Proc. Natl. Acad. Sci. USA
Vol. 74, No. 3, pp. 1162–1166, March 1977
Genetics

Isolation and characterization of conditional-lethal rho mutants of *Escherichia coli*

(temperature-sensitive and amber mutations/transcriptional read-through/growth of phages/rho factor)

HIDETOSHI INOKO*, KATSUYA SHIGESADA, AND MUTSUO IMAI†

Institute for Virus Research, Kyoto University, Kyoto, Japan

Communicated by Charles Yanofsky, December 8, 1976

ABSTRACT **Temperature-sensitive *nitA* (rho) mutants of *E. coli* were isolated; one of them was characterized as an amber mutant. These strains show the Nit phenotype (transcription of phage λ DNA independent of the *N* gene) at low temperatures and are inviable at high temperatures. The mutated sites appear to be between *cya* and *metE* on the chromosome. Temperature-sensitive *nitA* bacteria not only permit leftward transcription of the λ genome at a high rate in the absence of the λ *N* protein, but also allow λ growth at low temperatures. At high temperatures, phages λ and T4 are incapable of normal development in these cells, while growth of T7 is not affected. The production of thermally unstable rho by the *nitA* temperature-sensitive mutant suggests that *nitA* is the structural gene for rho.**

Studies of host mutants that specifically affect early gene expression of phage λ have provided useful information on the mechanism of transcription (1–4). We have taken advantage of the Spi phenomenon (5, 6)—the fact that bacteria lysogenic for phage P2 will not permit growth of wild-type λ but will allow λ*susN7nin5* to multiply—to develop a method for the isolation of bacterial mutants (*nit*) which permit *N*-independent transcription of the λ genome. Such mutants presumably have alterations that affect some stage in initiation or termination of transcription (4). Of the various *nit* mutants isolated, one class, designated *nitA*, is defective in termination of λ early transcription (4) and produces altered forms of the termination factor rho (K. Shigesada *et al.*, unpublished results). Mutants of this type have mutant sites that map between *ilv* and *metE* on the *Escherichia coli* chromosome. We believe that these findings provide evidence of a functional role for the rho factor in phage λ transcription *in vivo*. However, because not all our *nitA* mutations affected cell growth significantly (4), it was still uncertain whether rho is essential for bacterial processes. To establish that it is, we have isolated conditional-lethal *nitA* mutants. In this report we describe the isolation and genetic characterization of such temperature-sensitive (Ts) *nitA* mutants and an examination of the thermal stability of the rho factor they produce. Details of the purification and properties of the mutant rho factors will be presented elsewhere (K. Shigesada, H. Inoko, and M. Imai, unpublished results).

MATERIALS AND METHODS

Bacterial and Phage Strains. The bacterial strains used in this work are all derivatives of *E. coli* K-12. KH54 (F⁻ *thr*⁻ *trpE9829(am)* *his*⁻ *tyr(am)* *thy*⁻ *ilv*⁻ *metE*⁻ *sup-126*) was obtained from T. Horiuchi and T. Nagata (7). KY4792 is KH54 lysogenic for phage P2 at attachment site II (8) near *metE*. It was employed as the parent strain for isolation of *nitA* Ts mutants. Note that it contains *sup-126*, a temperature-sensitive variant of the amber suppressor *supD* (su_{I+}) (9). KY363 carries the amber suppressor *supE* (su_{II+}). All the other strains are isogenic with or derived from KY4792. Phages λ*CI857-susN7N53byp*, λ*susN7N53ptrp46* (10) (Fig. 1), and φ80d*cya* and φ80d*ilv* were kindly supplied by D. I. Friedman, N. Franklin, and L. Soll, respectively.

Rho Factor. Rho was purified by the method described in ref. 11. When purified from *nitA702*, the following changes were employed: Diisopropylfluorophosphate (5 mM) was added to crude cell lysates, and the glycerol concentration was increased to 20% (vol/vol) in all buffer solutions used for purification and storage of the factor. Rho factor activity was determined using the poly(C)-dependent ATPase assay, as in ref 12. Details will be described elsewhere (K. Shigesada, H. Inoko, and M. Imai, unpublished results).

Other procedures and material used in this work have been described (4). Biological experiments were carried out using a peptone–glucose medium, unless otherwise noted.

