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Biochemistry

Induction of sigma factor synthesis in *Escherichia coli* by the *N* gene product of bacteriophage lambda

(RNA polymerase/escape synthesis/sigma gene/transcription termination/rho factor)

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ABSTRACT **Thermoinduction of cells of *E. coli* carrying prophage λ*cI857* within the *bfe* gene brings about not only "escape synthesis" of core subunits of the DNA-dependent RNA polymerase (RNA nucleotidyltransferase, nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6), but also a striking stimulation of σ factor synthesis. The latter phenomenon, termed σ induction, is generally observed after λ phage infection or prophage induction. A series of experiments with various bacterial and phage strains led us to conclude that the *N* gene product of λ is directly involved in the σ induction. These and other results obtained with mutants defective in transcription termination factor ρ suggest the involvement of a ρ-sensitive site in the control of σ gene expression in *E. coli*.**

The DNA-dependent RNA polymerase (RNA nucleotidyltransferase, nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) of *Escherichia coli* consists of at least four subunits, α, β, β′, and σ (1). The specific role of σ factor in transcription initiation, as well as the central role of RNA polymerase in transcription, arouse a special interest in the problem of organization and expression of genes involved in biosynthesis of this protein. At present, neither the structural gene for σ nor the genes specifically regulating the synthesis of σ is known, though hyperproduction of this protein was observed recently with a mutant strain of *E. coli* (2). In the course of our studies on "escape synthesis" of the polymerase subunits (3), a striking stimulation of σ factor synthesis was noted after thermoinduction of lysogenic bacteria (4) carrying prophage λ*cI857* within the *bfe* gene, which is located near the structural genes for β and β′ subunits of RNA polymerase. This paper further exploits this phenomenon with special reference to the mechanisms involved.

MATERIALS AND METHODS

Bacterial and Phage Strains. *Escherichia coli* K12 and bacteriophage strains used are listed in Table 1.

Media and Chemicals. In most experiments, synthetic medium E (13) with 0.5% glucose was used with appropriate supplements. RM medium (14) was used in experiments involving phage infection. Chemicals used are generally those described previously (2).

Prophage Induction. Lysogenic bacteria were grown at 30° to 3 to 4 × 10^8 cells per ml in a 500 ml flask containing 40–50 ml of medium E, supplemented with 1 μg/ml each of biotin and thiamine, and 50 μg/ml each of required amino acids. A culture was divided into 5 ml portions, one kept at 30° and others shaken at 42° to induce phage growth.

Phage Infection. Bacteria were grown to 2 to 3 × 10^8 cells per ml in a 500 ml flask containing 40 ml of RM medium with 0.5% maltose and 1 μg/ml of thiamine. $MgSO_4$ was added to 10 mM, and cells were infected with phage at a multiplicity of infection of 10 to 20.

Radioactive Labeling of Cells. Cells were labeled with 10–20 μCi/ml of a synthetic mixture of 15 L-^{3}H-labeled amino acids (New England Nuclear Corp., NET-250) for 1 min, and were chased with 1% Casamino acids (Difco) for 3 min, unless otherwise indicated. Sodium azide (10 mM) was added in an ice bath to stop the incorporation. Cells were collected by centrifugation, and used without washing to minimize undue lysis in most experiments.

Preparation of Crude Extract. Cells were lysed by treatment with lysozyme-EDTA and Brij 58, and then by sonication as described previously (15). After digestion with DNase (deoxyribonuclease I, deoxyribonucleate 5′-oligonucleotidohydrolase, EC 3.1.4.5) and RNase [ribonuclease I, ribonucleate pyrimidinenucleotido-2′-transferase (cyclizing), EC 2.7.7.16], the supernatant was used as crude extract.

Determination of the Polymerase Subunits. The polymerase proteins were selectively precipitated from crude extract by treatment with antiserum against RNA polymerase holoenzyme [prepared by the published procedure (16)], and treated with sodium dodecyl sulfate ($NaDodSO_4$) as described previously (15). ^{35}S-Labeled RNA polymerase holoenzyme (about 3000 cpm) was added to the crude extract to facilitate estimation of the recovery of polymerase proteins, which was 70–90% for either σ or core subunits. Free σ as well as σ in complex with core could be efficiently precipitated under these conditions (2, 15).

