seems likely that *rpo*-rif.zero mutations affect the β structural gene directly or a locus that controls its expression. A simple screening test identifies the clones that have conditional mutations of the chain-terminating class (*rpo*-rif.zero amber mutations) (4). This allows the isolation of a haploid strain carrying only the mutant copy of the locus as long as a suitable amber suppressor gene is also present.

This paper describes the isolation of φ80d7-*rpo* phages that carry *rpo*-rif.zero amber mutations and the use of DNA extracted from them in the in vitro system. The results strongly support the supposition that the two major in vitro products of the wild-type *rpo* DNA are the β and β' subunits and provides proof that at least one of the *rpo* mutations used is an amber mutation within the structural gene for the β subunit (*rpoB*).

MATERIALS AND METHODS

Isolation of bacterial Rif^{0}-amber mutants. Starting strain AJ1001 *argH su36* ins_{567} *trpA36 glyS10 lac-2 araC*(U25) *recA strA* rpo^{+} carries an F' factor, KLF10 *argH rpoB7*, and is rifampin sensitive owing

to the dominance of the chromosomal *rpo*$^+$ gene over the (rifampin-resistant) *rpoB7* gene (3, 4). One hundred independent cultures were grown at 37 C in LB broth (13) starting from single-colony isolates. Approximately 2 × 10^8 cells from each log-phase culture were spread onto LB agar petri plates supplemented with 200 μg of rifampin (Sigma) per ml. Most cultures gave 4 to 20 resistant colonies/plate after overnight incubation at 37 C. One colony was picked from each plate, and a new single-colony isolate was made by streaking on the same medium. The colonies were tested for their ability to receive the F′ factors KLF1 *ara*$^+$ or F14 *supU*, either of which can give an Ara$^+$ phenotype if it can establish in the clone. This test consisted of cross-streaking single test colonies against log-phase streaks of suitable streptomycin-sensitive donor strains (4) on M63 arabinose agar supplemented with tryptophan (0.01%), streptomycin (100 μg/ml), and arginine (0.01%). Growth in this F′ superinfection test indicates the ability of the mutant clone to lose the resident KLF10 *rpoB7* episome on superinfection. In the case of clones that have a chromosomal *rpoB* rif.zero amber mutation, KLF10 *rpoB7* can be displaced only by F14 *supU*, since the amber mutation in the remaining *rpo* allele must be suppressed for the cells to survive (4). F14 *supU* does not itself carry the *rpoB* locus (4). Those mutant clones that received F14 *supU* but not KLF1 *ara*$^+$ were retested by the cross-streak method, and the F14 *supU* derivatives were purified by single-colony isolation. From 97 independent rifampin-resistant clones of AJ1001, seven putative *rpo* rif.zero amber mutants were recovered.

Lysogenization and induction using φ80 transducing phages carrying the *rpo* region. The selection of lysogens carrying φ80d7 transducing phages and their induction to give high-frequency transducing lysates are described elsewhere (12).

Isolation of recombinant φ80d7*rpo* rifampin zero phages. Figure 1 is a diagram of the steps used. Stationary-phase cultures of the seven putative *rpo* rif.zero amber strains were crossed (14) with Hfr donor strain KL16 *strA*$^+$ *rec*$^+$, selecting *rec*$^+$ recombinant recipient colonies by their ability to grow after being spread and irradiated with ultraviolet light on supplemented minimum agar plates containing streptomycin. The resulting clones were purified. They have the genotype *rpo*(rif.zero amber) *argH glyT, ins-576, trpA36 glyS10 lac-2 araC-*(U25)*rec*$^+$/F14 *argH*$^+$ *supU*. These strains were lysogenized by φ80d7*supM rpoB(rif*$^+$*)* selecting for suppression of the bacterial *lac-2* marker by the ochre *supM* suppressor (giving a Lac$^+$ phenotype). Lysates were prepared by induction of these lysogens. Rare recombinant phages, which had exchanged *supM* for the closely linked *glyT* marker, were selected by their ability to lysogenize and transduce strain W7002 *rpo*$^+$ *trpA36* to *trp*$^+$ by missense suppression (12).

