Isolation and Genetic Characterization of the *nitA* Mutants of *Escherichia coli* Affecting the Termination Factor Rho

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Summary. Taking advantage of the Spi (sensitivity to P2 interference) phenomenon, bacterial mutants seemingly resistant to phage $\lambda susN7nin5$, but sensitive to phage λspi, were isolated from a strain of *E. coli* K12 carrying no nonsense suppressor and lysogenic for P2. A class of these mutants, designated *nitA* (*N*-independent transcription), is described here.

Upon infection of the *nitA* mutants with a *trp* transducing phage $\lambda susN7N53ptrp46$ which carries the *E. coli trpE* and *D* genes in the *CIII-att* region of the λ genome, formation of anthranilate synthetase (ASase, a complex protein of *trp E* and *D* gene products) was clearly demonstrated. In contrast, no ASase formation was observed in the parent $nitA^+$ strain under the same conditions. The synthesis is subject to "turn off" control, and is completely repressed by the *CI* repressor of phage λ. The *nitA* cells lysogenic for $\lambda CI857susN7N53$ are killed by thermal induction much more efficiently than the parent cells lysogenic for the same phage. The *nitA* mutants support the growth of $\lambda susN7N53byp$ much better than the parent. These results suggest that the *nitA* mutation permits the early leftward and rightward transcription of the λ genome in the absence of the *N* gene product.

On the *E. coli* genetic map, *nitA* is located between *ilv* and *metE*, nearer to *ilv*. The mutant allele is recessive to the wild-type allele. The present evidence, together with results of biochemical investigations to be reported, suggests that *nitA* is a gene specifying the transcription termination factor rho.

Introduction

During the development of bacteriophage λ, the *N* and *Q* gene products are required as positive regulators for the expression of the early and late genes (Calendar, 1970; Szybalski *et al.*, 1970; Echols, 1972; Herskowitz, 1973). The presence of the *N* products allows comple-

tion of the early leftward and rightward transcriptions, which are initiated at the P_L and P_R promoters, respectively (Heineman and Spiegelman, 1970; Kumar *et al.*, 1970; Roberts, 1970). The *N* protein acts at three sites (t_L, t_{R1} and t_{R2}) on the λ genome (Szybalski *et al.*, 1970; Franklin, 1971), and permits further sequential transcription into the regions *CIII-att, y-O-P* and the late genes through *Q* (cf. Fig. 1). Transcription of these regions is generally prevented when the *N* protein is non-functional, though it must be noted that the prevention of the *y-O-P* transcription is less strict than the others (Ogawa and Tomizawa, 1968; Signer, 1969; Lieb, 1970; Brachet *et al.*, 1970). The control of the early expression of the λ genome was also studied in an *in vitro* transcription system. Roberts (1969, 1970) demonstrated that a bacterial protein factor, rho, permits termination of transcription of the λ DNA at sites possibly identical to those at which the *N* proteins is thought to act. Thus, shorter mRNAs corresponding to the *N* and *x* genes (and the *y-O-P* region when using *c17* DNA as a template) were produced in the presence of rho factor, when N was inactive. On the basis of these findings, the antitermination model (Roberts, 1969) was proposed; the action of the termination factor rho was thought to be antagonized by the *N* product at the genetically specified sites (Luzzati, 1970; Portier *et al.*, 1972).

Bacterial mutants in which transcription of the early genes of phage λ is specifically affected, such as *groN* (Georgopoulos, 1971), *ron* (Ghysen and Pironio, 1972) and *nus* (Friedman *et al.*, 1973), have already been isolated. They all restrict the growth of phage λ. Studies on these mutants have shown that bacterial factors, such as the DNA-dependent RNA polymerase, are involved in the control of viral transcription.

It is known that phage P2 interferes with the growth of wild-type phage λ (Bertani, 1958). The interference, known as Spi (sensitivity to P2 interference) phenomenon, is caused by the product of the P2 gene *old* when several λ genes (the *spi* genes; γ, β, exo and δ), located

in the *CIII-att* region, are functioning (Lindahl *et al.*, 1970; Zissler *et al.*, 1971; Cohen and Chang, 1971). Therefore, *E. coli* cells lysogenic for phage P2 are resistant[1] to wild-type *λ*. In contrast, these cells are sensitive to phage that lacks the *spi* genes. This is also the case for *susN7nin5*, because it does not express any *spi* genes owing to the defect in the *N* protein. This phage is capable of *N*-independent growth (Court and Sato, 1969; Mark, 1973) due to deletion of one of the *N*-acting sites, located between genes *P* and *Q* (Fiandt *et al.*, 1971). By looking for bacterial mutants resistant to *susN7nin5*, but still sensitive to *λspi*, from a P2 lysogen carrying no nonsense suppressor, we found some host mutants in which the rate of transcription of the early genes to the left of *N* (the *CIII-att* region) was much higher than in the parent cells in the absence of the *N* protein. We named these mutations *nit* (for *N*-independent transcription). Such *nit* mutants would be expected to possess one of the following characteristics: 1) the alteration of an element of the transcription machinery (*e.g.*, RNA polymerase, rho factor, other initiation or termination factors), or 2) no (or reduced) production of the host factor which is involved in the turn off control by the *tof* (or *cro*) gene product of phage *λ* as described before (Inoko and Imai, 1974; Oppenheim *et al.*, 1974).

In fact, the *nitA* class of *nit* mutants was found to produce an altered form of the termination factor rho (manuscript in preparation). In the present paper, we are mainly concerned with the genetic and physiological characteristics of the *nitA* mutation. The biochemical studies on the altered rho factor will be reported elsewhere.

