Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 5530–5534, September 1983
Biochemistry

A mutation in the *Escherichia coli rho* gene that inhibits the N protein activity of phage λ

(transcription termination/*nus* mutations)

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Communicated by Ira Pastan, June 9, 1983

ABSTRACT **Certain *Escherichia coli rho* mutations, exemplified by *rho026*, block the growth of phage λ by interfering with phage gene expression. The phage gene *N*, whose product suppresses transcription termination, appears to be expressed normally in the mutants, and the functional stability of the N protein is not affected. Our data suggest that these *rho* mutations allow transcription to terminate despite the presence of N. Other *E. coli* mutants displaying a similar phenotype (Nus⁻) fail to propagate wild-type λ but permit the growth of the λ variant λ*nin5*, which has undergone a deletion of the λ terminator t_{R2}. The phenotype of the *rho026* mutant differs: the growth of λ is only marginally improved by the *nin5* deletion. Interestingly, N activity at rho-independent terminators is not inhibited by the mutations, whereas its ability to suppress rho-dependent terminators is markedly reduced. The relevance of this specificity in terms of models of N action is discussed.**

The expression of the genes of the coliphage λ is regulated by several different mechanisms [reviewed by Herskowitz and Hagen (1)]. One of these is the suppression of transcription termination, brought about by the action of the λ N protein [for recent review, see Ward and Gottesman (2)]. N-mediated termination suppression is a complex process requiring: (*i*) A phage DNA sequence, *nut*, which must be located between the promoter and the terminator of the transcription unit. This is thought to be the site where N interacts with RNA polymerase and accessory proteins involved in transcription (3–7). (*ii*) Several *Escherichia coli* proteins, originally defined by host *nus* mutations that interfered with λ growth (8, 9). The *nus* mutations affect the RNA polymerase β subunit (*nusC*), L protein (*nusA*), ribosomal protein S10 (*nusE*), or a 14-kilodalton protein whose role in *E. coli* physiology is unknown (*nusB*). The *nus* mutations all block termination suppression by N (10–16). The *nusA*, *nusB*, and *nusE* mutations are most restrictive at 42°C. λ*nin5*, which has undergone a deletion of the t_{R2} terminator located between genes *P* and *Q* (ref. 17 and Fig. 1), grows well in Nus⁻ strains at 42°C. In contrast the growth of certain λ variants such as λ*r32*, which carries an additional IS2 terminator in the p_R operon (18, 19), is inhibited even at 32°C; the *nin5* deletion allows these bacteriophage to grow well at 32°C but not at 42°C.

Terminators in *E. coli* can be divided into two classes: those that require rho protein for activity and rho-independent terminators, which are active in the absence of rho *in vivo* and *in vitro* (20–24). N function suppresses transcription termination at both types of terminators (24).

A specific allele of *rho* (*HDF026* or *rho026*), originally selected as defective for coliphage T4 growth (25), has been shown to block λ development at the level of regulation by N (26). We present here evidence that *rho026* and similarly selected *rho* alleles act to inhibit the action of N specifically at rho-dependent terminators.

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MATERIALS AND METHODS

Bacterial and Phage Strains. All bacteriophage and bacterial strains are listed in Table 1. Bacteriophage P1 transduction procedures were as described by Miller (30).

Enzyme Assays. Galactokinase was assayed as described by Sherman and Adler (31), using toluene-treated extracts of cultures grown in LB medium (30).

N Decay Measurements. Decay of N was measured as described by Gottesman *et al.* (27).