Ten *trp*$^+$ W7002 lysogenic cultures derived from each *rpo* rif.zero parental lysogen were tested for *rpoB7* on the resident prophage by testing the presence of frequent rifampin-resistant (homogenote) clones on rifampin agar (12). Of 70 *trp*$^+$ lysogens

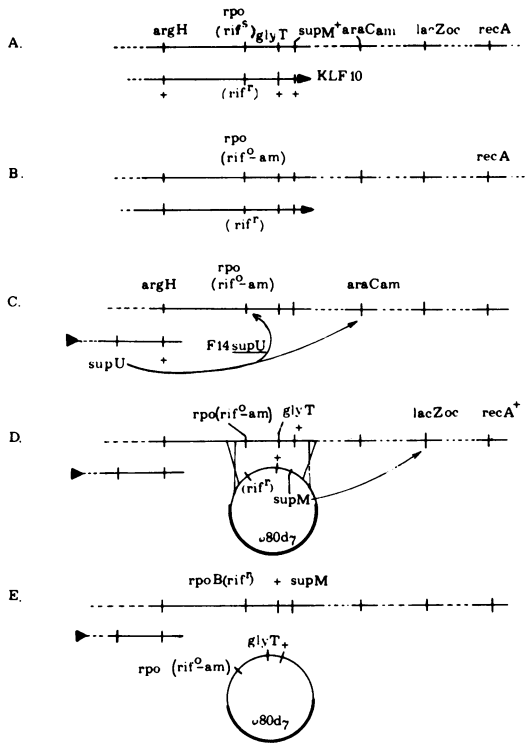

FIG. 1. *Steps in the construction of φ80d7rpo(rif. zero amber) transducing phages. (A) Starting strain E. coli K-12 AJ1001 showing relevant genetic markers. F′ factor KLF10 is maintained by selection by the Arg*$^+$ *phenotype. The strain is sensitive to rifampin due to the dominance of the rpo*$^+$ *(rifampin-sensitive) allele to the rpoB (rifampin-resistant) one. (B) Rifampin resistance selected. The rpo*$^+$ *allele is mutated to rpo-rifampin zero, which allows the expression of rifampin resistance. Recombination deficiency prevents the formation of Rif*^{r} *clones by the frequent homogenotization event. (C) F′ factor F14 supU introduced by mating, selecting for ara*$^+$ *by suppression. KLF10 must be displaced before F14 can establish itself. This can only happen if, as in this case, the rif*^{0} *mutation is amber and can thus be suppressed by supU. (D) Recombination deficiency eliminated by introduction of recA*$^+$ *by mating. φ80d7supM rpo(rif*^{r}*) was introduced by infection and lysogens were selected by the suppression of the ochre mutation lac-2 by supM to lac*$^+$*. On induction of the prophage, recombination can occur between the two rpo regions. (E) A recombination event exchanges rpoB(rif*^{r}*) supM for rpo-rif.zero amber and glyT. The glyT recombinant phages are selected from the lysate by their ability to transduce a recipient trpA36 strain to trp*$^+$ *by missence suppression. Most of the glyT recombinants also carry rpo(rif.zero amber) as shown.*

tested, nine had the prophage φ80d7*glyT rpoB7*. Those cultures that gave no Rif^r colonies (<1/10^6 viable cells) were chosen as candidates for lysogens containing recombinant phages with the genotype

ϕ80d7*glyT rpo*(rif.zero amber). Lysates of the prophage were made from certain of these ultraviolet induction.

Preparation of template DNA from phages. Phage DNA was extracted and purified as previously described (2). The preparation of bacterial S30 extracts was carried out as described by Wilcox et al. (17), except that French pressure cell lysis was carried out at a pressure of 600 lb/in^2. For certain experiments (see Results) a modified procedure was used for S30 preparation. This involves alumina grinding of cells, as described previously (2).

Protein synthesis in cell extracts. The composition of the disrupted cell system was as described by Wilcox et al. (17) omitting L-arabinose. For labeled protein synthesis leucine was replaced by [^{3}H]leucine (New England Nuclear Corp.; 0.3 mCi, 60 Ci/mmol). The 50-μl, cell-free mixtures were incubated for 60 min at 35.5 C with 10 μg of template DNA. The mixtures were then chilled on ice, diluted to 0.4 ml with buffer [0.01 M tris(hydroxymethyl)-aminomethane-hydrochloride, pH 8.4, 0.1 M NaCl], and decanted into a tube containing 20 μl of rabbit antiserum prepared against highly purified RNA polymerase. After 12 h at 4 C, the precipitate was collected by centrifugation at 40,000 $\times$ *g* for 30 min. The pellet was resuspended, without further washing, in 50 μl of sodium dodecyl sulfate sample buffer and run on polyacrylamide gels as previously described (2), except that slab gels (2 mm by 15 cm) were substituted for the cylindrical ones. The gels were stained and sliced, and the radioactivity was determined as previously described (2).