Materials and Methods

Phage and Bacterial Strains

The phage strains used are listed in Table 1. Phages *λsusN7nin5* and *susN7N53nin5* were obtained by crossing *λi434susN7nin5* with *λsusN7* and with *susN7N53*, respectively. Following Lindahl *et al.* (1970), phage *λsusN7nin5spi* was selected by plating a UV-induced *λsusN7nin5* lysate on an *E. coli* P2 lysogen carrying an amber suppressor, and was obtained at a frequency of ca. 6.4×10^{-6}. Phages *λptrp60-3* and *λpbio11* which have lost the *spi* genes were also used as *λspi*.

The bacterial strains used in this study were all derived from *E. coli* K12, and are listed in Table 2.

Media

Medium E (Vogel and Bonner, 1956) with 0.5% glucose was used as a minimal medium. Medium EA was medium E supplemented with 0.2% casamino acid (Difco). This was further supplemented with 50 µg of L-tryptophan per ml to make medium EAT. The peptone-glucose (PG) medium contained (per liter) 20 g of polypep-

Table 1. Phage strains

Phage	Source and/or reference	
λi434susN7nin5	K. Matsubara	Court and Sato (1969)
λsusN7nin5	this work	
λsusN7nin5spi	this work	
λsusN7N53nin5	this work	
λpbio11	laboratory stock	Zissler *et al.* (1971)
λptrp60-3	A. Matsushiro	Inoko and Imai (1974)
λsusN7N53ptrp46	N.C. Franklin	
λCI857susN7N53	Y. Takeda	
λCI857susN53sus029susP3	H. Uchida	
λCI857susN7N53byp	D. I. Friedman	Butler and Echols (1970)
P2	H. Yamagishi	
Pl*vir*	laboratory stock	

tone (Wako Drug Co.), 5 g of Nacl and 5 g of glucose and the pH was adjusted to 7.2 with NaOH. These media were all supplemented with 50 µg of thymine per ml when thymine-requiring bacteria were cultured. Titration of phage *λ* was carried out using the PG agar plate overlayed with the *λ* agar medium which contained (per liter) 10 g of polypeptone, 2.5 g of NaCl and 0.5% agar. The EMB agar plate (Lederberg, 1950) was used for the cross streak test to examine the sensitivity of bacterial strains to phage *λ* and its derivatives. For preparation of the Pl*vir* lysate, the L agar plate (Lennox, 1955) was employed. Phage adsorption buffer was 0.01M Tris-HCl ($_p$H 7.4) containing 0.15 g of $MgSO_4 \cdot 7 H_2O$, 0.08 g of $CaCl_2 \cdot 2H_2O$ and 0.01 g of gelatin per liter. For phage *λ*, this was further supplemented with 10^{-2}M $MgSo_4$.

Preparation and Purification of Phages

Phage lysates were obtained either by infection of bacteria with each phage at an appropriate multiplicity in the *λ* agar medium, or by induction of lysogens with UV-irradiation followed by incubation in the PG medium. Phage particles were concentrated and purified, if necessary, with NaCl-polyethyleneglycol (Yamamoto *et al.*, 1970) and subsequently by CsCl density equilibrium centrifugation.

Isolation of the nit Mutants

The *nit* mutants were isolated from *E. coli* strain KY4727 which is lysogenic for phage P2 and is carrying no nonsense suppressor. The general procedure for isolation is as follows. Exponentially growing cells were suspended with sodium acetate buffer and were treated with 700 µg/ml of *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine at 37° C for 45 min (10–25% survival) washed twice and incubated at 37° C in the PG medium overnight to allow for segregation. The treated cells were then plated on peptone agar plates.

After incubation at 37° C overnight, colonies were replica-plated onto a set of EMB agar plates, each of which had been seeded with *λsusN7nin5* or *λsusN7nin5spi* beforehand. Colonies seemingly resistant[2] to *λsusN7nin5* but sensitive to *λsusN7nin5spi*

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[1] See footnote in Materials and Methods.

[2] In this paper we use the term "resistant" to describe no visible cell lysis in, for example, a cross-streak. P2-lysogenic cells do not fully survive after infection with *λ*, rather, 50–90% are killed (Lindahl *et al.*, 1970). However, the small number of *λ* progeny produced is not sufficient to bring about such an extent of lysis that can be seen upon replica-plating or cross streaking on EMB agar plates.

