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Recognition Properties of the β Subunit of *Escherichia coli* Ribonucleic Acid Polymerase

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Changes in the phage protein patterns obtained by gel electrophoresis of extracts from phage S13 and φX174 infection of rifampin-resistant hosts suggest that the β subunit of ribonucleic acid polymerase of *Escherichia coli* has a function in the recognition of promoter or terminator sites or both. The altered protein patterns also provide information on the location of some ribonucleic acid polymerase recognition signals in S13 deoxyribonucleic acid. There is a promoter site before gene A, which lies either in gene H or between H and A. There is evidence for a promoter between genes C and D or in gene C. There is either a terminator or a promoter somewhere between the end of gene D and the beginning of gene F.

It is known that the σ subunit of ribonucleic acid (RNA) polymerase confers transcription selectivity on the core enzyme (1, 2, 4, 10, 15). Selectivity means transcription initiation at specific promoter sites, asymmetric transcription of deoxyribonucleic acid (DNA), and termination at correct sites (4). In the absence of σ, selectivity is lost. The only known instance of transcription selectivity in the absence of σ appears to be the case of the RNA polymerase of *Azotobacter vinelandii* (6), for which the core enzyme lacking σ can carry out asymmetric transcription when the DNA template is circular supercoiled DNA (from phage S13). This result is not found in vitro for core enzyme from *Escherichia coli*. It is possible that in *E. coli* one or all of the subunits of the core enzyme acting together with σ may be required for transcription selectivity.

One approach to determining whether a given subunit of RNA polymerase has a role in transcription selectivity would be to alter that subunit and to see if there is a change in the amount of transcripts produced from a given DNA template. This approach has been used by Mailhammer et al. (13) using, however, an in vitro protein synthesis assay instead of a transcription assay. These authors have shown that the small degree of modification of the α subunit produced by T4 infection produces very large changes in amount of transcription of *E. coli* DNA. To study the effect of a change in β, we have performed an experiment with altered β by analyzing in vivo protein synthesis by phage S13 and φX174 DNA in cells whose RNA polymerase is mutated in β, i.e., in *rif*^{r} mutants (17). These phages are suitable templates for detecting changes in recognition properties of RNA polymerase subunits, since they contain several recognition signals. Phages φX174 and S13 have multiple promoter sites and probably multiple transcription terminator sites (5, 9, 16).

A multi-promoter template is convenient for detecting relative changes in recognition of different promoter sites, whereas a template with a single promoter requires the more difficult detection of absolute changes in recognition and subsequent gene expression.

The altered phage protein patterns obtained from infection of the *rif*^{r} strains also provide information on the location of some of the RNA polymerase recognition signals in phage S13.

MATERIALS AND METHODS

Media. HFS medium consists of the HF basal medium of Gelfand and Hayashi (7) made 0.2% in glycerol, 5×10^{-3} M in $NaKPO_4$, and 10^{-5} M in $FeCl_3$. This medium was used for the infected cultures from which extracts were obtained for gel electrophoresis. Resuspension buffer was 0.05 M tris(hydroxymethyl)aminomethane-acetate, pH 9.1-1% sodium dodecyl sulfate-1% 2-mercaptoethanol. M9 minimal agar (14) supplemented with methionine (40 μg/ml) was used for the selective plates in transduction experiments.

Bacterial and phage strains. The strains used were *E. coli* C *rif*^{s} and a spontaneous rifampin-resistant mutant, *E. coli* C *rif*^{r}7; *E. coli* C AP1 *Rif*^{s} (Hcr$^-$); and a spontaneous rifampin-resistant mutant derived from it, *E. coli* C AP1 *rif*^{r}*245* (Hcr$^-$). The phage used was the S13 lysis mutant *am*En15. T4*h* was a spontaneous mutant of T4 wild type that gives larger plaques on *E. coli* C than does T4 wild

type. The transducing phage P1CM*clr100* was obtained from M. Levinthal, and the K12 strain CSH70 (*metB argE*) was obtained from Cold Spring Harbor Laboratories. Transduction was performed by the methods of Rosner (18) and Goldberg et al. (8).

Drugs. Rifampin and mitomycin C (MT) were obtained from Calbiochem.