***supF*-enriched tRNA.** *supF*("suIII")-enriched transfer RNA (tRNA), prepared as described by Zubay et al. (19), was a generous gift of Elizabeth Bikoff.

Assays for β-galactosidase activity. Assays for β-galactosidase activity were carried out as described by Zubay et al. (18).

Bacterial strains. Strain AJ1001 is a derivative of W7001, obtained from B. Konrad. The *lac-2* (ochre), *araC* (U25) (amber), and *recA56* markers were introduced by crosses with Hfr strains from the collection of J. Beckwith. Strains KL16, W7001, and W7002 are from the same collection.

Bacteriophages. ϕ80d7*glyT rpoB* (*rif* resistant) (11) and ϕ80d7*supM rpoB*$^+$ were obtained from B. Konrad. λc*I857St68*h80d*lac*$^+$ and λc*I857St68*h80-d*lac*-545 were obtained from J. Beckwith (19).

RESULTS

RNA polymerase subunits synthesis in vitro. The basic experimental protocol that I have used to study the synthesis of RNA polymerase subunits in vitro is described in detail above. Briefly, DNA extracted from a ϕ80 transducing phage that carries a fragment of DNA derived from the *rpoB,C* region of the host chromosome was used as a template in a subcellular transcription-translation system similar to that described by Zubay et al. (18). Since the cell extract used in the in vitro system necessarily contains large quantities of active RNA polymerase, a physical rather than enzymatic assay for newly synthesized subunits was used. Proteins synthesized in vitro were labeled with tritiated leucine, and the polymerase subunits were purified from the system by precipitation with rabbit antiserum prepared against highly purified RNA polymerase. After washing, the radioactively labeled products were identified by sodium dodecyl sulfate-polyacrylamide electrophoresis. The excess of co-purifying unlabeled polymerase subunits that come from the cell extracts gives stainable marker bands that act as internal standards for identification of the radioactive subunits.

Specificity of the antibody precipitation step. Figure 2 shows visual scanning data from a typical stained gel subjected to electrophoresis with the immunoprecipitate from the disrupted cell system. Arrows indicate the positions characteristic of the polymerase subunits, taken from a control gel, subjected to electrophoresis with purified polymerase. Two diffuse bands correspond to the light- and heavy-chain proteins of rabbit immunoglobulin. The immunoprecipitation step gives a good one-step purification of RNA polymerase from the in vitro system. The recovery of σ, a protein that is a cyclically reused cofactor of RNA polymerase (7), is relatively poor. Moreover, the antiserum does not precipitate purified σ protein (data not shown). Thus, the assay system is unable to resolve the question of whether σ protein is a product of the *rpoB,C*-directed in vitro system.

ϕ80d7*rpoB* DNA directs the synthesis of β and β' in vitro. Figure 3a shows profiles of radioactivity obtained from the products of syn-

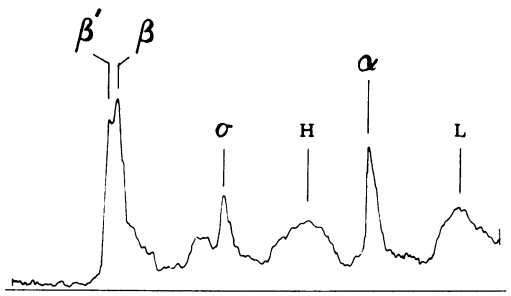

FIG. 2. *Facsimile of a densitometer tracing of a typical stained polyacrylamide gel, subjected to electrophoresis with the immunoprecipitate from the in vitro protein synthesis system. The indicated positions for RNA polymerase subunits were taken from a parallel experiment on the same gel using purified RNA polymerase. H and L are the broad bands formed by heavy and light chains of rabbit immunoglobulin.*