RESULTS

Isolation of Temperature-Sensitive *nitA* Mutants. Since *nitA* maps close to *ilv* (81% cotransduction, using phage P1) (4), we could use *ilv* as the linked selective marker in the localized mutagenesis technique (13). The basic procedure employed was the same as that used before (4); mutants resistant to λ*susN7nin5* were selected in an *E. coli* K-12 strain that was lysogenic for phage P2. The starting bacterial strain (KY4792) was also auxotrophic for isoleucine and valine and carried a temperature-sensitive amber suppressor, *sup-126* (9). The presence of the suppressor made it possible to recover amber-type *nitA* mutants that could subsequently be tested for temperature sensitivity. It is important to note that *sup-126* does not suppress the *N7* amber mutation of λ, even at a low temperature (30°) (9). Thus, *sup-126* has no effect on the sensitivity of KY4792 to λ*susN7nin5* because λ does not exhibit Spi functions in the absence of *N* function. Therefore, the presence of *sup-126* does not hinder the isolation of *nit* mutants on the basis of the Spi phenomenon (4).

After treating strain KY4792 with 700 μg/ml of *N*-methyl-*N*-nitrosoguanidine at 37° for 60 min (20–30% survival), Ilv⁺ revertants were selected at 30° on agar lacking isoleucine and valine. Temperature-sensitive (growth) mutants were then identified from among these Ilv⁺ clones (*ca* 18,000) by replica plating. Ts mutants unable to grow at 42° in the haploid state were tested for ability to grow at the high temperature after introduction of an F′ plasmid (F 16) covering the *ilv-nitA* region of the *E. coli* chromosome (Fig. 2). The individual Ts mutants thus recognized were examined for the Nit phenotype,

Abbreviations: Ts, temperature-sensitive; *am*, amber; *nit* (phenotype, Nit), gene-*N*-independent transcription; Spi, sensitivity of λ phage growth to interference by phage P2 lysogeny; e.o.p., efficiency of plating. Gene symbols for bacteria and phage are those described in refs. 30 and 32, respectively.

* Present address: Department of Molecular Biology, School of Medicine, Keio-gijyuku University, Tokyo, Japan.
† To whom reprint requests should be addressed.

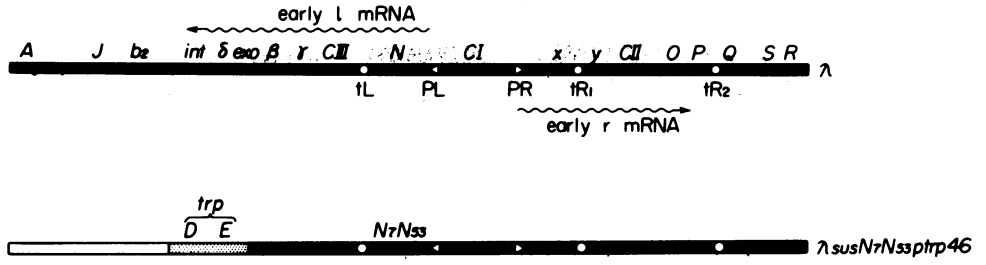

FIG. 1. Genetic map of phages λ and λ*susN7N53ptrp46*. White, black, and stippled spaces represent genomes of φ80, λ, and *E. coli*, respectively. Open triangles (P_L and P_R) show promoters for λ early operons. Open circles (t_L, t_{R1}, and t_{R2}) indicate putative *N*-sensitive transcription termination sites. The map is not drawn to scale.

resistance to λ*susN7nin5*, and sensitivity to λ*susN7nin5spi*, by cross-streaking. We ultimately obtained four independent isolates that clearly showed the Nit⁻ phenotype at 30° and were temperature-sensitive for cell growth. The mutations all mapped between *ilv* and *metE*. Two mutants, HD136 (*nitA112*) and HD138 (*nitA702*), were characterized further.

Characterization of the *nitA* Mutations. In P1 transduction experiments, the *nitA112* and *nitA702* mutations were cotransduced 61% and 82% with *ilv*, and 11% and 9% with *metE*, respectively. Throughout these recombination experiments the mutant characteristics Nit⁻ (resistance to λ*susN7nin5*) and temperature-sensitive growth never separated from one another. The genetic location of these *nitA* mutations was more precisely determined to be between *cya* and *metE* (closer to *cya*) by isolation of φ80d*nitA* (H. Inoko, *et al.*, unpublished results). This transducing phage carries *ilv-cya-nitA* from the *E. coli* chromosome and complements the mutant phenotypes upon infection. By contrast, φ80d*cya*, the parental phage that carries only *ilv-cya*, and several φ80d*ilv* phage that carry *ilv* and the adjacent region in the opposite direction from *cya*, did not complement *nitA*.