Isolation of *rif*^{r} strains. Spontaneous rifampin-resistant mutants of *E. coli* C and of *E. coli* C AP1 (Hcr$^-$) were obtained by picking parental colonies into individual broth tubes, growing up the cultures, and then plating each culture with top agar that contained rifampin at 1 mg/ml. The rifampin was dissolved in 0.05 N NaOH at 20 mg/ml and had to be made fresh daily since it is unstable at high pH. No rifampin was added to the bottom agar. Two resistant colonies were picked from each rifampin plate and grown up. It was found that all the *rif*^{r} strains permitted growth of wild-type S13 and T7 but that the strains varied greatly in ability to support growth of T4*h*. Strains that were *gro*$^-$ for T4*h* were analyzed by gel electrophoresis, and two mutants that gave altered S13 protein gel patterns were chosen. Only *rif*^{r} mutants that severely affected the efficiency of plating of T4*h* were found to give altered gel patterns, among about 12 *rif*^{r} strains tested. The two mutants used here, *rif*^{r}*7* and *rif*^{r}*245*, gave large clear plaques of λ CI857 when incubated at 37°C.

Reduction of host protein synthesis. To reduce labeled host protein in the gel patterns, labeling of infected cultures was done under two types of growth conditions. In the first type there was no pretreatment of the cells, but by labeling late (20 min) after infection at 37°C most host protein synthesis was already shut off (M. Hayashi, personal communication). MT pretreatment of a *rif*^{r} strain that was Hcr$^-$ was used to further eliminate host protein synthesis (12). This procedure was effective using some but not all batches of MT. The test of effectiveness of a batch of MT was the ability of an *am*A extract to eliminate the A protein peak from the gel. In untreated cells infection with *am*A105 phage does not eliminate counts in the A region from the gel, because a large amount of labeled host protein comigrates with the A protein. In cells treated with an effective batch of MT, co-electrophoresis of a wild-type and an *am*A105 extract shows a missing A peak in the *am*A extract. The analogous test for effectiveness of reduction of host protein in the C region of the gel depends on distinguishing phage C protein from host protein. This was technically difficult because the one nonsense mutation available in gene C of S13 causes only a small shift in the C peak (3).

Infection and labeling. Cells were grown in HFS medium to 2×10^8 cells per ml. In experiments without MT pretreatment, $MgSO_4$ was added to 2×10^{-2} M, and phage was added to a multiplicity of infection of 15 to 25. At 19 min after infection cold lysine (0.25 μg/ml) was added to the culture that was to receive [^{3}H]lysine (53 Ci/mmol), but no cold lysine was added to the culture that was to receive [^{14}C]lysine (300 mCi/mmol). After 1 min radioactive lysine was added to both cultures, and aeration was continued at 37 or 42°C for 5 to 10 min. [^{3}H]lysine was added to give 20 μCi/ml of culture, and [^{14}C]lysine was added to give 1 μCi/ml of culture. The cultures (5 ml or 10 ml) were then chilled, centrifuged, suspended in resuspension buffer, and boiled for 2 min. A 0.2-ml portion of extract was applied to tube gels.

For cultures receiving MT pretreatment, 50 μg of MT per ml was added to Hcr$^-$ cells in HFS medium at 2×10^8/ml and incubated at 37°C for 30 min without aeration. The cells were centrifuged and suspended at 2×10^9/ml in HF buffer lacking MT. $MgSO_4$ was added to 2×10^{-2} M, and phage was added to a multiplicity of infection of 10. Adsorption was for 10 min, and then the infected cells were diluted 10-fold into HFS medium. The time of dilution was called time zero. Label was added to the cultures at 10 min, and then they were incubated at 37°C until 40 min and then chilled. Cold lysine (0.15 μg/ml) was added to cultures 1 min before the addition of [^{3}H]lysine. This amount of cold lysine permitted incorporation of label throughout the whole growth period.

Gel electrophoresis. Our current methods of preparation of protein extracts and gel electrophoresis on ethylene diacrylate cross-linked tube gels are described in detail elsewhere (18a).

RESULTS

Transduction mapping of the *rif*^{r} mutations. To prove that the rifampin-resistant mutations used were conventional *rif*^{r} mutations and not permeability mutations, they were mapped by P1-mediated transduction, using as the recipient strain *E. coli* K-12 CSH70, which is *metB argE*. The *rif*^{r} locus is known to be closely linked to *argE* on the K-12 map. The cotransduction frequency of *rif*^{r}*7* and also of *rif*^{r}*245* with *argE* was of the order of 20%. Therefore it is concluded that *rif*^{r}*7* and *rif*^{r}*245* are genuine *rif*^{r} mutations.