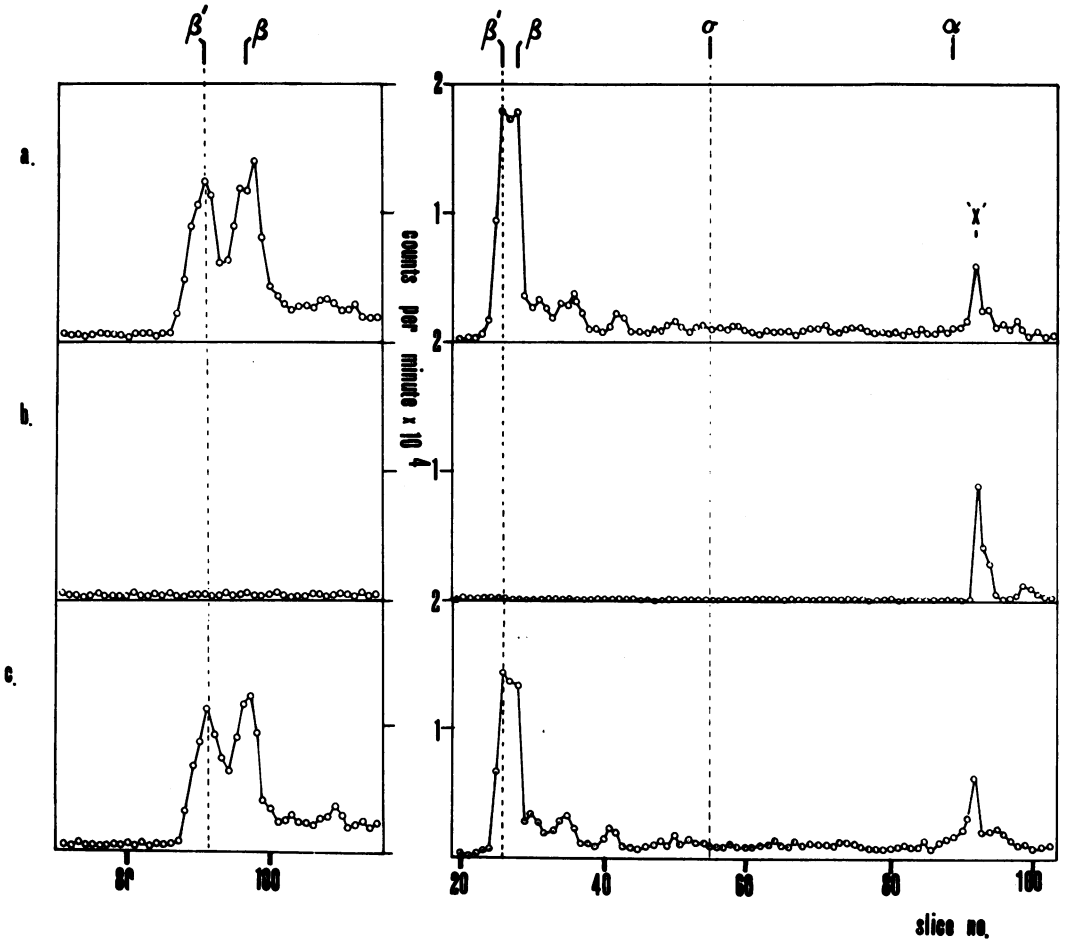

FIG. 3. *Radioactivity in sequential slices of 15-cm sodium dodecyl sulfate-polyacrylamide slab gels subjected to electrophoresis with the total resuspended immunoprecipitate from the in vitro protein-synthesizing system. (³H counts per minute were normalized to 10^7 total trichloroacetic acid-precipitable counts synthesized in the system. Total trichloroacetic acid counts varied between 0.6×10^7 and 1.8×10^7.) The marked positions of the RNA polymerase subunits are those of the stained bands derived from the RNA polymerase present in the S30 extracts. The in vitro mixtures contained: (a) φ80d7rpoB(rif^r) DNA; (b) φ80 wild-type DNA; (c) φ80d7rpoB(rif^r) DNA and 20 pmol of supF-enriched tRNA per ml. The left-hand panels were taken from a gel subjected to electrophoresis for 28 h at a constant voltage of 4 V/cm and cut into 0.5-mm slices. The right-hand panels are from a gel subjected to electrophoresis for 12 h at the same potential and cut into 1-mm slices. The origin of each gel was slice no. 1 (left-hand side).*

thesis directed by φ80d7*rpoB(rif^r)*. The long-term gel (left) resolves the β',β region well and suggests that both these subunits are synthesized by the system. The short-term gel (right) resolves a wider molecular-weight range (ca. 20,000 to 200,000).