In some experiments, logarithmic-phase wild-type cells labeled for 1 hr with [^{14}C]leucine (6 to 7 × 10^5 cpm) were used as internal reference; they were added to ^{3}H-labeled cells before preparing crude extract. Polyacrylamide gel electrophoresis with $NaDodSO_4$, and estimation of differential rate of synthesis of polymerase subunits was carried out by a modification (2) of the method described previously (15). The differential synthesis rate is defined as the percentage of ^{3}H counts associated with each protein band among total acid-insoluble radioactivity incorporated. When ^{14}C-labeled cells were used as reference, the ^{3}H/^{14}C ratio for each protein band was divided by that for bulk protein to estimate the relative differential synthesis rate.

RESULTS

RNA polymerase synthesis after prophage induction

Synthesis of RNA polymerase subunits was first examined with a lysogenic strain of *E. coli* after thermoinduction of prophage λ*cI857* inserted at the *bfe* gene. As seen in Fig. 1a, rates of synthesis of core subunits, α, β and β′, as judged by incorporation of labeled amino acids into precipitable RNA polymerase, decrease for initial 10 min, and increase markedly followed by a gradual decrease. Lysis begins at about 45 min under these

Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; *am*, amber; Δ, deletion. Gene symbols for bacteria and phage are those described in refs. 37 and 38, respectively.

Table 1. Bacterial and phage strains

Strain	Genotype	Reference or source
λ*vir*		This laboratory
λ*Nam7 nin5*		Ref. 9
λ*trp48 Nam*	λ*trp48 Nam7 Nam53 i*λ*ts h*$^{-80}$	Ref. 10
HD138	*nitA702 trpE9829(am) tyr(am) thy his metE thr sup-126* (P2)	Ref. 11
KY4792	*trpE9829(am) tyr(am) thy his metE thr sup-126 ilv* (P2)	Ref. 11
psu 1	*psu1 trpR lacZ val azi trpE9829(am) trpA9761*	Ref. 12
psu$^+$	*trpR lacZ val azi trpE9829(am) trpA9761*	Ref. 12
KY3304	HfrH Δ(*gal att*λ *chlA uvrB*) *thiA bfe*(λ*cI857* in *bfe*)	Ref. 4
KS505	HfrH Δ(*gal-bio*) *thiA ilv*(λ*cI857* in *ilv*)	Ref. 5
HfrH	HfrH *thiA*	K. Shimada
HfrH(λ*cI857*)	HfrH (λ*cI857*) *thiA*	K. Shimada
KY1404	HfrH (λ*cI857 Nam7 Nam53*) *thiA*	This paper
KY1406	F$^-$ *lac strA supF* (λ*cI857 Nam7 Nam53* ΔH1)	This paper
KY1408	F$^-$ *lac strA supF* (λ*cI857 Nam7 Nam53* ΔF3)	This paper
groN785	*groN785 galE sup*$^+$	Ref. 6
K-37	*nus*$^+$ *gal str*	Ref. 7
nusAB27-1	*nusAB27-1*	D. Friedman
376	*suA thr(am) trpE9851(am) trpR*	Ref. 8
247	*suA*$^+$ *thr(am) trpE9851(am) trpR*	Ref. 8

conditions. Synthesis of σ factor behaved similarly except that the final decrease was not observed. The rate of bulk protein synthesis did not change appreciably during this period. In all experiments involving λ induction or infection reported here, the polymerase subunits synthesized formed bands at the positions that coincided with those of the reference enzyme in $NaDodSO_4$/gel electrophoresis. This was shown by the comparisons of ^3H-labeled bands with ^{35}S- or ^{14}C-bands, taking the ratio of radioactivity for each fraction. Thus, no evidence was found for appreciable degradation, premature termination or modification that affects gel mobility of these polypeptides during phage growth. The differential synthesis rate of each subunit in the uninduced culture was also similar to that found previously with nonlysogenic wild-type bacteria (2, 15).