Growth properties and phage-yielding characteristics of the *rif*^{r} mutants. Strain *rif*^{r}*7* and strain *rif*^{r}*245* were compared with the parental *rif*^{s} strain with respect to generation time, and strain *rif*^{r}*7* was compared with *rif*^{s} with respect to minimum latent period of phage S13$^+$-infected cells, rate of phage production, final phage yield, and rate of protein synthesis after infection. The generation time at 37°C was 63 min for *rif*^{r}*7* and also for *rif*^{r}*245*, as opposed to 47 min for the *rif*^{s} strain, a difference of 1.3. The minimum latent period was 15 min for wild-type S13 at 37°C for both *rif*^{r}*7* and the *rif*^{s} strains. The rate of phage production was the same for both strains, and the final phage yield in both strains was about 200 particles per cell at 36 min.

Rate of protein synthesis in the infected cells was determined by measuring cumulative up-

take of [^{3}H]leucine into acid-insoluble material beginning at a time when host protein synthesis had largely shut off. Points were taken from 10 to 35 min postinfection, and the slope of the curve for uptake of [^{3}H]leucine was found to be 15% greater for infected strain *rif*^{r}7 than for infected strain *rif*^{s}.

Phage protein gel patterns. Phage protein gel patterns were obtained from extracts of both *rif*^{r}7 and parental-type (*rif*^{s}) cultures infected with S13 *am*En15 and labeled with [^{14}C]lysine or [^{3}H]lysine from 20 to 25 min after infection. The resulting patterns obtained from electrophoresis on polyacrylamide tube gels were arbitrarily normalized at the G peak. The main result of the gel patterns is that the D peak is increased in height in the *rif*^{r}7 extract relative to the F, G, and H peaks (Fig. 1). The same result was obtained when the labels were reversed (data not shown). Figure 2 shows a control gel in which two samples of a single infected culture (*E. coli* C) have each been labeled with a different isotope. The control gel shows that the gel patterns are superimposable, independent of which isotope is used to label the proteins, except for a small discrepancy in the case of the B peak. In similar controls for other experiments it was often found that the B peaks were not superimposable. The isotope used did not determine which extract gave the higher B peak. It is concluded that the amount of B protein varies in different extracts, probably because of lability of this protein. Therefore it is not possible to determine the effect of the *rif*^{r} mutations on the amount of B protein from these gel patterns.

By contrast, however, the increase in the D peak in the *rif*^{r}7 strain was observed consistently in more than 10 repetitions of this experiment. The increase in D was about 1.6 times. The figures shown are for cultures labeled at 42°C for 5 min, but the same result was obtained for cultures labeled at 37°C for 5 and also for 10 min. Phage ϕX174 *am*E3 (lysis mutant) was used to infect strains *rif*^{r}7 and *rif*^{s}. Labeling was from 20 to 30 min after infection at 37°C as in many of the S13 infections. A 1.45-fold enhancement in the D peak was observed, with the rest of the protein pattern being unaltered (data not shown). It is not known if the increase in D in both S13 and ϕX174 infections is absolute or only relative, but the conclusions would be the same in either case.

We wished to know which genes showed coordinate increase or decrease of expression in the *rif*^{r} host to deduce the locations of recognition