Table 2. Bacterial strains

Strain	Genetic character	Origin
KY363	F⁻ lac suII	C600 × HfrH, H. Ozeki
W3350	F⁻ gal1 gal2 lac	H. Ozeki
KY817	F⁺ trpE	Y-mel
KY4710	F⁻ thr tonB-trp deletion his thy str	derived from KY817 through several steps
W3350(P2)	F⁻ gal1 gal2 lac(P2)	W3350
KY363(P2)	F⁻ lac suII(P2)	KY363
KY4727	F⁻ thr tonB-trp deletion his thy str(P2)	KY4710
KY4737	Hfr pro trpE(P2) gene sequence of transfer: origin-str-arg-thr-trp-	derived from KY817 through several steps
KY4738	Hfr pro trpE(P2) gene sequence of transfer: origin-trp-his-str-arg-	derived from KY817 through several steps
KY4742	F⁻ thr tonB-trp deletion his thy metE str(P2)	KH39---- × HD12
KY4744	F⁻ thr tonB-trp deletion his thy ilv str	KY4710
KY4745	F⁻ thr tonB-trp deletion his thy metE str	KH39---- × KY4744
KY4754	F14 pro trp ilvD metE str recA	AB1206
KY4757	F⁻ thr trpE his thy str(P2)	KY817---- × KY4727
KY8022	F16 proA his ilvC argE thi	T. Miki
HD1	F⁻ nitA6 other markers same as in KY4727	KY4727
HD6	F⁻ nitA16 other markers same as in KY4727	KY4727
HD7	F⁻ nitA18 ilv(leaky) other markers same as in KY4727	KY4727
HD10	F⁻ nitA6 ilv other markers same as in KY4727	HD1
HD12	F⁻ nitA18 thr tonB-trp deletion his thy ilv str(P2)	HD7
HD15	F⁻ nitA18 thr trpE his thy ilv(leaky) str(P2)	KY817---- × HD7
HD16	F⁻ nitA6 thr tonB-trp deletion his thy str	HD1---- × KY4744
HD19	F⁻ nitA16 thr tonB-trp deletion his thy str	HD6---- × KY4744
HD21	F⁻ nitA18 thr tonB-trp deletion his thy ilv(leaky) str	HD7---- × KY4745
HD33	F⁻ nitA18 thr trpE his thy ilv(leaky) str	KY817---- × HD21
HD43	F⁻ nitA18 thr trpE his thy ilv(leaky) str(λsusN7N53)	HD33
KH39	F⁻ thr trpE his tyr thy metE sup126	T. Horiuchi
KH50	KHF50 pro trp ilv metE str recA	T. Horiuchi
AB1206	F14 pro his ilv-argH deletion thi str	E. Adelberg

For symbol nitA (N-independent transcription) see text. Other gene symbols are according to Taylor and Trotter (1972). Transduction was carried out with phage Plvir: donor---- × recipient.

were picked and reexamined by cross streak tests to confirm this phenotype on another EMB agar plate.

Mating Experiment

Bacterial crosses were carried out in Difco Penassay broth for 1 or 2 hr at 37° C, and cells were plated on a selective agar medium after appropriate dilution. The recombinant clones obtained were streaked with sterile toothpicks on appropriate agar media to examine unselected marker.

Transduction

Transduction with phage Plvir was carried out by a modification (Hiraga, 1969) of the method described by Lennox (1955).

Measurement of Anthranilate Synthetase Activity in Phage-Infected Bacteria

Anthranilate synthetase (ASase) formed in phage-infected bacteria was assayed as described in a previous paper (Inoko et al., 1974).

Results

Isolation of the nit Mutants

The growth of wild-type phage λ is blocked in E. coli cells lysogenic for P2: P2 lysogens are "resistant"[3] to wild type λ, and λ is said to be sensitive to P2 interference or Spi⁺ (Bertani, 1958). In contrast, λsusN7nin5 (or λsusN7N53nin5), a mutant capable of N-independent growth (Court and Sato, 1969), can grow normally in P2 lysogens carrying no nonsense suppressors (Su⁻). It is Spi⁻ because its spi genes (mapped in the CIII-att region of the λ genome) are not expressed owing to the defective N protein. Consequently, strain W3350 (Su⁻) when carrying a P2 prophage is sensitive to λsusN7nin5 (Table 3). However, λsusN7nin5 becomes Spi⁺ in suppressor carrying hosts

[3] See footnote 2 to Materials and Methods for the use of this word.

Table 3. Plating of *λnin5* derivatives on various strains

Bacterial strain	Relevant properties	Phage			
		λsusN7nin5		*λsusN7N53nin5*	
W3350	Su⁻	S	(1.0)	S	(1.0)
W3350	Su⁻, (P2)	S	(0.70)	S	(0.78)
KY363	SuII⁺	S	(1.0)	S	(1.0)
KY363	SuII⁺(P2)	R	(1.5×10^{-5})	R	(5.8×10^{-6})

Sensitivity of bacterial strains to phage was examined by cross streak test on EMB agar plates. Symbols S and R in the table denote sensitivity and resistance to these phages, respectively. The values in parentheses represent the efficiencies of plating of these *λnin5* derivatives. The assay was carried out by plating phage suspensions on PG agar plates overlayed with *λ* agar medium, together with aliquots of the indicator bacteria grown overnight in PG medium. The efficiency of plating is expressed as the ratio of the phage titers obtained on the P2 lysogen to that on the non-lysogenic host.

due to the presence of *N* protein. Accordingly, the P2 lysogen of strain KY363 (SuII⁺) is resistant to *λsusN7nin5*. A *λsusN7nin5spi* phage is Spi⁻ regardless of whether the P2 lysogen is Su⁺ or Su⁻.

An attempt was made to isolate bacterial mutants which are resistant to *λsusN7nin5*, but sensitive to *λsusN7nin5spi*, from strain KY4727 which is a P2 lysogen carrying no suppressor. Such mutants would be expected to permit the expression of the *λ* early genes in the absence of the *N* protein, unless they are amber suppressor mutants. After treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (details in Materials and Methods), seven such mutants were obtained at a frequency of 10^{-3} to 10^{-4}. Three of these mutants (*nit6*, *nit16* and *nit18*) were tested against *λnin5* derivatives (Table 4a).

The *λnin5* phages plated with various efficiencies on these *nit* mutants, and formed minute plaques, especially on the *nit6* and *nit18* strains. In additon, the e.o.p. of *λsusN7N53nin5* on *nit6* and *nit18* but not on *nit16* was much lower than that of *λsusN7nin5*. All the *nit* mutants tested were also resistant to *λi434susN7nin5* which carries the early promoters from phage 434. No significant difference was observed between these *nit* mutants and the parent KY4727 in respect to growth rate under various cultural conditions.