Different results were obtained when another isogenic lysogen carrying prophage within the *ilv* gene was examined; no abrupt increase of core subunit synthesis was observed, whereas σ synthesis was greatly stimulated (Fig. 1b). These results suggest that the marked increase of core subunit synthesis observed in the *bfe* lysogen (Fig. 1a) is related to the close linkage of the prophage with the cluster (17) of structural genes for β and β′ subunits, and may be explained at least in part by "escape

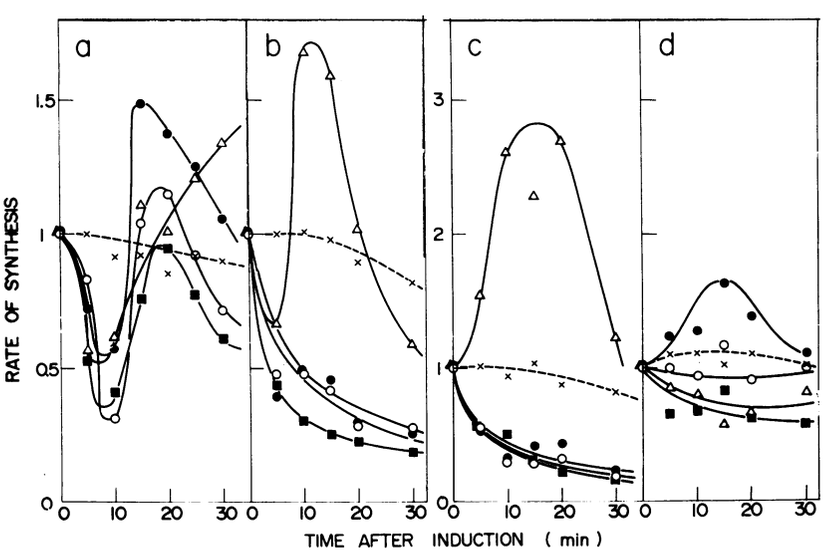

FIG. 1. RNA polymerase subunit synthesis upon thermoinduction of λ lysogens. General procedures and conditions are as described in *Materials and Methods*. Cultures were grown at 30° and divided into several portions; one was pulse-labeled at 30° with ^3H-labeled amino acids (10–15 μCi/ml), whereas others were labeled after shift to 42° at the times indicated. Crude extracts were treated with antiserum to precipitate RNA polymerase proteins, and were analyzed by $NaDodSO_4$-gel electrophoresis. The differential synthesis rate of each subunit, and the rate of bulk protein synthesis are presented relative to that for zero-time (30°) control. The zero-time cells contained 3.0 to 5.5 × 10^6 cpm of acid-insoluble radioactivity. β′ (○), β(●), α(■), σ(△), bulk protein (×).(a) KY3304, (b) KS505, (c) HfrH(λ*cI857*), and (d) KY1404.

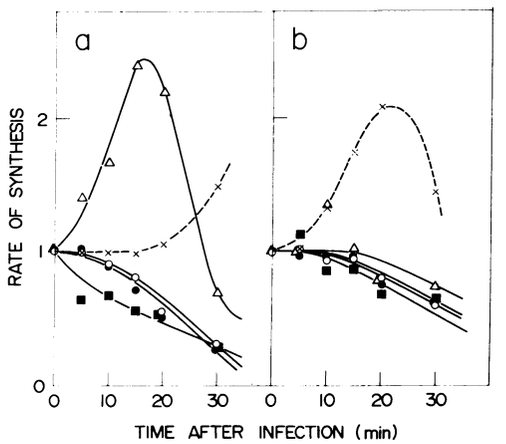

FIG. 2. RNA polymerase subunit synthesis in λ-infected cells. Cultures (HfrH) were grown at 37°, and divided into several portions; one portion was treated with 20 μCi/ml of ^{3}H-labeled amino acids (uninfected control), whereas others were infected with λ*vir* (a) or λ*Nam7 nin5* (b) at an input ratio of 20, and shaken further at 37°. Survivors decreased exponentially with a half-life of about 2 min after λ*vir* infection. Phage-infected cells were labeled at varying periods of time after infection. Other conditions and procedures are as described in *Materials and Methods* and in Fig. 1. The zero-time cells contained 1.1×10^7 and 4.9×10^6 cpm of acid-insoluble radioactivity for (a) and (b), respectively. Symbols are as in Fig. 1.

synthesis" that has been studied in other systems (18, 19). The stimulation of σ synthesis, on the other hand, is apparently unrelated to the location of prophage sites. The latter finding was further confirmed with other lysogens including the one carrying prophage at the normal attachment site (*att*λ) (Fig. 1c). No such increase of σ production was observed either with a lysogen carrying λ*Nam* prophage (Fig. 1d) or with a nonlysogen (data not shown) under the same conditions. In addition, stimulation of σ synthesis was found during induction of a wild-type λ lysogen with mitomycin C (data not shown). Thus, both the marked stimulation of σ factor synthesis and the rapid decrease of core enzyme synthesis seem to be generally observed upon induction of λ lysogens. The lack of stimulation of σ synthesis with the λ*Nam* lysogen suggests that the *N* gene product, a positive regulatory factor for early gene expression, is somehow involved in bringing about an increased rate of σ synthesis during phage development.