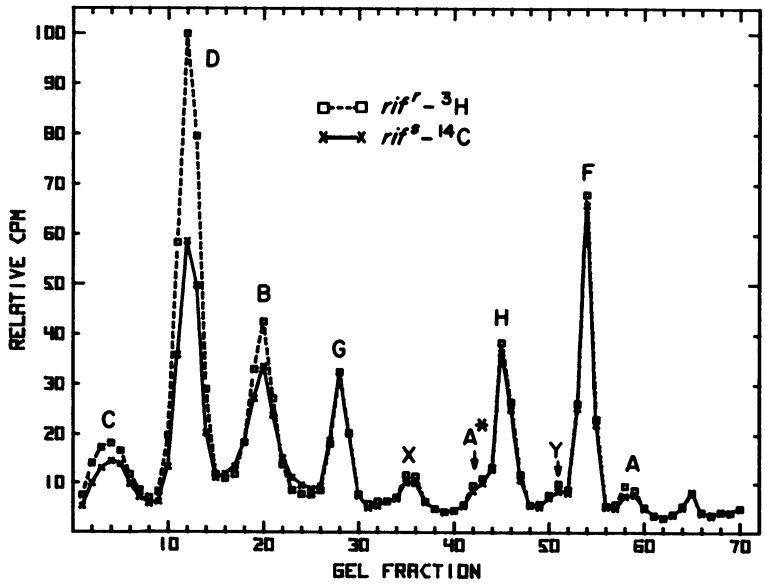

FIG. 1. *Gel electrophoregram of labeled proteins extracted from E. coli C rif*^{r}*7 infected with S13 amEn15 and from E. coli C rif*^{s} *infected with amEn15. Procedures are described in Materials and Methods. Infection was at 42°C, phage multiplicty of infection was 20, labeling was from 20 to 25 min postadsorption, and host cells were untreated. Cold lysine (0.25 μg/ml) was added at 19 min to the culture to be pulsed with [^{3}H]lysine. The letters designate phage gene products, except A and C, which refer to the gel regions where the phage A and C proteins are located rather than to the pure proteins, and X and Y, which are host proteins. In untreated cells the A and C regions always contain host protein in addition to the phage protein. A* denotes the smaller of the two proteins coded for by the A gene (12).*

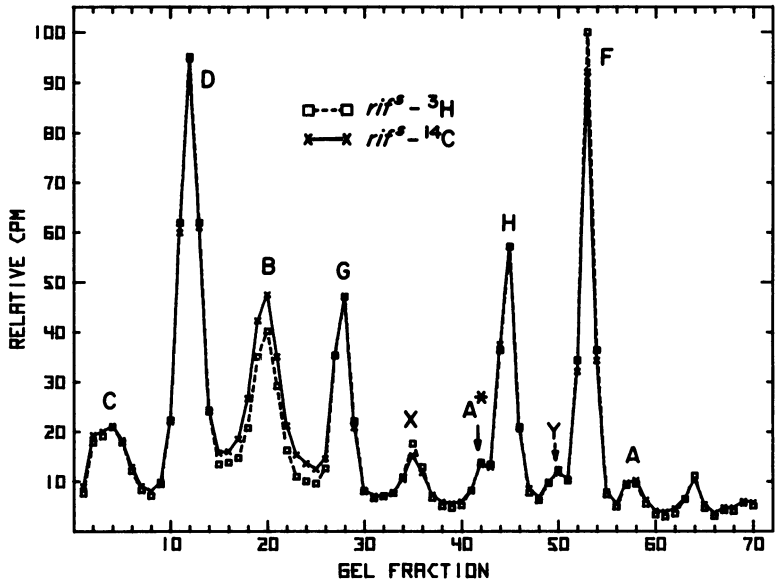

FIG. 2. *Gel pattern of labeled proteins extracted from two cultures of E. coli C rif^s infected with amEn15, one culture being labeled with [^{3}H]lysine and the other with [^{14}C]lysine. Infection procedures are as described in the legend to Fig. 1.*

signals for RNA polymerase. To determine whether the A, A* (12), and C peaks were changed in amount relative to the F, G, and H peaks in the *rif^r* host, it was necessary to use host cells that had been pretreated to reduce host protein synthesis. The strain used was *rif^{r}245* (Hcr$^-$), which gives the same increase in the D peak as *rif^{r}7*. The standard ultraviolet pretreatment of Hcr$^-$ hosts when applied to *rif^{r}245* resulted in an aberrant gel pattern, and therefore MT pretreatment was used (11). MT pretreatment very effectively eliminates host protein radioactivity throughout most of the gel and makes possible visualization of the A protein peak, as determined by co-electrophoresing a mixture of a ^{3}H-labeled S13 wild-type extract with a ^{14}C-labeled *am*A105 extract (data not shown). Other evidence of elimination of host protein from an extract is the disappearance in the gel of the host protein peaks X and Y that are indicated in Fig. 1. In the C peak region of the gel, some host protein appears to persist in MT-treated cells (T. J. Pollock, personal communication). Our data on the effect of the host *rif^r* mutation on size of the C peak are variable: there is little or no increase in C seen in the experiment shown in Fig. 3 and several repetitions of this experiment, whereas other experiments show some increase in the C peak. However, we find that the less there is residual host protein evident in regions X and Y of the gel, the less the increase in the C peak. Therefore the evidence to date favors noncoordinate increase in expression of genes C and D.