Construction of Plasmids. The λ $o_L p_L$*nut*L *N* region is flanked by a *Hin*dIII site in *c*I and a *Bam*HI site beyond the *N* gene (24, 32). This *Hin*dIII/*Bam*HI fragment was cloned in pBR322 by using the *Hin*dIII and *Bam*HI sites in the *tet* gene (33). The recombinant plasmid, pAD284, complements a λ N^- prophage for galactokinase escape synthesis. At the *Pvu* II site of pAD284, a 1.7-kilobase pair *Pvu* II fragment containing *galK* (7) was inserted to produce the pAD329 plasmid (see Fig. 3). This plasmid allows the synthesis of galactokinase only upon induction of the p_L promoter. The *Hin*dIII-N^+-*Sal* I fragment of pAD329 was substituted with *Hin*dIII-*N*am7*N*am53-*Xho* I and with *Hin*dIII-*N*am7*N*am53-*Sal* I fragments to give rise to pAD355 and pAD348, respectively. In each case the *N* fragment was derived from a λ*c*I857Sam7*N*am7*N*am53 *gal313* phage constructed by S. Adhya. The construction of pAD3485 was done as follows: pAD348 was digested with *Bam*HI and *Xho* I and briefly treated with *Bal*31 nuclease to generate flush ends, and the large fragment was purified and ligated. Details of the plasmid construction and analysis will be published elsewhere. The rho dependence of terminators t_{L1} and t_{L2} has been confirmed in these plasmids (34).

RESULTS

The *rho026* and *rho4008* Mutations Block λ*N* Action. A variety of *E. coli* mutations that block phage development have been characterized. The strain with the *nusA1* mutation was selected by resistance to λ induction; strains with *rho* mutations *rho026* and *rho4008* are unable to support the growth of phage T4 (25). In addition, these latter also inhibit λ propagation (ref. 26 and this work). The effect of the *nusA1* and *rho* mutations on the ability of λ to form plaques is shown in Table 2. The *rho* mutations, unlike *nusA1*, permit the growth of λ at 42°C, although with a plaque size that is significantly reduced compared to the *rho*⁺ parent. The λ derivative, λ*r32*, fails to grow on either *nusA1* mutants at 32°C or the *rho* mutants; the presence of the rho-dependent IS2 terminator between genes *c*II and *O* may increase its dependence on N function (refs. 17 and 18 and Fig. 1).