σ factor synthesis after phage infection

It was then examined whether σ factor synthesis can be stimulated upon infection of phage-sensitive bacteria with phage λ. As seen in Fig. 2, infection by λ*vir* phage induced a pronounced increase in the rate of σ production, just as after prophage induction, showing that the effect of λ is exerted in *trans*. In contrast, no such effect was observed when the same host was infected with λ*Nam nin* phage. This phage can grow in the absence of *N* gene product, because the *nin* mutation (ref. 20; deletion covering tR_2) permits limited expression of genes located to the right of tR_2 (see Fig. 3). The fact that λ*Nam nin*

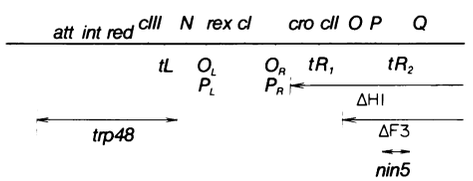

FIG. 3. Genetic map of the early region of λ. Arrows indicate the region deleted from the respective phage genome, or substituted by the bacterial *trp* region in λ*trp48*.

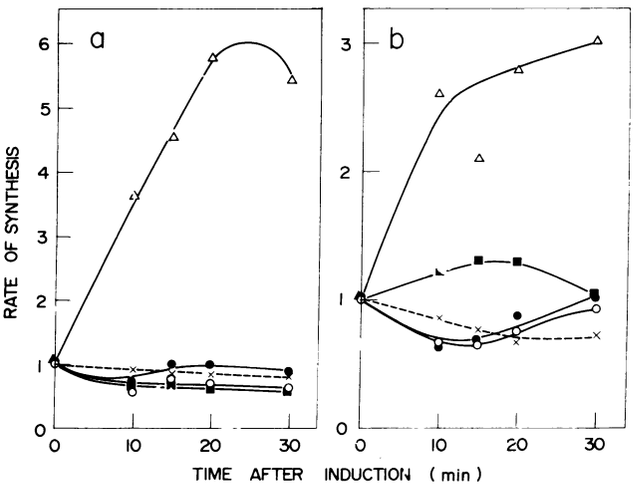

FIG. 4. RNA polymerase subunit synthesis upon induction of λ deletion prophage. Procedures and conditions are essentially as described in Fig. 1 and *Materials and Methods*. Cells labeled with L-[^{14}C]leucine were used as internal reference. The zero-time cells contained 2.6×10^6 and 3.2×10^6 cpm of acid-insoluble ^{3}H radioactivity for (a) and (b), respectively. Symbols are as in Fig. 1. (a) KY1406(ΔH1), and (b) KY1408(ΔF3).

phage, which is unable to stimulate σ production, can grow at least to some extent suggests that the observed induction of σ factor synthesis is not a prerequisite for vegetative growth of phage λ. Conversely, vegetative growth of the phage is not sufficient for σ induction to take place.

Involvement of the *N* gene in σ induction

The following experiments were carried out to determine the λ gene(s) responsible for stimulation of σ production. First, two lysogens each carrying a λ*cI857* prophage deleting most of the genes on the "right arm" of the λ genome (ref. 21; Fig. 3) were examined in which heat inactivation of *cI* repressor triggers expression of only *N* (ΔH1) or *N* and *cro* genes (ΔF3). As seen in Fig. 4, σ synthesis was markedly stimulated with either strain which indicates that the genes deleted from these lysogens are *not* essential for σ induction. The isogenic control strains lacking the amber suppressor (*supF*) failed to exhibit σ induction presumably due to the amber mutations in the gene *N*. The extent of σ induction with the ΔH1 lysogen was significantly higher than that with the ΔF3 lysogen, perhaps because the former lacks the active *cro* gene product. In a separate experiment, it was found that infection of a sensitive host with λ*trp48 Nam* lacking the λ genes to the left of *tL* (Fig. 3) induces σ production provided that the host carries an amber suppressor (data not shown). This suggests that none of the genes to the left of *tL* are required for σ induction.