A control experiment in which φ80 DNA was used showed no labeled peaks in the β,β' region (Fig. 3b). The peak marked "X" appeared in both experiments and must therefore be contaminating material not specified by the *rpo* region. The background "noise" and some minor peaks in Fig. 3a probably represent incompleted immunologically active fragments of β' or β. As expected of the product of a gene not present on the *rif* region DNA, α protein does not appear as a radioactive band.

***rpo*-rifampin zero amber mutations eliminate β synthesis.** The effect of *rpo*-rif.zero amber mutations on in vitro synthesis were studied. The *rpo*-rif.zero mutations used were characterized as chromosomal mutations and then transferred to φ80d7 by recombination. The steps involved in the construction are illus-

trated in Fig. 1 and are described in detail in Materials and Methods. DNA was extracted from lysates of two of the putative *rpo*-rif.zero transducing phages. Figure 4a shows the results obtained with one of these, *ϕ*80d7-*rpoB115*. Unlike the results obtained with the *rpoB(rif^r)* DNA, no *β* peak is seen, and a new peak (labeled Pn) appears in a position on the gel equivalent to a molecular weight of approximately 70,000.

Another *rpo*-rif.zero amber mutation, *rpo-197*, gave the results shown in Fig. 4c. In this case, the *β* peak is not replaced by any significant new peak.

A well-characterized amber mutation can be efficiently suppressed in vitro. Amber mutations can be suppressed in the in vitro system by the addition of tRNA isolated from *E. coli*, which is enriched for the *supF* amber suppressor tRNA (19). Table 1 shows the effect of added *supF*-enriched tRNA on the synthesis of *β*-ga-

TABLE 1. *β-Galactosidase assays on the products of λcI857t68h80dlac$^+$ DNA and its derivative carrying the amber Z-gene mutation 545*[a]

Additional tRNA	λcI857t68h80dlac$^+$	λcI857t68h80dlac$^-$545
None	25	<0.01
20 pmol/ml extracted from *sup$^+$* cells (suppressor negative)	22	<0.01
20 pmol/ml enriched for *supF*	23	26

[a] The in vitro system used the same components as used for the synthesis of *β* and *β'* subunits of RNA polymerase (see text), except for the addition of 1.0 *μ*mol of cyclic adenosine 3',5'-monophosphate per ml. The use of the modified in vitro system gave essentially the same results but with somewhat lower *β*-galactosidase activities (data not shown).