Table 1. Bacterial and bacteriophage strains

Strain	Relevant genotype	Source or ref.
Bacteria		
C388	F⁻ *his nusA1 ilv*	Douglas Ward
N5032	F⁻ *his rho026 rpsL*	Ref. 26
SG13060	F⁻ *his ilv proC rpsL*	Ref. 26
SG13081	F⁻ *his proC rho4008 rpsL*	SG13060 + P1(HDF4008); select *ilv*⁺
SG13504	F⁻ *lon Δ100 his ilv galE*::Tn*10 Δ8* (Δ*Bam* λcI*857* Δ*H1*) *rpsL*	Ref. 27
N5340	F⁻ *proC his ilv galE*::Tn*10 Δ8* (Δ*Bam* λcI*857* Δ*H1*) *rpsL*	Ref. 27
N5117	F⁻ *lon* Δ100 *his rho026 arg*::Tn*10 rpsL*	Ref. 26
SG13505	F⁻ *lon Δ100 his rho026 galE Δ8*::Tn*10* (Δ*Bam* λcI*857* Δ*H1*) *rpsL*	SG13504 + P1(N5117)
SG13506	F⁻ *proC his rho026 galE*::Tn*10 Δ8* (Δ*Bam* λcI*857* Δ*H1*) *rpsL*	N5340 + P1(N5117)
SG13508	F⁻ *proC his ilv Δ8* (Δ*Bam* λcI*857* Δ*H1*) *rpsL*	N5340 to Gal⁺
SG13531	F⁻ *proC his ilv nusA*1 *zgi*::Tn*10 Δ8* (Δ*Bam* λcI*857* Δ*H1*) *rpsL*	SG13508 + P1(C308)
SG13573	F⁻ *his rho4008 Δ8* (Δ*Bam* λcI*857* Δ*H1*) *proC rpsL*	SG13508 + P1(HDF4008)
SG13574	F⁻ *proC his rho026 Δ8* (Δ*Bam* λcI*857* Δ*H1*) *rpsl*	SG13508 + P1(N5117)
SG13582	F⁻ *proC his rho4008 galE*::Tn*10 Δ8* (Δ*Bam* λcI*857* Δ*H1*) *rpsL*	SG13573 + P1(N5340);
SG13583	F⁻ *proC his rho026 galE*::Tn*10 Δ8* (Δ*Bam* λcI*857* Δ*H1*) *rpsL*	SG13574 + P1(N5340)
SG13593	F⁻ *proC his ilv nadA*::Tn*10 Δ482* λcI*857* Δ*H1 rpsL*	SG13060 + P1(SG13586)
SG13586	F⁻ *nadA*:: Tn*10 Δ482* λcI*857* Δ*H1 rpsL*	N5382 + P1(NK6969)
N5382	F⁻ *Δ482* λcI*857* Δ*H1 his proC rho026 rpsL*	Ref. 28
SG13594	F⁻ *proC his rho4008 nadA*::Tn*10 Δ482* λcI*857* Δ*H1 rpsL*	SG13081 + P1(SG13586)
SG13621	F⁻ *proC his ilv galE*::Tn*10 Δ8* (Δ*Bam* λcI*857* Δ*H1*) *rpsL*	SG13508 + P1(N5340)
C308	F⁻ *his ilv nusA zgi*::Tn*10 Δ8* (*chl–blu*)	Douglas Ward
NK6969	F⁻ *nadA*::Tn*10 gal*⁺	Nancy Kleckner
SG13619	F⁻ *proC his nadA*::Tn*10 Δ482* λcI*857* *rho026*	SG13593 + P1(N5117)
SG13520	F⁻ *proC his ilv zgi*::Tn*10 nusA1 Δ482* λcI*857* Δ*H1*	SG13593 nad⁺ Tet^s + P1(C308)
N5503	F⁻ *galOP*::IS*1 Δ8* (Δ*BAM N*⁺ λcI*857* Δ*H1*)	Ref. 29
N4831	F⁻ (ΔBAM *N*am7*N*am53 λcI*857* Δ*H1*) *his ilv*	Ref. 24
AD5700	F⁻ (ΔBAM *N*am7*N*am53 λcI*857* Δ*H1*) *his rho026*	N4831 + P1(N5117)
AD5622	F⁻ *galOP*::IS*1 Δ8* (Δ*BAM N*⁺ λcI*857* ΔH1) *rho026*	N5503 + P1(N5517)
Bacteriophage		
λcI*857*		NIH phage collection
λcI*857 nin5*		NIH phage collection
λcI*857 int6 r32*		NIH phage collection
λcI*857 int6 r32 nin5*		NIH phage collection

The λ variant λ*nin5*, in which the t_{R2} terminator has been deleted, grows well on *nusA1* hosts at 42°C; and λ*r32nin5* forms normal plaques on the *nusA1* mutant at 32°C. In contrast, the growth of λ*r32* on the *rho* mutants is not restored to wild-type levels by the *nin5* deletion. Where the efficiency of plating is increased by *nin5*, plaque size is minute (λ*r32* on *rho4008*).

To simplify the analysis of the effect of *rho* mutations on N action, we used lysogens bearing fusions of *galK* to the λ p_L promoter. In this system, assay of galactokinase enzyme activity reflects the level of expression of *galK* from p_L (35). Three such fusions were constructed (Table 3). In fusion 1, the expression of *gal* from p_L does not require N activity; we assume that the *Δ482* deletion removes all terminators between the p_L promoter and the *galK* cistron (28). The *Δ8* and *ΔBAM* deletions of fusion 2 are less extensive, and at least one terminator remains between *galK* and p_L. This terminator(s) is rho-dependent (24). Fusion 3, derived from fusion 2, carries additional rho-dependent terminators, introduced by a Tn*10* insertion element (24). The expression of *galK* from p_L in fusions 2 and 3 has been shown to be entirely dependent on a functional *N* gene (24). Measurement of galactokinase activity after thermal induction of these lysogens permits us to quantitate the effects

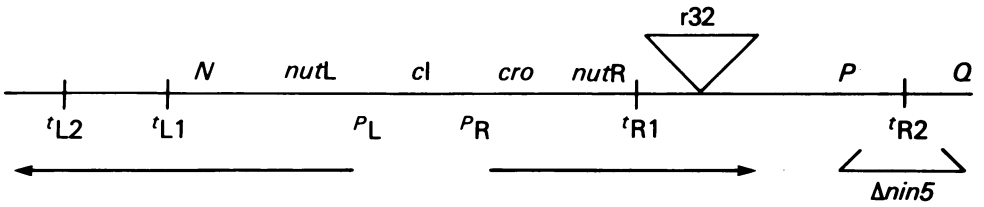

FIG. 1. Transcription termination sites in phage λ.

of the *nus* mutations on N activity.