The *cI* repressor and the *rex* gene product do not appear to be required for σ induction, because (*i*) the rate of σ synthesis is not altered by uninduced prophage λ, (*ii*) σ induction occurs upon inactivation of *cI* repressor, and (*iii*) σ induction does not occur after infection of cells lacking suppressor with λ*Nam nin* or λ*trp48 Nam* phage where *cI* repressor and the *rex* gene product are produced. These results taken together suggest that the *N* gene product itself is directly responsible for induction of σ factor synthesis.

Effect of host mutations on σ induction

To gain further insight into the mode of involvement of the λ*N* gene in σ induction, two host mutants, *groN* (6) and *nus* (7), which permit synthesis but not functioning of N protein, were

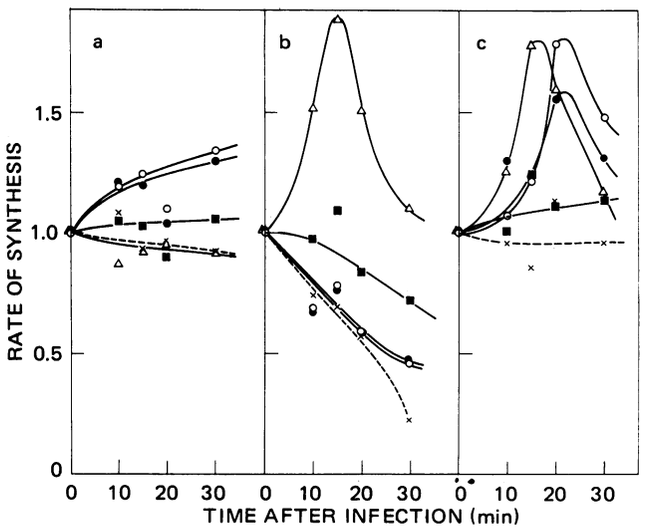

FIG. 5. RNA polymerase subunit synthesis in λ*vir*-infected host mutants. Procedures and conditions are as described in Fig. 2 and in *Materials and Methods*, except that cells were grown at 42° in RM medium containing 50 μg/ml of all amino acids except leucine, and labeled with L-[^{3}H]leucine (10 μCi/ml, 54 Ci/mmol) for 2 min followed by a chase with 200 μg/ml of unlabeled L-leucine for 2 min. No appreciable lysis took place under these conditions except for strain K-37 which showed marked lysis. Cells labeled with [^{14}C]leucine were used as internal reference. The zero-time cells contained 2.0×10^6, 1.6×10^6, and 2.0×10^5 cpm of acid-insoluble radioactivities for (a), (b), and (c), respectively. Symbols are as in Fig. 1. (a) *groN785*, (b) K-37(*nus*$^+$), and (c) *nusAB27-1*.

examined after infection with λ*vir* phage. Each of these mutants carries two mutations responsible for the mutant phenotype observed (22, 23), and one mutation present in the *groN* mutant probably affects β or β′ subunit of RNA polymerase (6). As shown in Fig. 5, infection with λ*vir* phage at 42°, where the mutant phenotype is maximally expressed, failed to cause σ induction with the *groN* mutant. In contrast, σ induction seems to occur with the *nus* mutant, though synthesis of β and β′ subunits also increases for some unknown reason. Hence, the necessity of functional activity of the N protein for σ induction cannot be assessed rigorously from these results alone, but the data suggest that interaction between N protein and host RNA polymerase is involved.

σ factor synthesis in mutants defective in termination factor ρ

Because the N protein of λ should act by antagonizing transcriptional termination mediated by ρ factor (9, 24), mutational defects in ρ might exhibit similar effects leading to an increased rate of σ synthesis. As seen in Table 2, the differential synthesis rates of σ in several mutants defective in ρ factor (cf. 8, 11, 12, 25, 26) were found to be about two times higher than those with the corresponding parental strains. In a separate experiment, the temperature shift from 30 to 42° of a mutant that produces heat-labile ρ (11) caused doubling in the rate of σ synthesis within an hour (data not shown). These results suggest that a ρ-sensitive termination site may be present early in the transcription unit containing the structural gene for σ, although other possibilities such as σ induction caused by some other bacterial gene under ρ control are not excluded.

DISCUSSION

The antitermination activity of the N protein of λ plays an essential role in transcriptional regulation during phage growth.