Table 4. Plating of various *λ* derivatives on *nit* mutants

(a)

Bacterial strains	Relevant properties	Phage					
		λsusN7nin5		*λsusN7N53nin5*		*λi434susN7nin5*	
HD 1	*nit6* (P2)	R	(1.5×10^{-2})	R	(4×10^{-4})	R	
HD 6	*nit16* (P2)	R	(0.25)	R	(0.25)	R	
HD 7	*nit18* (P2)	R	(1.0×10^{-2})	R	(4×10^{-5})	R	
KY4727	*nit⁺* (P2)	S	(1.0)	S	(1.0)	S	

(b)

Bacterial strain	Relevant properties	*λpbiol1*		*λptrp60 − 3*		*λsusN7nin5spi*	
HD 1	*nit6* (P2)	S	(1.02)	S	(0.71)	S	(1.14)
HD 6	*nit16* (P2)	S	(0.97)	S	(1.26)	S	(1.34)
HD 7	*nit18* (P2)	S	(0.73)	S	(0.98)	S	(1.04)
KY4727	*nit⁺* (P2)	S	(1.0)	S	(1.0)	S	(1.0)

(c)

Bacterial strain	Relevant properties	*λsusN7nin5*		*λ*	
HD 16	*nit6*	S	(0.95)	S	(1.08)
HD 19	*nit16*	S	(1.04)	S	(1.36)
HD 21	*nit18*	S	(0.90)	S	(1.39)
KY4710	*nit⁺*	S	(1.0)	S	(1.0)

Sensitivity of *nit* mutants to phage was examined. See legend to Table 3. The efficiency of plating (shown in parenthesis) of each phage is normalized to the phage titer obtained with the parent *nit⁺* strain.
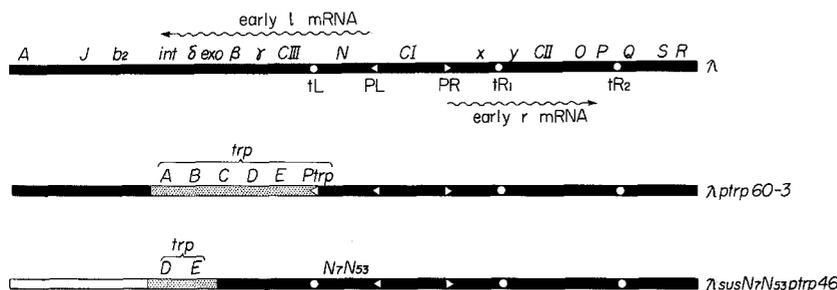
Fig. 1. Genetic maps of bacteriophages λ, $\lambda ptrp60-3$ and $\lambda susN7N53$ ptrp46. The λtrp phages are plaque formers carrying the *trp* region of the *E. coli* chromosome. White, black and dotted spaces represent genomes of $\phi 80$, λ and the bacterial chromosome respectively. Open triangles (P$_L$, P$_R$ and *Ptrp*) show promoters for λ early tryptophan operons. Open circles (t$_L$, t$_{R1}$ and t$_{R2}$) indicate putative *N*-sensitive transcription termination signals. Gene symbols for bacteriophage λ are according to Szybalski *et al.* (1970) and are also used for phage $\phi 80$. The map is not drawn to scale

Confirmation of the nit Charcter

The *nit* mutants were characterized more precisely, since their resistant phenotype could be attributed to several causes. Generally, the growth of phage λ lacking the *spi* genes is not blocked by P2. This was also the case for the *nit* mutants when tested by infection with λspi such as $\lambda pbioll$, $\lambda ptrp60-3$ and $\lambda susN-7nin5spi$ (Table 4b). This implies that resistance of the *nit* strains to $\lambda susN7nin5$ is accomplished through expression of the *CIII-att* region of the infecting phage.

We next examined the effect of phage P2 on the *nit* phenotype by using *nit* mutants free from P2. Nonlysogenic *nit* mutants were easily prepared by transducing the *nit* gene with phage P1*vir* into a recipent strain nonlysogenic for P2 (see later). Absence of prophage P2 made the *nit* mutants no longer resistant to $\lambda susN-7nin5$; it permitted the normal growth of this phage (Table 4c). The resistant phenotype was established in these sensitive *nit* bacteria, when they were lysogenized with P2. These results provide the most convincing evidence that the resistance of the *nit* mutants to $\lambda susN-7nin5$ requires the presence of prophage P2.

The possibility that the *nit* strains may have aquired a suppressor enabling the amber *N* mutant to function normally, was excluded as we showed that none of the *nit* mutants was able to suppress any T4 and λ amber mutations tested, while supporting normal growth of wild-type phages T4 and λ.

N-Independent Expression of the early leftward operon of λ in the nit Mutants

To obtain more direct evidence for *N*-independent transcription, the extent of early gene expression was determined with regard to the *CIII-att* region of the genome by measuring the formation of anthranilate synthetase (ASase, a complex of proteins specified by

trpE and *D* genes) upon infection of the *nit* mutants with phages $\lambda ptrp60-3$ or $\lambda susN7N53ptrp46$. These are *trp* transducing phages; the former carries the whole tryptophan operon and the latter only the *trpE* and *D* genes (without *trp* promoter) in the *CIII-att* region of the λ genome (Fig. 1). The $\lambda susN7N53ptrp46$ phage is actually a λ-$\phi 80$ hybrid; all late genes (*A-att*) are derived from the $\phi 80$ genome. In either case, the *l* strand of the phage DNA is the sense strand. Expression of the *trp* genes on the $\lambda ptrp60-3$ genome, only under repression conditions (excess L-tryptophan) depends on the phage transcription system, initiating at the P$_L$ promoter (Inoko and Imai, 1974). On the other hand, expression of the *trp* genes on the $\lambda susN7N53ptrp46$ genome is obliged to follow the λ transcription under any condition, and absolutely requires the presence of the *N* gene product (Franklin, 1971).