We determined that the Nit and the Ts phenotypes are due to single mutations by picking spontaneous Ts⁺ revertants of the *nitA* Ts mutants. All six independent revertants of *nitA702* and six of nine revertants of *nitA112* were found to lose the Nit⁻ (resistance to λ*susN7nin5*) and temperature-sensitive phenotypes simultaneously. These results suggest that the Nit and Ts phenotypes of both *nit* mutants are due to single mutations in the *nitA* gene.

The *nitA112* mutant became Ts⁺ when amber suppressors su_{I}^{+} or su_{III}^{+} were introduced by transfer of the F′ plasmid KHF16 (7) or by infection with φ80psu_{III}^{+}, respectively. By contrast, *nitA702* could not be made temperature-resistant by these means. This result demonstrates that the *nitA112* mutation is of the amber, suppressible type, while the *nitA702* mutation is not. Both of these mutants lose their temperature sensitivity upon infection with φ80d*nitA*.

When these *nitA* Ts mutants were grown at 30° in a peptone–glucose medium the number of viable cells increased exponentially, with a doubling time of about 85 min (Fig. 3). (Incidentally, the mutant cells showed abnormally slow growth in a minimal-salts *liquid* medium supplemented with required ingredients.) When the cells were shifted to 42°, the number of survivors began to decrease exponentially at 30–60 min, whereas the optical density of the cultures gradually increased for the first 3 hr after the shift-up. During this period at least, bulk RNA and protein synthesis continued and the formation of β-galactosidase was normally induced by isopropylthiogalactoside (an inducer) at the restrictive temperature (data not shown). However, microscopic observations indicated that the number of filamentous cells increased markedly under these conditions.

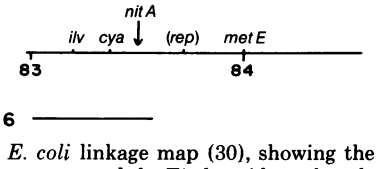

FIG. 2. The *E. coli* linkage map (30), showing the location of *nitA* on the chromosome and the F′ plasmid employed.

***N*-Independent Expression of the λ Genome in the *nitA* Ts Mutants.** The *nitA* Ts strains are nonpermissive for λ*susN7nin5* infection at 30°. When quantitatively assayed, the efficiency of plating (e.o.p.) of the phage was found to be 1.0×10^{-5} and 3.2×10^{-5} on the *nitA112* and *nitA702* mutants, respectively (Table 1). This indicates that growth of λ*susN7nin5* is much more strongly restricted on the *nitA* Ts strains than on the *nitA* Ts⁺ strains used before, on which the e.o.p. of the phage ranged from 0.25 to 0.01 (4). However, these *nitA* Ts mutants were as permissive as their parent strain for λ*susN7nin5spi* and became non-restrictive for λ*susN7nin5* upon removal of the P2 prophage (Table 1). Therefore, the

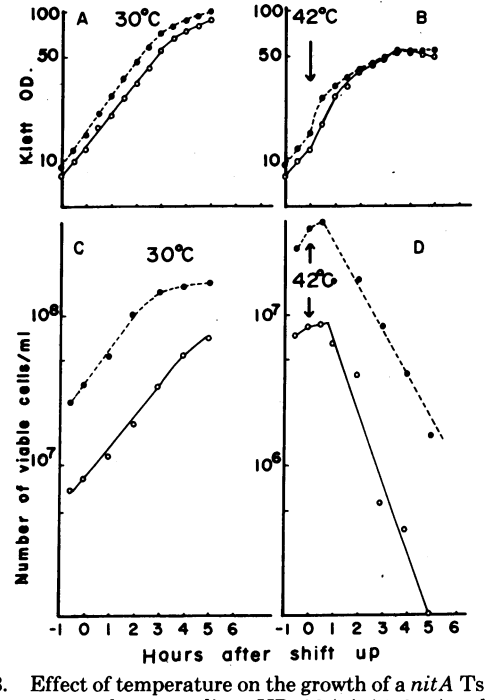

FIG. 3. Effect of temperature on the growth of a *nitA* Ts mutant strain in a peptone–glucose medium. HD136 (*nitA112*, ○) and HD138 (*nitA702*, ●) cultures exponentially growing at 30° were each divided in half. One half was kept at 30° (A and C), while the other was transferred to 42° for further incubation (B and D). Cell growth was measured by optical density (A and B) using a Klett–Summerson colorimeter and by colony formation at 30° (C and D).