Table 2. Synthesis of RNA polymerase subunits in ρ mutants

Exp. no.	Strain	β′	β	σ	α
I a	376(*suA*)	0.45	0.26	0.29	0.35
	247(*suA*$^+$)	0.49	0.41	0.11	0.26
b	HD138(*nitA702*)	0.40	0.38	0.28	0.27
	KY4792(*nitA*$^+$)	0.52	0.46	0.15	0.20
II a	376(*suA*)	1.02	0.87	1.80	0.90
	247(*suA*$^+$)	0.80	0.76	1.10	0.75
b*	HD138(*nitA702*)	0.45	0.57	2.72	0.70
	KY4792(*nitA*$^+$)	0.76	0.79	1.38	0.77
c	*psu1*	1.01	0.95	2.22	0.76
	psu$^+$	1.01	1.00	1.37	0.99

Logarithmic-phase cells were labeled at 37° with ^{3}H-labeled amino acid mixture as described in *Materials and Methods*, except in Exps. Ib and IIb, where cells grown at 30° were incubated at 42° for 1 hr prior to labeling. The labeled cells containing 1 to 4×10^6 cpm of acid-insoluble radioactivity were washed before preparing crude extracts. In Exp. II, the labeling was done for 2 min followed by a 3 min chase, and cells of each parental strain labeled with L-[^{14}C]leucine were used as internal reference. RNA polymerase proteins were precipitated by antiserum, and analyzed by NaDodSO$_4$-gel electrophoresis. Values represent differential synthesis rates (in %) (Exp. I), or relative differential synthesis rates (Exp. II), estimated as described in *Materials and Methods*.

* Pulse-labeled with L-[^{3}H]leucine (10 μCi/ml, 54Ci/mmol) for 2 min and chased with unlabeled leucine (200 μg/ml) plus isoleucine (50 μg/ml) for 3 min. The decreased rates of core subunits synthesis in HD138 may be due to the temperature-sensitive growth of this strain (cf. 16).

Such an activity could also affect normal termination of transcription of host bacterial operons. The present results reveal that N protein increases the rate of synthesis of σ, but not other subunits of RNA polymerase. This provides clear evidence that the N protein can act on at least one *E. coli* operon. A recent report also indicates that N protein exerts trans antitermination activity at ρ-sensitive sites in the *gal* operon of *E. coli* (27). However, N protein apparently does not work at all ρ-sensitive sites. For example, it does not affect termination within the structural genes or at the attenuator of the *trp* operon, when transcription is initiated from the *trp* promoter (28). Such a differential effect of N protein on various operons appears to reflect the promoter specificity rather than specificity of termination sites (10, 29).

A number of host mutations are known which either affect N function (*groN*, *nus*, *ron*) (6, 7, 30) or bypass the requirement for N protein in λ growth (*nitA*, *nitB*, *sun*) (9, 31). Whereas *groN*, *ron*, and *nitB* presumably affect the β or β′ subunit of RNA polymerase, *nitA* (and probably *sun*) affects ρ factor. Thus, transcription termination appears to be a process involving interaction of a number of components: RNA polymerase, ρ factor, termination sequences, regulatory proteins like N, and possibly promoter sequences.

Previous work indicated that the rate of σ factor synthesis is relatively constant, and is controlled by a mechanism separate from that for the core enzyme (15, 16). However, σ synthesis was recently shown to be enhanced under several different situations involving structural alteration of RNA polymerase. Thus, a mutation (*sig-1*) presumably affecting β or β′ subunit leads to hyperproduction of σ during steady-state growth (2). An antibiotic rifampicin also induces transient stimulation of σ synthesis at certain concentrations (32). The σ induction described here presumably involves interaction (28) between N

protein of λ and host RNA polymerase. In addition, higher levels of σ production were observed with mutants defective in ρ factor (Table 2). All these observations might be explained in terms of defects in transcriptional control at a presumptive ρ-sensitive site located between the promoter and the structural gene for σ.

In view of these considerations, we propose that an attenuator, such as those found recently for the *trp*, *his*, and other operons (33, 34), is involved in regulation of σ gene expression in *E. coli*. Because σ is specifically involved in transcription initiation and presumably acts "catalytically" (35), relatively small amounts of σ may be needed for active growth of cells. In fact, the molar ratio of σ to core enzyme synthesized *in vivo* is rather low (about 0.3) (2, 16, 36). Such a limited synthesis of σ may be a consequence of ρ-mediated regulation at the level of σ gene transcription. Furthermore, existence of regulatory proteins functionally analogous to λ N protein may be implicated as part of the regulatory circuits in bacteria.

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