Upon infection with $\lambda ptrp60-3$ in the presence of excess tryptophan, ASase activity appeared at essentially the same rate both in the mutant HD7 (*nit18*) and in the parental strain, KY4727 (*nit*$^+$). The activity leveled off at around 8 minutes after infection (Fig. 2), due to the turn off control (Pero, 1970), as was observed previously (Inoko and Imai, 1974). Similar results with almost the same kinetics were obtained with strains carrying *nit6* or *nit16* upon infection with $\lambda ptrp60-3$ (data not shown). These results indicate that the early leftward transcription of the λ genome occurs normally in the *nit* mutants tested as well as in KY4727. At the same time, the results seem to eliminate the possibility that some of the *nit* mutants carry a mutation affecting the host factor involved in the turn off control by the *tof* gene product of phage λ (Inoko and Imai, 1974; Oppenheim *et al.*, 1974).

In the next experiment, KY4757, a *tonB*$^+$ derivative of KY4727, was infected with $\lambda susN7N53ptrp46$. This phage was found to be wild-type in the tail gene
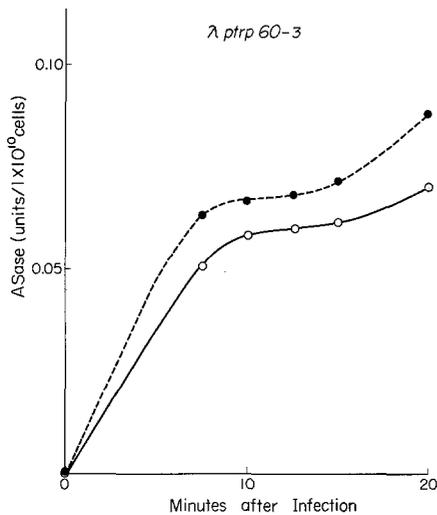
Fig. 2. Formation of ASase in cells of the strains *nit18* and its parent *nit⁺* after infection with *λptrp60−3* in the presence of tryptophan. These bacterial strains carry a deletion covering the whole *trp* operon. Cells grown in EAT medium to 2×10^9/ml were spun down and resuspended in phage adsorption buffer with 50 μg/ml L-tryptophan, mixed with a purified phage suspension at m.o.i. of 1 followed by incubation at 37° C for 20 min. After removal of unadsorbed phages, infected cells were resuspended in EAT medium and cultured at 37° C by shaking. Samples were removed and chilled in ice at the times indicated, and 50 μg/ml of chloramphenicol was added. Enzyme assays and other detailed procedures were as described previously (Inoko *et al.*, 1974). Host strains used were o——o, HD7 (*nit18*) and ●——●, KY4727 (*nit⁺*)

(*i.e.*, h^{+80} instead of h^{-80}) and failed to adsorb to TonB⁻ cells, in contrast to the original description (Franklin, 1971). As seen in Fig. 3a, no detectable ASase activity appeared even after 60 minutes from infection because of the absence of the *N* protein. In contrast, when the *nit18 tonB⁺* cells (lysogenic (HD15) or non-lysogenic (HD33) for P2) were infected with

the same phage, a significant increase in ASase activity was observed which continued until about 10 minutes after infection (Figs. 3a and b). The increase stopped thereafter, probably due to the turn off control. Essentially the same results were obtained with mutant cells carrying *nit6* or *nit16* upon infection with the same transducing phage (data not shown). Irrespective of the P2 lysogenicity of the *nit* mutants, the extent of ASase formation was approximately the same, amounting to about 2% of that observed in the presence of the *N* protein (that is, the ASase activity observed in KY363, a strain carrying *suII⁺*) as shown in Fig. 3a and b. The enzyme formation was completely inhibited when the *nit18 tonB⁺* strain which is lysogenic for *λsusN7N53* was used as the host (Fig. 3b). These results strongly suggest that in these *nit* mutants the early leftward transcription of the *CIII-att* region of the infecting λ genome takes place in the absence of the *N* product. The P_L promoter may be the initiation site for this *N*-independent transcription, since the ASase formation observed appears to be subject to the turn off control and is completely repressed by the *CI* repressor.