Table 1. Plating of λ*nin5* derivatives on *nitA* Ts mutants at a permissive temperature

Bacterial strain	Relevant property	Phage efficiency of plating λ*susN7nin5*	λ*susN7nin5spi*
HD136	*nitA112*(P2)	R (1.0×10^{-5})	S (0.43)
HD138	*nitA702*(P2)	R (3.2×10^{-5})	S (0.77)
KY4792	*nitA*$^+$(P2)	S (1.00)	S (1.00)
HD149	*nitA112*	S (0.97)	
HD152	*nitA702*	S (1.00)	
KH54	*nitA*$^+$	S (1.00)	

Sensitivity of bacterial strains to phage was examined by cross streak tests on eosin/methylene blue (EMB) agar plates. Symbols S and R denote sensitivity and resistance to these phages, respectively. The values in the parentheses represent the efficiencies of plating (e.o.p.) of these λ*nin5* derivatives. The assay was carried out at 30° using the indicator bacteria listed. The e.o.p. is normalized to the phage titer obtained with the parental NitA$^+$ strain. Removal of phage P2 from *nitA* Ts mutants was performed by P1 transduction using an isogenic P2 sensitive strain as donor, followed by selection of methionine-independent clones. It was done because P2 was lysogenized at site II near *metE*.

stringent nonpermissiveness of the *nitA* Ts mutants for λ*susN7nin5* even at 30° is clearly associated with the Spi phenomenon. This implies a considerable *N*-independent leftward transcription proceeding into the *CIII-att* region (*spi* genes) without cessation at the t_L terminator.

To test more directly for this nonterminated transcription, the formation of anthranilate synthetase (the product of the *trpE* and *D* gene(s) was assayed at 30° after infection of *nitA* Ts cells (*trpEam*) with λ*susN7N53trp46* (Fig. 1) (10). As shown in Fig. 4, only slight anthranilate synthetase activity was detected in the parent strain. [This is probably due to weak suppression of the *trpE* amber mutation of the host strain by *sup126*, because no anthranilate synthetase activity was detected in host strains that were free of nonsense suppressors (4).] In contrast, the *nitA112* and *nitA702* mutants allowed production of a fairly large amount of the synthetase under the same conditions. Anthranilate synthetase production amounted to about 30% in *nitA112* cells and more than 20% in *nitA702* cells, respectively, in contrast to about 2% in *nitA* Ts$^+$ cells (4), when calculated on the basis of the synthetase produced in KY363 in which the phage *N* gene was suppressed by su_{II}^+. These results demonstrate that at least 20–30% of leftward transcription fails to stop at the t_L terminator of λ*susN7N53trp46* in *nitA* Ts cells.

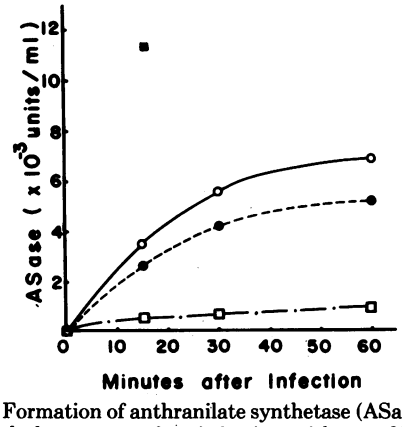

FIG. 4. Formation of anthranilate synthetase (ASase) in *nitA* Ts mutants and the parent after infection with λ*susN7N53*p*trp46*. Culture media and solution used were all supplemented with 50 μg/ml L-tryptophan. Cells grown to 100 Klett units in the peptone–glucose medium were spun down, resuspended in phage adsorption buffer, and mixed with a suspension of the purified phage at the multiplicity of infection of 5, followed by incubation at 30° for 20 min. After removal of unadsorbed phages, infected cells were resuspended in the minimal medium supplemented with 0.2% casamino acids and aerated at 30°. Samples were removed and chilled on ice at the times indicated and 50 μg/ml of chloramphenicol was added. The enzyme assay and other procedures have been described (31). Host strains used were ○—○, HD136 (*nitA112*); ●- - -●, HD138 (*nitA702*); □-·-□, KY4792 (parent *nitA*$^+$); and ■, KY363 (*nitA*$^+$ su_{II}^+).