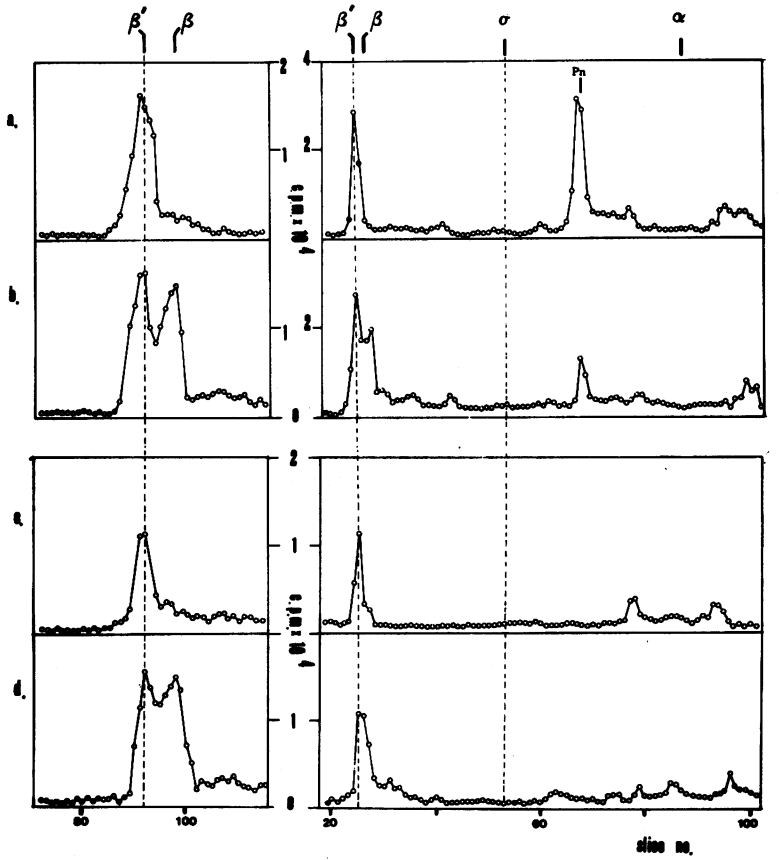

FIG. 4. *Radioactive profiles of sodium dodecyl sulfate-polyacrylamide gels. In vitro synthesis directed by: (a) ϕ80d7rpoB115 DNA; (b) ϕ80d7rpoB115 DNA plus supF-enriched tRNA; (c) ϕ80d7rpo-197 DNA; (d) ϕ80d7rpo-197 DNA plus supF-enriched tRNA. (Left-hand panels) Electrophoresis for 28 h; 0.5-mm slices. (Right-hand panels) Electrophoresis for 12 h; 1-mm slices.*

lactosidase in vitro. The mixture contained the same components as those used for the polymerase subunit synthesis, except that λ*c*I857*t*68*h*80d*lac*$^+$ DNA was used as a template. If the *lac* genes contained the amber *Z*-gene mutation *lacZ545*, synthesis of β-galactosidase was dependent on *supF* tRNA addition. The suppression obtained was approximately 100% relative to the product from the wild-type *lac* genes, on the (untested) assumption that the two DNA preparations used are potentially of equal efficiency as templates.

In vitro suppression of *rpoB115* restores β synthesis and reduces the synthesis of the Pn material. Figures 2c, 4b, and 4d show the effect of *supF*-enriched tRNA on wild type-, *rpoB115*- and *rpo-197*-directed products. It had no effect on β and β′ synthesis from wild-type *rpoB* DNA, but with *rpoB115* DNA it allowed the reappearance of the β component and reduced the amount of the Pn product. Thus, Pn is probably a β fragment produced in response to the unsuppressed *rpoB115* mutation. In the case of the *rpo-197* template, β production was also restored efficiently by the addition of *supF* tRNA (Fig. 4d).

Uncoupling of β and β′ synthesis confirms that Pn is a fragment of β. The surprising discovery was made that apparently minor modifications to the in vitro system (see Materials and Methods) led to its inability to synthesize β′ subunits, whereas β synthesis remained normal (1). The two types of system also discriminated in a similar way between the synthesis of the products of two cistrons of the *ara* operon of *E. coli* (1, 17). The basis for the physiological discrimination between β and β′ synthesis in vitro remains obscure. However, it provides a tool for the study of β synthesis in the absence of β′ synthesis. Figure 5 shows the results obtained with the modified system, using φ80d7*rpoB-rif*^{r} and φ80d7*rpoB115* templates. The results are essentially identical to those obtained above, except for the complete absence of the β′ subunit. The results help to confirm that the Pn product is of β gene origin. In the absence of any β′ material, a small amount of β protein is revealed as a product of the unsuppressed *rpoB115* mutant DNA preparation. This can be accounted for by the presence of recombinant φ80d7*rpo*$^+$ DNA in the preparation. This is the result of growing the mutant phage in an *rpo*$^+$ host (data not shown).

DISCUSSION

Amber mutations are those that give rise to the "nonsense" codon UAG within the DNA nucleotide sequence of the structural gene for a protein (5). This leads to the premature termination of the polypeptide chain at the nonsense codon, and a truncated product results ("nonsense fragment"). This can often be recognized by its immunological cross-reaction with the full-length product (9). In certain cases, protein synthesis can restart beyond the nonsense codon to produce a second fragment of the protein ("restart fragment") (15). Amber mutations can be suppressed by a mutant tRNA that can recognize the "nonsense" codon and catalyze incorporation of a suitable amino acid at this point in the growing polypeptide chain. Thus, the synthesis of the full-length polypeptide chain is substantially restored, and the production of truncated products is correspondingly reduced (16).