Pleiotropic Effect of the nit Mutation on Early Rightward Gene Expression in λ

Assuming that the *nit* mutation indeed alters some element of transcription related to the *N* function, the mutation should also affect the early rightward gene expression of infecting phage λ, since sites for *N*-action are known for both left and right transcription of the λ genome. Therefore, we investigated the effect of the *nit* mutation on the expression of the *y-O-P* region, in which transcription also depends,
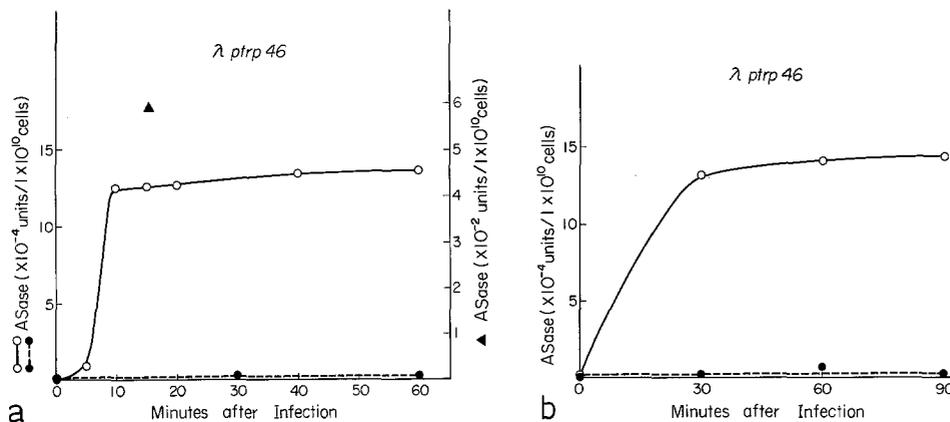
Fig. 3. (a) Formation of ASase after infection with *λsusN7N53ptrp46* in cells of the strains *nit18*, its parent *nit⁺* and a *nit⁺* carrying a suppressor in the presence of tryptophan. Procedures were the same as in Fig. 2 except that the m.o.i. was 5. Host strains used were o——o, HD15 (*nit18 trpE tonB⁺*), ●——●, KY4757 (*nit⁺ trpE tonB⁺*) and ▲, KY363 (*nit⁺ suII⁺*) (b) Formation of ASase after infection with *λsusN7N53ptrp46* in cells of the strains *nit18* lysogenic and non-lysogenic for *λsusN7N53* in the presence of tryptophan. Neither bacterial strain is lysogenic for P2. Procedures were the same as was in Fig. 2, except that the m.o.i. was 5. Host strains used were o——o, HD33 (*nit18 P2ˢtrpE tonB⁺*) and ●——●, HD43 (*nit18 P2ˢ trpE tonB⁺* (*λsusN7N53*))

though to less extent than other regions, on the *N* protein.

It is known that the products of genes *O* and *P* of phage λ may act in initiation of the phage DNA replication (see review, *e.g.* Kaiser, 1971). It is also known that induction of a λ prophage carrying *susN* results in cell killing without phage development, and that the killing is mainly due to the function of genes *O* and *P* in the absence of the *N* product (Pereira da Silva *et al.*, 1968; Sly *et al.*, 1968; Brachet *et al.*, 1970). Thus, the extent of the *O* and *P* expression can be determined by measuring viability of bacteria, following heat induction of the λ*CI857susN7N53* lysogens (Friedman *et al.*, 1973).

Emploxing this procedure, the surviving bacteria were scored after thermal induction of the prophage λ*CI857susN7N53* in the *nit18* mutant strain HD21 and in the *nit*⁺ parent strain KY4710, both non-lysogenic for P2. In KY4710, the ratio of colony formers at 42° C to those at 30° C was 3.0×10^{-3}, but it was hundred times lower in the *nit18* mutant (Table 5). When corresponding bacterial strains lysogenic for λ*CI857susN53sus029 susP3* were tested in the same manner as a control, thermal induction hardly affected their growth (Table 5).

To confirm the above result, we performed another experiment in which phage λ*CI857susN7N53* carrying *byp* was used. The *byp* mutation (Butler and Echols, 1970), which is located between *P* and *Q* in the λ genome, permits some synthesis of the *Q* product in the absence of the *N* protein. However, λ*susN7N53byp* cannot grow well in bacteria carrying no suppressor, because of an insufficient supply of the *Q* protein (Mark, 1973), unless an additional promoter mutation *c17*, which maps in the *y* region, is present (Pereira da Silva and Jacob, 1968). Based upon the above knowledge, we examined the growth of λ*CI857susN7N53byp* in the three *nit* mutants. As seen in Table 6, e.o.p. of the phage on KY4710 was found to by 6.6×10^{-8}. In contrast, the *nit* mutants were able to support growth of the same phage much better, the e.o.p. increasing about hundred fold.

The results obtained in these two experiments clearly indicate that *N*-independent transcription of the *y-O-P* region takes place in the *nit* mutants at a much higher rate than in the *nit*⁺ strain.

Location of the nit Gene on the E. coli Chromosome

Preliminary mapping was performed by crossing each of the *nit* mutants with an Hfr strain, such as KY4737 or KY4738, which are lysogenic for P2, and were derived from KY817, the ancestor of the *nit* mutants. The results indicated all the three *nit* mutations to

Table 5. Survival at 42° C of a nit mutant lysogenic for defective λ possessing thermo-inducible repressor

Bacterial strain	Relevant pro-perties	Prophage	
		λ*CI857susN7N53*	λ*CI857susN53sus029susP3*
HD 21	*nit18*	3.5×10^{-5}	0.66
KY4710	*nit*⁺	3.0×10^{-3}	0.81

Lysogens grown overnight at 30° C in PG medium were diluted and plated in duplicate on PG ager plates, which were then incubated at either 42° C or 30° C. The table gives the ratio of number of colony formers at 42° C to the number at 30° C.

Table 6. Efficiency of plating of λ*CI857susN7N53byp* on the nit mutants

Bacterial strain	Relevant properties	
HD 16	*nit6*	1.0×10^{-5}
HD 19	*nit16*	5.0×10^{-6}
HD 21	*nit18*	5.0×10^{-6}
KY4710	*nit*⁺	6.6×10^{-8}

See legend to Table 3.