Table 2. Efficiency of plating of λ*susN* derivatives on *nitA* Ts mutants at a permissive temperature

Bacterial strain	Relevant property	Phage efficiency of plating λ*sus-N7N53byp*	λ*susN7*	λ*susN7N53*
HD149	*nitA112*	0.75	3.7×10^{-2}	3.0×10^{-2}
HD152	*nitA702*	0.56	6.6×10^{-2}	2.5×10^{-2}
KH54	*nitA*$^+$	1.7×10^{-4}	4.7×10^{-7}	$< 2.5 \times 10^{-7}$

The assay was carried out at 30°. The e.o.p. is normalized to the phage titer obtained with KY363 (*nitA*$^+$ su_{II}^+). All the bacterial strains used were nonlysogenic for P2.

For rightward transcription of the λ genome, the two sites of action of rho are the terminators, t_{R1} (between *x* and *y*) and t_{R2} (between *P* and *Q*) (4, 11, 15) (Fig. 1); P_R-promoted transcription *in vivo* is apparently prevented at these sites unless the *N* product acts as an anti-rho factor. To investigate the effects of the *nitA* Ts mutations on termination of rightward transcription, expression of early indispensable genes, such as *O*, *P* (13), and *Q* (14), was examined in terms of growth of phages λ*susN7N53byp*, λ*susN7*, and λ*susN7N53* at 30° in *nitA* Ts cells nonlysogenic for P2. The *byp* mutation close to t_{R2} does not allow sufficient *N*-independent synthesis of the *Q* product for normal growth of *N* defective phage (17). As shown in Table 2, the *nitA* Ts mutants permitted almost normal growth of λ*sus7N53byp*. Similar results were obtained with λ*susN7* and λ*susN7N53*, whose e.o.p. values on the *nitA* Ts mutants increased about 10^5-fold compared with those on the parent strain KH54 (nonlysogenic for P2). These results indicate that *N*-

Table 3. Burst size of phages on *nitA* Ts mutants at a non-permissive temperature

Bacterial strain	Relevant property	Phage efficiency of plating λ	T4	T7
HD149	*nitA112*	1.4	12.2	143
HD152	*nitA702*	0.017	1.0	62
KH54	*nitA*$^+$	34.1	76.5	173

Bacterial cells exponentially growing in the peptone–glucose medium at 30° were harvested and infected with λ at the multiplicity of infection of 6 in the presence of 10 mM Mg^{2+}, followed by incubation for adsorption at 30° for 20 min. After removal of unadsorbed phages by centrifugation, a portion was diluted 2×10^{-2} into the same medium and was further aerated at 42°. After 2.5 hr, the culture was treated with chloroform and the phage yield was determined with KY363 as indicator bacteria. In experiments with T4 and T7, phage which grow rapidly, cells growing at 30° were first transferred to 42° for 60 min and then infected with each phage at a multiplicity of infection of 1, followed by incubation at 42° for 5 min. A portion was diluted 10^{-4} into prewarmed medium and was further aerated at 42° for 60 min. A shift to a higher temperature after infection of cells with these phages gave essentially the same results as shown above. Determination of phage yield was the same as used for λ. The bacterial strains employed were all nonlysogenic for P2.

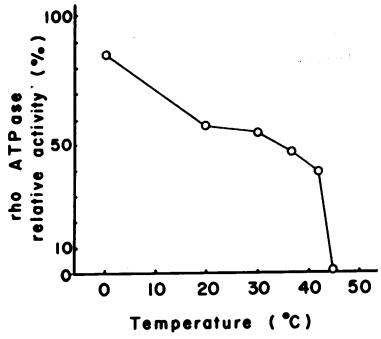

FIG. 5. Temperature sensitivity of *nitA702* rho. Aliquots of about 5 μg/ml rho purified from both the parent and *nitA702* cells were subjected to heat treatment for 10 min at the temperatures indicated, followed by assay at 37° of the residual poly(C)-dependent ATPase activity. Under the enzyme assay conditions, the ATPase activity of the mutant rho, as well as that of wild-type rho, was not affected. Throughout the experimental process, 20% glycerol was present. Values obtained with mutant rho relative to those with wild-type rho were plotted at each temperature used for the heat treatment.

independent rightward transcription, without termination at t_{R1} and t_{R2}, occurs at a much higher rate in the mutants than in the parent cells.