As shown in Table 4, neither *nusA1* nor the two *rho* mutations tested, *rho026* and *rho4008*, reduce *galK* expression from p_L in fusion 1. This suggests that the activity of the λ p_L promoter is unaffected in the Nus$^-$ or *rho* mutant strains. In fusion 2, the *nusA1* mutation essentially abolishes galactokinase synthesis (94 units vs. 4 units), while partial inhibition by *rho026* and *rho4008* is seen (94 units vs. 37 and 46 units). We had previously demonstrated that *rho026* reduced, but did not eliminate, *galK* expression in a type 2 fusion strain (26). In fusion 3, however, galactokinase synthesis is fully inhibited by both *rho* mutations (61 units vs. 2 units). These data are consistent with the measurements of λ plating efficiency described above. Whereas *nusA1* blocks N action at 42°C in the type 2 fusion, the N-limiting phenotype of the *rho* mutants is clear only in the type 3 fusion, in which additional rho-dependent terminators are inserted between *galK* and p_L.

Basis of the Phenotype of *rho026*. There are several mechanisms by which *rho026* might inhibit N activity.

(*i*) Inhibition of N synthesis. Because the activity of p_L, the promoter that controls the synthesis of N, is not reduced by the *rho* mutation (Table 4), this possibility is unlikely, although defects in N translation have not been excluded.

(*ii*) Increased rate of N protein decay. Because the *rho026* mutation accelerates the degradation of some abnormal proteins—e.g., puromycyl peptides—we had previously suggested this possibility (26). To measure the effect of *rho026* on N decay, a λ*cI857N*$^+$ lysogen was transiently induced by heating the culture at 42°C for 10 min; the culture was then returned to 32°C to repress further synthesis of N protein. At various times thereafter, the cells were infected with λ$i^{434}N^-$ phage, and the burst size of the superinfecting phage was determined. By this complementation assay (Fig. 2), we confirmed the instability of N in wild-type *E. coli* (27). This experiment also indicates that, although the initial level of N activity is lower in the *rho026* strains, the functional half-life of N is identical in the mutant and *rho*$^+$ parent.

Table 2. Growth of λ on Nus strains

Genotype	Temp. of plating, °C	Efficiency of plating: λcI857	λcI857 *nin5*	λcI857 *r32*	λcI857 *nin5 r32*
nus$^+$ *rho*$^+$	32	1.0	1.0	1.0	1.0
	42	1.0	1.0	1.0	1.0
nusA1	32	1.0	1.0	10^{-3}	1.0
	42	10^{-4}	1.0	$<10^{-5}$	10^{-5}
rho026	32	1.0	1.0*	10^{-4}	$<10^{-4}$
	42	0.1†	<0.1†	$<10^{-5}$	$<10^{-5}$
rho4008	32	1.0	1.0	10^{-4}	0.1*
	42	1.0†	0.8†	10^{-4}	10^{-4}*

Efficiencies of plating are expressed relative to plating of phage on *nus*$^+$ *rho*$^+$ at 32°C. Strains used: *nus*$^+$ *rho*$^+$, SG13060; *nusA1*, C388; *rho026*, 15032; *rho4008*, SG13081.
* Small plaques.
† Minute, poorly defined plaques.

The bacterial mutation *lon* increases the stability of λ N protein (27). The stabilization of N by *lon* is seen in both the *rho*$^+$ and *rho026* backgrounds (Fig. 2). As was seen in the *lon*$^+$ parent, the initial N activity is lower in the *rho* mutant, but the rate of decay is equivalent to that in the *rho*$^+$ parent. The increased N levels found in the *lon* mutant strains result in partial compensation of the defect in N activity. Similar results are seen in *nusA1 lon* doubly mutant strains (unpublished results), indicating that the block in N activity in at least these two mutants may be partially suppressed by raising the intracellular level of N protein.