Table 7. Transductional mapping of the *nit6* mutation by phage P1*vir*

(a)

| Donor: | KH39 | (*ilv*⁺ *nit*⁺ *metE*) |
| Recipient: | HD10 | (*ilv nit6 metE*⁺) |

Selected marker	Unselected markers		Number of transductants	Frequency (%)
	nit	*metE*		
ilv⁺			100	
	1	1	2	2
	1	0	79	79
	0	0	19	19
	0	1	0	0

(b)

| Donor: | HD10 | (*ilv nit6 metE*⁺) |
| Recipient: | KY4742 | (*ilv*⁺ *nit*⁺ *metE*) |

Selected marker	Unselected markers		Number of transductants	Frequency (%)
	ilv	*nit*		
metE⁺			100	
	1	1	48	48
	0	1	9	9
	0	0	43	43
	1	0	0	0

Recipient cells were infected with phage P1*vir* grown on donor cells and were plated out on an appropriate agar medium for selection. The *nit* character among the transductants was determined by cross straking against λ*susN7nin5* on EMB agar plates. Symbols 1 and 0 stand for donor's or recipient's allele, respectively.
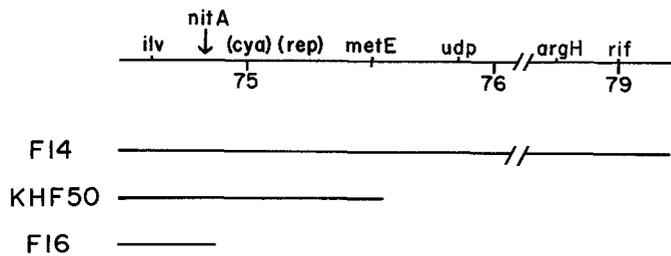
Fig. 4. The *E. coli* linkage map (Taylor and Trotter, 1972) showing the region of the *nitA* gene on the chromosome and the F'episomes employed. Assignment of the positions of *nitA*, *cya* and *rep* is tentative

be located somewhere in the vicinity of ilv. In fact, in Pl transduction experiments *nit* markers resulted to be well cotransducible with *ilv* and *metE* (Table 7 for *nit6;* data not given for *nit16* and *nit18*).

These results indicate that the three *nit* mutations are located between *ilv* and *metE* (Fig. 4), each presumably representing a single mutation which occurred in the same or in neighboring cistrons. To distinguish this class of *nit* mutations from the others, the *nit* gene presently defined will be hereafter designated *nitA*.

It was then examined whether the *nitA* mutation is dominant to the wild-type allele or not. The answer to this question should provide information for understanding the mechanism of *N*-independent transcription. To prepare strains partially diploid with respect to the *ilv-nitA* region, the F' episomes F14, KHF50 and F16 were each transfered into strain HD12 (*ilv nitA18*) by cross, followed by selection of the Ilv⁺ clones. The genetic constitution of F' episomes used is illustrated in Fig. 4. The F'-derivatives of the *nitA18* strain thus prepared were sensitive to male specific RNA phage, and had the ability to transfer the F' episomes further to another recipient strain carrying the *recA* mutation. In respect to their response to phage *λsusN7nin5*, most of these partially diploid, *nitA18/ nit⁺* strains were found to have lost the mutant phenotype (resistance) which characterized the original haploid *nitA18* strain. It is, therefore, concluded that the *nitA* mutation is recessive to the wild-type allele, implying that a certain regulatory component of transcription is functionally defective in the *nitA* mutant

Discussion

From an *E. coli* strain which is lysogenic for P2 and carries no suppressor, *nitA* mutants resistant[4] to phage *λsusN7nin5* were isolated. In these mutants the early gene expression of phage λ occurs even in the absence of the *N* protein. Although the *nitA* mutants were first recognized by their sensitivity to P2 interference, the mutation shows a pleiotropic effect on *N*-independ-

ent expression of both the *CIII-att* and the *y-O-P* regions. These regions are situated on the early left and right operon, respectively, and their transcription normally requires the *N* function. However, no effect of the *nitA* mutation was detected for the third *N*-acting site, which is located between *P* and *Q* (presumably within the *nin* deletion, Fiandt, 1970) and is necessary for expression of *Q* and the late genes therewith. This was demonstrated by the fact that the *nitA* mutants tested did not support growth of phage *λN⁻ nin⁺*, such as *λsusN7N53*, *λsusN7* and *λsusN7c17* (unpublished results). Therefore, it is not clear whether the *nitA* mutation is able to promote *Q* expression. Presently we do not have a more sensitive method at hand to detect the activity of the *Q* product, which seems to function in a stoichiometric manner for expression of the late genes.

More direct evidence for the *N*-independent transcription of the *CIII-att* region was obtained by measuring ASase formation after infection with *λsusN7N53ptrp46*. It wws quite convenient to use this *trp* transducing phage for the present purpose, since genetic studies (Franklin, 1970) had established that the *E. coli trp* genes are integrated into the *λCIII-att* region in such a form that they behave as if they were members of the early gene class with regard to transcription regulation. In addition, ASase is an enzyme whose activity can be measured with high sensitivity, so that even a small increase in the enzyme formation can be detected. In fact, the experimental results clearly demonstrated ASase formation due to *N*-independent transcription in *nitA* cells after infection, even though the increase in enzyme activity was only about 2% of that found in the presence of the *N* product. However, this value is presumably an underestimation, when the following facts are taken into consideration. For reason unknown, the presence of the *N* product further stimulates the *trp* enzyme formation by *λsusN7N53ptrp48* (Franklin, 1974). This *trp* transducing phage lacks the tL terminator (an *N*-acting site for the early leftward transcription, Fig. 1) and therefore does not require the *N* protein for the *trp* gene expression. Besides, the present conditions used for ASase formation may permit replication of the DNA of *λsusN7N53ptrp46* in SuII⁺ cells, while they do not

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[4]   See footnote to Materials and Methods for the use of this word in this paper.

in Su⁻ cells. Still, the present *nitA* mutants exhibit a low degree of *N*-independence. Efforts have been made to obtain a *nitA* mutant with much higher *N*-independence, without success so far. This may imply that such mutants become lethal on account of the *nitA* function being essential for the viability of the *E. coli* cell. Accordingly, experiments are in progress to isolate some conditional type of the *nitA* mutants.