Development of Phages in the *nitA* Ts Mutants at High Temperature. The discovery of conditionally lethal *nitA* mutants suggests that rho plays an indispensable role in *E. coli*, presumably as a transcription termination factor (11). To determine whether this is also the case for normal development of different phages, we measured burst sizes of phages λ, T4, and T7 in *nitA* Ts cells at the nonpermissive temperature. As seen in Table 3, the growth of λ and T4 was greatly inhibited, especially in *nitA702* cells at the high temperatures, while that of T7 was scarcely affected in either *nitA* Ts strain under these conditions. These results suggest that rho is an essential factor for normal development of phages λ and T4 but is not important for the growth of phage T7.

Temperature-Sensitivity of the Mutant Rho. Rho was purified from two strains, the parent, KY4792, and the mutant *nitA702*. The mutant rho was found to be quite unstable. We therefore added high concentrations of glycerol to all the buffers used in purification and storage (see *Materials and Methods*). To investigate the thermal sensitivity of the mutant rho in relation to that of wild-type rho, the purified preparations were subjected to heat treatment at various temperatures and then assayed for poly(C)-dependent ATPase activity (12) (Fig. 5). Rho from strain *nitA702* showed considerable sensitivity to temperatures above 10° and was completely inactivated at 45°. Under the same conditions, wild-type rho was only slightly affected at 37°, and retained about half of its activity at 45°. This result clearly indicates that the *nitA702* strain produces a temperature-sensitive rho. The temperature sensitivity observed here, as well as that observed in transcription inhibition tests *in vitro* (K. Shigesada, H. Inoko, and M. Imai, unpublished results), are consistent with the thermal sensitivity of *nitA702*.

DISCUSSION

Mutant *nitA702* shows the Nit phenotype at low temperatures and produces thermally unstable rho. These facts strongly suggest that *nitA* is the structural gene for rho and that rho-mediated regulation of transcription is essential for normal growth in *E. coli*. These conclusions are supported by our recent finding that in mutant *nitA112* (*am*) the rate of synthesis of rho is greatly reduced when cells are exposed to a temperature at which the product of the temperature-sensitive amber suppressor is inactivated (K. Shigesada, H. Inoko, and M. Imai, unpublished results). Our findings also suggest that rho is essential for the development of phages λ and T4 but not for T7.

Recently, mutants (designated *sun*) that partly suppress the *N* requirement of λ as do the *nitA* Ts^+ mutants have been isolated (18). *sun* is also genetically linked to *ilv*, suggesting that it is allelic with *nitA*. In contrast to *sun* or *nitA* Ts^+ mutations, however, *nitA* Ts mutations show much greater suppression and have a pronounced effect on cell viability, presumably because their rho is more severely altered. In the *nitA* Ts mutants, *N*-independent rightward transcription proceeds at a high rate beyond both t_{R1} and t_{R2}; *Q* is consequently expressed. We think that *Q* is still essential for late transcription because λ*susQ112* does not propagate in mutant cells. The present findings allow us to conclude that rho functions at t_{R1} but do not establish that it acts at t_{R2}. Thus, we cannot exclude the possibility that t_{R2} is merely subject to read-through when P_R-initiated transcription is increased in the *y-O-P* region due to the inability of the mutant rho to function properly at t_{R1} (4). The nature of the presumed termination event at t_{R2}, therefore, is not yet clear.

Recently, *suA*, *psu*, and *rho* mutants, selected for suppression of polarity, were found to produce altered rho factors (12, 21, 22). These mutations all map close to *ilv* (22, 23). The *psu* mutations are thought to be of the same type as *suA*, but show increased polarity suppression (21). Though the genetic identity of *nitA* and *suA* is unproven, it seems likely that both mutations are in the same gene or at least in the same operon, judging from the following similarities: (*i*) their effects on rho, (*ii*) map position, (*iii*) λ*N*-independent transcription by *sun* and *psu* mutations ([—= 2](= (*iv*) polarity suppression in the *trp* operon by *nitA702* (H. Inoko, unpublished results) and (*v*) the relief of polarity by the λ *N* protein, an anti-rho factor (24, 25, 27). The genetic location of *rho* has been reported to be between *ilv* and *cya* (22). This location differs from the site we have assigned to *nitA*, despite the close similarities of the various types of mutants studied. We suspect that the mapping (22) may be in error.