(*iii*) Block of N activity. The above data suggest that *rho026* and *rho4008*, like *nusA1*, block N activity rather than N synthesis or decay. We will refer to the effect of these *rho* mutations on λ development as the NusD$^-$ phenotype. Although both NusA$^-$ and NusD$^-$ reduce N activity, deletion of the λ t_{R2} terminator permits phage growth in the former but not in the latter strain. These data can be explained if different terminators become resistant to N action in NusA$^-$ and NusD$^-$ strains. The *rho026* mutation might interfere specifically with N activity at terminators that require rho for activity; rho-independent terminators might still be suppressed by N.

We tested this hypothesis by measuring the effect of *rho026* on N activity in *galK*–p_L fusions constructed *in vitro* in a pBR322-derived plasmid (Fig. 3). All fusions contained the N recognition site, *nut*L. Between *nut*L and *galK* the fusions carried either no terminator (plasmid A), the rho-dependent terminator, t_{L1} (plasmid B), or the rho-independent terminator, t_{L2} (plasmid C). The plasmids were introduced into λ*cI857N*$^+$-*ΔH1ΔBAM* or λ*cI857N*$^-$*ΔH1ΔBAM* lysogens bearing *rho*$^+$ or *rho026* alleles, and the galactokinase levels were determined after thermal induction (Table 5).

Results obtained with *rho*$^+$ strains show that plasmid A produces similar amounts of galactokinase in the N^- and N^+ lysogens (1,081 and 1,102 units, respectively). This indicates that plasmid A in fact does not bear any functional terminator between *galK* and p_L. Plasmids B and C, on the other hand, yield galactokinase only in the N^+ lysogens (983 and 942 units vs. 54

Table 3. Construction of fusions

Fusion 1: N-independent	*galK*		*Δ482*	*N*$^+$	p_L	*cI857*	*ΔH1*
Fusion 2: N-dependent	*galKTE*	*Δ8*	*ΔBAM*	*N*$^+$	p_L	*cI857*	*ΔH1*
Fusion 3: N-dependent	*galKTE*::Tn*10*	*Δ8*	*ΔBAM*	*N*$^+$	p_L	*cI857*	*ΔH1*

Fusion	Strains: *rho*$^+$	*nusA1*	*rho026*	*rho4008*
1	SG13593	SG13620	SG13619	SG13594
2	SG13508	SG13531	SG13574	SG13573
3	SG13621	—	SG13583	SG13582

A further description of these strains is found in Table 1.

Table 4. Expression of *galK* from λp_L in Nus$^-$ mutants

Mutation	Galactokinase units		
	Fusion 1	Fusion 2	Fusion 3
nus$^+$	59	94	61
nusA1	63	4	ND
rho026	53	37	2
rho4008	69	46	2

Strains were grown at 32°C in LB medium and shifted to 42°C for 60 min. The assay of galactokinase is as in ref. 31. Uninduced cultures had galactokinase values of 2 units or less. ND, not determined.

and 33 units, respectively), reflecting the presence of terminators in the *galK*–p_L fusion.

The expression of *galK* from plasmid A is not reduced when *rho026* is substituted for *rho*$^+$, consistent with our conclusion that the mutation does not affect p_L activity. The *rho026* mutation, however, reduces the capacity of N to suppress the rho-dependent terminator, t_{L1}. Galactokinase expression from plasmid B falls from 983 units to 315 units with the introduction of the *rho* mutation. In contrast, the rho-independent terminator in plasmid C, t_{L2}, is suppressed by N with equal efficiency (942 units vs. 923 units) in the *rho*$^+$ and *rho026* strains. Note that both t_{L1} and t_{L2} terminators are active in the *rho* mutant; without N no galactokinase expression from p_L is observed. These data indicate that *rho026* is Nus$^-$ because it specifically inhibits the ability of N to suppress rho-dependent terminators of transcription; N-mediated suppression of rho-independent terminators is not affected by the mutation. In this respect, *rho026* is unique, because both *nusA1* and *nusB5* can block N action at both terminator classes (34).