In the *nitA* strains used, the *N*-independent transcription of the early left operon of phage λ is definitely affected by the *tof* product, and is completely repressed by the *CI* repressor. These two proteins are known to act at (presumably by binding to) the *v2* (or *virL*) operator; they shut off or repress the leftward mRNA synthesis which is initiated from the P_L promoter (Chadwick *et al.*, 1970; Steinberg and Pashne, 1971; Pero, 1971; Sly *et al.*, 1971; Koga and Horiuchi, 1971; Matsubara, 1972). Therefore, we exclude the possibility that the *nitA* mutation may allow a new transcription initiation for the *CIII-att* region from some site neighboring *CIII*, and that the *nitA* gene specifies some host factor which is involved in the turn off control by the *tof* product of phage λ (Inoko and Imai, 1974; Oppenheim *et al.*, 1974).

The *nitA* gene was found to reside somewhere between *ilv* and *metE* on the *E. coli* linkage map. The gene is different from *rep* (Calendar *et al.*, 1970) and *cya* (Yokota and Gots, 1970), which have all been mapped in that region (Fig. 4), because the *nitA* mutants do not inhibit the growth of phage P2, and ferment sugars normally. With respect to *suA* which is closely linked to *ilv*, a brief discussion may be necessary as to its possible identity with the *nitA* gene. The *suA* function is known to degrade immediately an untranslatable mRNA which corresponds to a region distal to an amber polar mutation (Morse and Primakoff, 1970; Kuwano *et al.*, 1971). Assuming that early genes are once transcribed throughout the whole left operon, but the mRNA distal to *N* is subject to immediate degradation by the *suA* product unless the *N* protein is present, it is not unreasonable to speculate the *nitA* strain to be a type of *suA* mutant. However, we tentatively rule out this possibility, because the P2 lysogen with the *suA* mutation, originally isolated by Beckwith (1963) (obtained from M. Kuwano), did not show any *nitA* phenotype, that is, the resistance to λ*susN7nin5*. Therefore, we believe that *nitA* is a newly identified gene which affects transcription regulation in *E. coli* cells, at least, upon infection with phage λ.

A single mutation occurring in the *nitA* gene pleiotropically promoted the expression of the *y-O-P* as well as of the *CIII-att* region of the λ genome in the absence of the *N* function. Judging from these results, it is likely that the *nitA* product is involved in transcrip-

tion termination which operates at the genetically specified stites, t_L between *N* and *CIII*, and t_R between *x* and *y*, of the λ genome (Fig. 1). These sites are interpreted to be identical to the sites at which the transcription termination factor rho was found to act in the *in vitro* transcription experiment (Roberts, 1969 and 1970). Besides, the *nitA* mutation was found to be recessive to the *nitA*⁺ allele in our genetic experiment. Therefore, the simplest possibility would be that *nitA* is a gene concerned with the rho factor.

This possibility is supported by recent biochemical investigations of a *nitA* mutant in our laboratory. The termination factor rho was purified from both the *nitA18* and its parent strains to characterize its biochemical and immunological properties. We found that the mutant rho, purified by the method of Roberts (1969), had a much reduced activity when assayed in terms of both the transcription inhibition (Roberts, 1969) and the poly (C) dependent ATPase activity (Lowery-Goldhammer and Richardson, 1974). In addition, it exhibited markedly different molecular size and antigenicity from the normal rho factor which was purified from the parent strain. By employing an improved method of purification, it was found that the altered properties of the *nitA18* rho may be the consequence of its increased sensitivity to protease, presumably due to an altered conformation of the rho protein by mutation. Details of these studies will be described elsewhere (manuscript in preparation). In combination with the results of these genetic and biochemical investigations, we assume that *nitA* is the structural gene which specifies the rho protein, though other possibilities remain to be tested.

Information on the action of the rho factor has so far been available mainly from the *in vitro* transcription experiments, and very little proof is there for its role *in vivo*. The isolation of the *nitA* mutants has now provided the first evidence that the rho factor actually functions in the transcription termination, at least, of the λ genome in bacterial cells. However, it remains still unknown whether the factor is effective only in the control of transcription of infecting phage genomes, or is essential in the regulation of cellular transcription, because the mutation does not distinctly affect the growth of the cell itself, as far as the present *nitA* mutants are concerned. For the present, we prefer to postulate that the rho factor is essential for the cellular transcription regulation, but the *nitA* mutants so far obtained are leaky, and not so defective in the rho function as to affect cell growth. This presumption may be justified by our finding that *nitB* (a conditional lethal mutation), representing another class of *nit* mutations, which is very closely linked to *rif* and alters the structure of RNA polymerase, neither affects the cell growth distinctly under the conditions showing

the *nit* phenotype (manuscript in preparation). Isolation and characterization of *nitA* mutants that are conditionally lethal would be most useful for further investigation of the role played by the rho factor in transcription of the bacterial genes in general.

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Note Added in Proof. Conditionally lethal *nitA* mutants are recently isolated, one of which is characterized as an amber type mutant. These strains show the *nit* phenotype at low temperature and lose their viability at high temperature. The fact implies that rho is an essential factor for cell growth.

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