The two mutations in the *rpo* region studied here are amber mutations since their corresponding phenotypes are suppressible by a known amber suppressor, *supU*. In vitro, the mutations cause the loss of production of a protein product that can be identified as the β-subunit of RNA polymerase, whereas another product of the same operon, β′, is unaffected. *rpoB115* causes a new, shorter length product (Pn) to appear, which is immunologically related to RNA polymerase. In a modified system that normally produces only the β protein, only Pn is produced from the *rpoB115* DNA. Thus, Pn is probably a β fragment. On adding the product of another known amber suppressor gene (*supF*) to the in vitro system, β synthesis from *rpoB115* DNA is efficiently restored and the production of Pn is much reduced. Thus, Pn is a nonsense (or restart) fragment of β. I conclude that *rpoB115* is an amber mutation within the structural gene for the β subunit of RNA polymerase.

The *rpo-197* mutation does not lead to the production of a significant nonsense fragment. It may be that *rpo-197* is also a β structural mutation but that any nonsense fragment produced is too small to be resolved by the gel, or it is not an antigen for the antiserum, or that it is too unstable to be detected.

The combined use of in vitro synthesis and fractionation of immunoprecipitates used in this study should prove to be of use for the study of mutations in other vital cell functions. In vitro synthesis from a phage template allows the study of the expression of lethal mutations in essential functions in the absence of other alleles. The fractionation of immunoprecipitates allows the detection of truncated or full-length products of mutant genes. The products need not be enzymatically active.