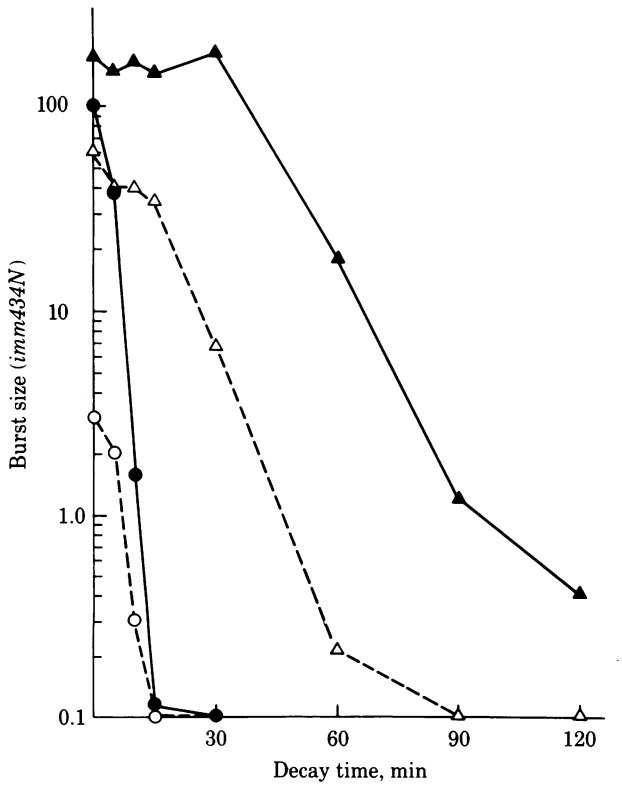

FIG. 2. Functional half-life on N in *rho* mutant strains. The experimental protocol is described in the text and by Gottesman *et al.* (27). ▲, SG13504 (*lon*▽100, *rho*$^+$); △, SG13505 (*lon*▽100, *rho026*); ●, N5340 (*lon*$^+$ *rho*$^+$); ○, SG13506 (*lon*$^+$ *rho026*). Burst size is expressed as phage produced per infected cell.

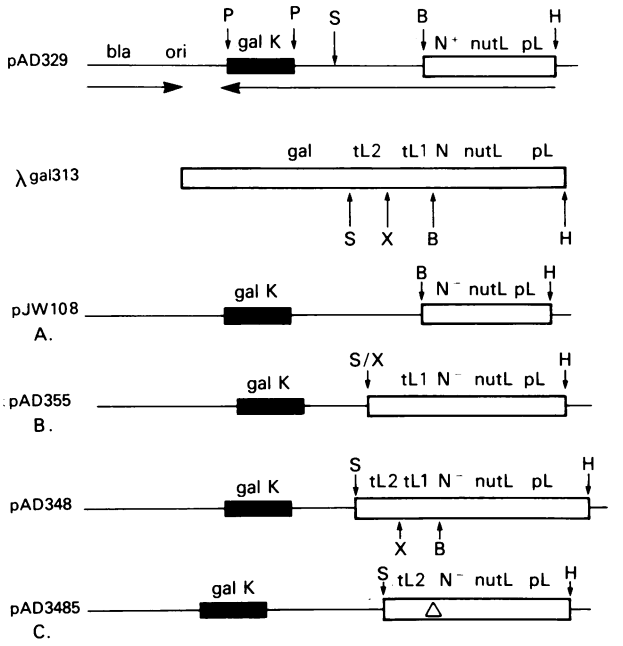

FIG. 3. Structure of plasmids expressing galactokinase from p_L. All plasmids were derived from pBR322 (34). Arrows indicate cleavage by the following restriction endonucleases: S, *Sal* I; P, *Pvu* II; B, *Bam*HI; X, *Xho* I; H, *Hin*dIII. Plasmids pJW108, pAD355, and pAD348 were formed by substitution of fragments from λ*gal313* into pAD329. pAD3485 is derived from pAD348. Labels A, B, and C refer to plasmids used in the experiment described in Table 5.