The A and A* peaks are both increased relative to F in the *rif^r* strain (Fig. 3). This result was obtained in two additional experiments. It is difficult to determine if the A gene products are increased as much as D, because of the small size of the A and A* peaks.

We conclude that in the *rif^r* strains used, expression of genes A and D is definitely noncoordinate with expression of F, G, and H and is probably noncoordinate with expression of C.

DISCUSSION

In the present work we used *E. coli* mutants (17) with altered β subunits of the RNA polymerase (*rif^r* mutants) to attempt to answer two questions: do changes in the β subunit change the ability of the enzyme to recognize promoter or terminator sites, and if so where are these recognition signals in the small DNA phage S13 located.

It is well known that *E. coli* polymerase in the absence of σ subunit has very limited transcriptional selectivity (4). There is no tight binding of the core enzyme to DNA and transcription of various DNA templates is symmetric, indicating lack of specific recognition properties. An important exception to the requirement for σ factor in transcriptional selectivity is the finding by Domingo et al. (6) that core

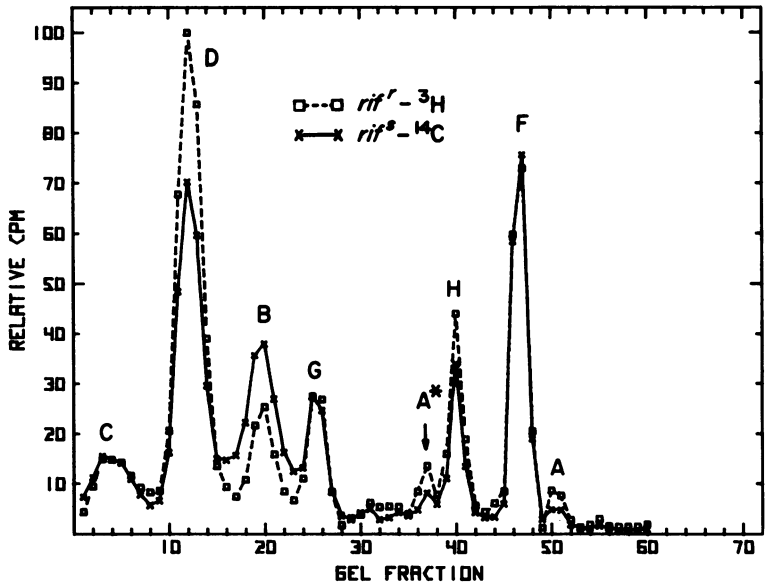

FIG. 3. *Gel pattern of pulse-labeled extracts obtained from infection of MT-treated cells. MT treatment is described in Materials and Methods. A culture of E. coli C AP1 rif^r245 (Hcr^-) was infected with amEn15 at a multiplicity of infection of 10 at 37°C, and at 10 min [³H]lysine was added. Cold lysine was added at 9 min to give 0.15 μg/ml. A culture of E. coli AP1 (Hcr^-) rif^s was similarly infected and labeled with [¹⁴C]lysine, with no cold lysine being added. The cultures were chilled at 40 min after infection. The letters designate phage gene products, with A designating the phage A protein free of host protein. The C region may still contain some host protein even in MT-treated cells (Pollock, personal communication).*

polymerase of *A. vinelandii* can asymmetrically transcribe supercoiled replicative form DNA of S13. These authors found that this selective transcription without σ did not occur if the template was in the relaxed form or if *E. coli* enzyme lacking σ was used.

Our aim here was to determine whether the β subunit contributed to the recognition properties of *E. coli* polymerase when σ was present rather than to learn whether β has recognition properties in the absence of σ. Therefore, we studied in vivo phage protein synthesis in host mutants with altered β subunits. It would seem to be irrelevant here whether the in vivo template is supercoiled or relaxed, since presumably all in vivo transcription initiations occur with σ bound to the RNA polymerase.

Our present result of alterations in the protein gel pattern from phage S13-infected extracts of *rif^r* cells suggests that the β subunit has a role in selectivity of transcription in vivo. We are assuming that *rif^r* mutations affect only transcription and have no selective effects on translation.

The use of the alterations in the phage protein patterns to determine the location of promoter and terminator sites on the phage DNA is based on the following reasoning: when a relative increase in a given gene product is observed in the gel pattern, the increase could be due either to increased promotion or decreased termination at sites before that gene, or to both of these factors. Decreased termination in the *rif^r* host implies relatively strong termination in the parental-type host; a strong termination signal is very likely to be followed by a promoter. Therefore we interpret an increase in the amount of a given gene product as evidence that a promoter is located before that gene.