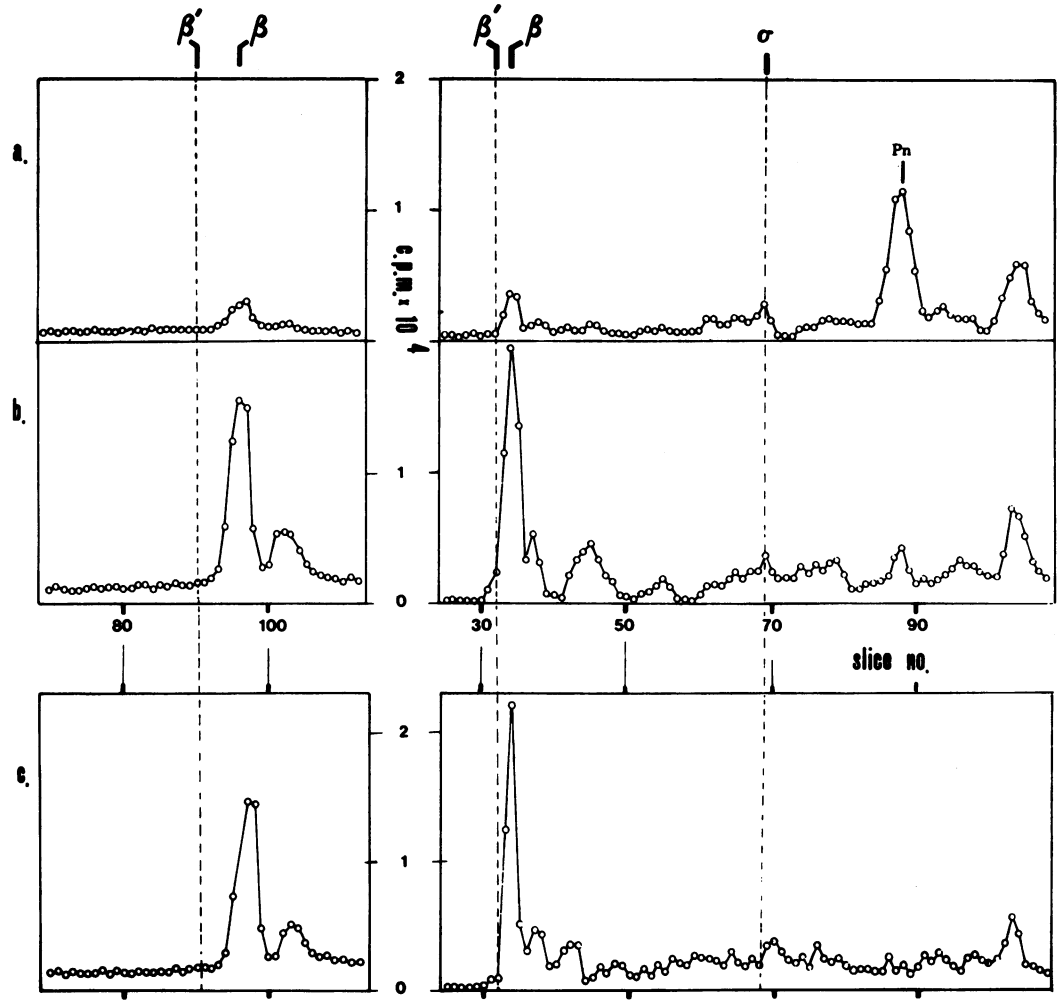

FIG. 5. *Radioactive profiles of sodium dodecyl sulfate-polyacrylamide gels run with the products of a modified in vitro protein synthesis system that is unable to synthesize the β' subunit. (a) φ80d7rpoB(rif^r) DNA; (b) φ80d7rpoB115 DNA; (c) φ80d7rpoB115 DNA plus supF-enriched tRNA.*

LITERATURE CITED

1. **Austin, S.** 1974. Coordinate and differential *in vitro* synthesis of two RNA polymerase subunits. Nature (London) **252**:596–597.
2. **Austin, S., and D. McGeoch.** 1973. The synthesis *in vitro* of RNA polymerase subunits of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **70**:2420–2432.
3. **Austin, S., and J. Scaife.** 1970. A new method for selecting RNA polymerase mutants. J. Mol. Biol. **49**:263–267.
4. **Austin, S., I. P. B. Tittawella, R. S. Hayward, and J. G. Scaife.** 1971. Amber mutations of *Escherichia coli* RNA polymerase. Nature (London) **232**:133–136.
5. **Brenner, S., A. D. W. Stretton, and S. Kaplan.** 1965. Genetic code: the "nonsense" triplets for chain termination and their suppression. Nature (London) **206**:994–998.
6. **Burgess, R. R.** 1969. Separation and characterization of the subunits of ribonucleic acid polymerase. J. Biol. Chem. **244**:6168–6176.
7. **Burgess, R. R., A. A. Travers, J. J. Dunn, and E. K. F. Bautz.** 1969. Factor stimulating transcription by RNA polymerase. Nature (London) **221**:43–46.
8. **Errington, L., R. E. Glass, R. S. Hayward, and J. G. Scaife.** 1974. Structure and orientation of an RNA polymerase operon in *Escherichia coli*. Nature (London) **249**:519–522.
9. **Fowler, A. V., and I. Zabin.** 1966. Co-linearity of β-galactosidase with its gene by immunological detection of incomplete polypeptide chains. Science **154**:1027–1029.
10. **Heil, A., and W. Zillig.** 1970. Reconstitution of bacterial DNA-dependent RNA polymerase from isolated subunits as a tool for the elucidation of the role of the subunits in transcription. FEBS Lett. **11**:165–171.
11. **Jaskunas, S. R., R. R. Burgess, and M. Nomura.** 1975. Identification of a gene for the α-subunit of RNA polymerase at the *str-spc* region of the *Escherichia coli* chromosome. Proc. Natl. Acad. Sci. U.S.A. **72**: 5036–5040.

12. **Konrad, B., J. Kirschbaum, and S. Austin.** 1973. Isolation and characterization of $\phi 80$ transducing bacteriophage for a ribonucleic acid polymerase gene. J. Bacteriol. 116:511-516.
13. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology 1:190-206.
14. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 82-107. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Newton, A.** 1966. Re-initiation of polypeptide synthesis and polarity in the *lac* operon of *Escherichia coli*. J. Mol. Biol. 41:329-339.
16. **Stretton, A. O. W., and S. Brenner.** 1965. Molecular consequences of the amber mutation and its suppression. J. Mol. Biol. 12:456-465.
17. **Wilcox, G., P. Meuris, R. Bass, and E. Englesberg.** 1974. Regulation of the L-arabinose operon BAD *in vitro*. J. Biol. Chem. 249:2946-2952.
18. **Zubay, G., D. A. Chambers, and L. C. Cheong.** 1970. Cell-free studies on the regulation of the *lac* operon, p. 375-391. *In* J. R. Beckwith and D. Zipser (ed.), The lactose operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Zubay, G., L. C. Cheong, and M. Gefter.** 1971. DNA-directed cell-free synthesis of biologically active transfer RNA: su^{+}_{III} tyrosyl-tRNA. Proc. Natl. Acad. Sci. U.S.A. 68:2195-2197.