The detection and isolation of conditionally lethal *nitA* mutants makes it clear that rho plays an essential role in *E. coli in vivo*. This is plausible in view of the recent observations that rho functions as a termination protein in several transcription systems (26, 28). Consistent with this view is the finding that the cellular content of rho is high (ref. 29; K. Shigesada, H. Inuko, and M. Imai, unpublished results), particularly in *nitA* mutant cells; in wild type rho constitutes no less than 0.1% of the total cellular protein. This value is equivalent to a rho content 1/5th to 1/10th the cellular content of RNA polymerase molecules.

The isolation method employed in the study described in this paper yields other classes of *nit* mutants (H. Inoko *et al.*, unpublished results). Studies of these mutants and the *nitA* strains described here should provide useful information on various aspects of RNA synthesis and its regulation.

We express our deep gratitude to Drs. N. C. Franklin and C. Yanofsky for their critical reading of the manuscript.

1. Georgopoulos, C. P. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 2977–2981.
2. Ghysen, A. & Pironio, M. (1972) *J. Mol. Biol.* **65**, 259–272.
3. Friedman, D. I., Jolly, C. T. & Mural, R. J. (1973) *Virology* **51**, 216–226.
4. Inoko, H. & Imai, M. (1976) *Mol. Gen. Genet.* **143**, 211–221.

5. Bertani, G. (1958) *Adv. Virus Res.* **5,** 151–193.
6. Zissler, J., Signer, E. & Schaefer, F. (1971) in *The Bacteriophage* λ, ed. Hershey, A. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), pp. 469–475.
7. Horiuchi, T. & Nagata, T. (1973) *Mol. Gen. Genet.* **123,** 89–110.
8. Kelly, B. (1963) *Virology* **19,** 32–39.
9. Nagata, T. & Horiuchi, T. (1973) *Mol. Gen. Genet.* **123,** 77–88.
10. Franklin, N. (1971) in *The Bacteriophage* λ, ed. Hershey, A. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), pp. 621–638.
11. Roberts, J. W. (1969) *Nature* **224,** 1168–1174.
12. Lowery-Goldhammer, C. & Richardson, J. P. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 2003–2007.
13. Oeschger, M. R. & Berly, M. K. B. (1975) *Mol. Gen. Genet.* **134,** 77–83.
14. Roberts, J. W. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35,** 121–126.
15. Kaiser, D. (1971) in *The Bacteriophage* λ, ed. Hershey, A. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), pp. 195–210.
16. Herskowitz, I. (1973) *Annu. Rev. Genet.* **7,** 289–324.
17. Butler, B. & Echols, H. (1970) *Virology* **40,** 212–222.
18. Brunel, F. & Davison, J. (1975) *Mol. Gen. Genet.* **136,** 167–180.
19. Richardson, J. P., Grimley, C. & Lowery, C. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1725–1728.
20. Korn, L. J. & Yanofsky, C. (1976) *J. Mol. Biol.* **103,** 395–409.
21. Korn, L. J. & Yanofsky, C. (1976) *J. Mol. Biol.* **106,** 231–241.
22. Das, A., Court, D. & Adhya, S. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1959–1963.
23. Morse, D. E. & Primakoff, P. (1970) *Nature* **226,** 28–31.
24. Franklin, N. C. (1974) *J. Mol. Biol.* **89,** 33–48.
25. Segawa, T. & Imanoto, F. (1974) *J. Mol. Biol.* **87,** 741–745.
26. de Crombrugghe, B., Adhya, S., Gottesman, M. & Pastan, I. (1973) *Nature New Biol.* **241,** 260–264.
27. Adhya, S., Gottesman, M. & de Crombrugghe, B. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 2534–2538.
28. Shimizu, N. & Hayashi, M. (1974) *J. Mol. Biol.* **84,** 315–335.
29. Ratner, D. (1976) *Nature* **259,** 151–153.
30. Bachmann, B. J., Low, K. B. & Taylor, A. (1976) *Bacteriol. Rev.* **40,** 116–167.
31. Inoko, H., Naito, S., Ito, K. & Imai, M. (1974) *Mol. Gen. Genet.* **129,** 37–48.
32. Szybalski, W., Bøvre, K., Fiandt, M., Hayes, S., Hradecna, Z., Kumar, S. Lozeron, H. A., Nijkamp, H. J. & Stevens, W. F. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35,** 341–353.