In contrast, a decrease in the amount of a protein could be due to enhanced termination at a weak promoter or diminished promotion at a strong promoter. A distinction between these two cases cannot be made.

The alterations in phage protein pattern for extracts from the infected *rif^r* strains are very specific, showing an increase in D protein relative to F, G, and H. Also, there is a smaller but definite increase in A and A* relative to F, G, and H. It is concluded from the gel patterns that the *rif^r* mutations cause expression of the D gene to be noncoordinate with expression of genes F, G, and H. Expression of A and A* is also noncoordinate with F, G, and H. It should be noted that the genes of phages S13 are arranged on the map in alphabetical order and are transcribed and translated in that order

(20, 21). A recognition signal for RNA polymerase must therefore be located somewhere between the end of gene D and the beginning of gene F. This signal could be either a terminator that is recognized better or a promoter that is recognized more poorly in the *rif*^{r} strains. Also, a promoter must be located before gene A, either between genes H and A or in gene H. Another signal probably lies between genes A and C. There is also evidence suggesting that a promoter lies between genes C and D or within gene C.

Our present finding that genes F, G, and H constitute a group of coordinately expressed genes, indicating that there is no promoter in this stretch of DNA (until perhaps the end of H), is in agreement with polarity data (9, 19).

Chen et al. (5) used direct physical methods to locate RNA polymerase binding sites on the DNA of φX174 and found that such sites are located before genes A, D, and G of φX174. Previous to the work of these authors, we (19) had found evidence of another type for a promoter in front of gene A, using polar mutants in genes F and G that do not decrease the amount of A protein. Up to now no supporting evidence has been obtained for the result of Chen et al. (5) that a promoter site exists near gene G in phages φX174 or S13. On the contrary, the occurrence of gene F polar mutants in both phages affecting expression of genes G and H makes the existence of such a promoter unlikely (9, 19).

We have examined various explanations for our altered gel patterns other than a change in the recognition ability of the mutated RNA polymerase for promoter and terminator sites. The observed 1.3-fold difference in growth rates of the *rif*^{r} and *rif*^{s} strains cannot account for the observed alteration in the *rif*^{r} protein pattern. A greatly altered growth rate might under conditions of short pulse-labeling produce a gel pattern whose alteration consists of an enhancement in amount of small proteins and a decrease in amount of large proteins; this alteration can be shown to depend on the length of the pulse. However, in our experiments there is no correlation of peak enhancement with protein size, and the enhancement is independent of pulse length over the range 5 to 30 min. Therefore, growth rate differences do not explain our altered gel patterns. Another explanation is that an altered β subunit might cause misreading in transcription that would in turn yield abnormal, unstable proteins, resulting in an altered protein gel pattern. We have searched for misreading in strain *rif*^{r}7 by looking for suppression of numerous S13 nonsense mutants, with negative results. Thus there is no generalized misreading of the DNA, at least in strain *rif*^{r}7. An explanation involving altered recognition is that, in the *rif*^{r} strain, DNA sequences that normally have no promoter or terminator function are now recognized as valid promoters or terminators by the mutated RNA polymerase. This possibility seems unlikely because such extensive alterations in recognition would be expected to severely disrupt regulation of gene expression in the host cell, with lethal consequences. Our explanation of changed affinity of the mutated RNA polymerase for an already existing promoter or terminator seems to us the most likely explanation of our results.

The main conclusion of this work is that the β subunit of RNA polymerase is involved in recognition of promoter or terminator sites or of both, at least when σ is present, as it is in vivo.

ADDENDUM IN PROOF

Locations of promoter sites for φX174 have recently been identified by two groups, using direct methods. L.H. Smith and R.L. Sinsheimer (J. Mol. Biol. 103:699-710, 1976) have determined that in vitro promoter sites are located in front of genes A, B, and D. In vivo promoters have been located at these same positions by M. Hayashi, F.K. Fujimura, and M.N. Hayashi (Proc. Natl. Acad. Sci. U.S.A., in press). These latter authors have also identified in vivo termination sites after genes E, F, G, and H. Since no promoter has been found between D and F but a terminator has been found, our results suggest that the β subunit is involved in recognition of termination signals.

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