DISCUSSION

We have shown that a set of *E. coli rho* mutations interfere with the expression of the bacteriophage λ genome. The mutations alter the transcription termination factor rho so that the λ N protein can no longer suppress termination. This phenotype, called Nus$^-$, is displayed by a number of quite distinct *E. coli* mutants. The properties caused by the *rho026* and *rho4008* mutations, referred to as NusD$^-$, differ in several respects from the Nus$^-$ phenotype previously described.

(*i*) The ability of N to suppress transcription termination is less defective in NusD$^-$ strains than in NusA$^-$, NusB$^-$, NusC$^-$,

Table 5. NusD$^-$ is terminator specific

Plasmid	Galactokinase, units/min per ml culture			
	N^- lysogen		N^+ lysogen	
	rho$^+$	*rho026*	*rho*$^+$	*rho026*
None	1	2	99	30
A (t_{L0})	1,081	1,056	1,102	1,080
B (t_{L1}, rho-dependent)	54	65	983	315
C (t_{L2}, rho-independent)	33	37	942	923

Plasmid A is pJW108 (34); plasmid B is pAD355, and plasmid C is pAD3485. Their structures are shown in Fig. 2. Lysogens were λ*cI857ΔBamΔH1* derivatives: N^+ *rho*$^+$, N5503; N^+ *rho026*, AD5622; N^- *rho*$^+$, N4831; N^- *rho026*, AD5700. They are described in Table 1. The various plasmids were introduced into the four strains by transformation and selection for ampicillin resistance at 32°C. For galactokinase assays (28), cells were grown at 32°C in LB to OD_{600} = 0.3 and induced for 50 min at 40°C. Unit values represent nmol of D-[^{3}H]galactose converted to [^{3}H]galactose 1-phosphate by extracts of cultures of OD_{600} = 1.0 (approximately 5 × 10^8 cells). The galactokinase found in N5503 and AD5622 (N^+, no plasmid) is expressed from the chromosomal *galK*–p_L fusion in this strain. Chromosomal *galK* adds, presumably, about 10% to the galactokinase levels seen in plasmid-containing strains.

or NusE$^-$ mutants. Whereas λ fails to propagate on the latter at 42°C, it will form small plaques on *rho026* or *rho4008*. Complete inhibition of phage growth is seen only under conditions of increased stringency—e.g., when an additional terminator (*r32*) is introduced into the λ p_R operon. In some N-requiring *galK*–p_L fusions, in which the *nusA1* mutation completely blocks galactokinase synthesis, *rho026* reduces expression by 70% (Tables 4 and 5). The presence of a Tn*10* terminator(s) in the fusion eliminates the residual *galK* expression.

(*ii*) In NusA$^-$ and NusB$^-$ mutants, N fails to suppress transcription termination at either rho-dependent or rho-independent terminators (34). In contrast, NusD$^-$ mutants affect N activity only at specific terminators; suppression of three rho-dependent terminators (t_{L1}, *Tn*10, and unmapped terminators present in the SG13508 fusion) is inhibited, whereas suppression of the rho-independent terminator t_{L2} is not.

(*iii*) The λ variant λ*nin5*, from which t_{R2} has been deleted, grows normally on the previously isolated Nus$^-$ strains. The *nin5* deletion only partially improves the growth of λ or λ*r32* in NusD$^-$ mutants (Table 2). This suggests that other *nus*$^-$ mutations prevent λ growth by causing termination to occur at t_{R2}. In NusD$^-$ strains, the inability of N to suppress the rho-dependent terminator t_{R1} may be the principal cause of growth inhibition.

The pleiotropic properties of the NusD$^-$ mutants—i.e., the inability to support the growth of phage T4 or λ and the rapid degradation of abnormal bacterial proteins—are all due to mutation in *rho*. Revertants selected as NusD$^+$ simultaneously restore the stability of abnormal proteins and the capacity to propagate T4. The responsible mutations are genetically linked to *rho* (data not shown). Furthermore, *rho026* is partially complemented by a λ *rho*$^+$ transducing phage for both T4 growth and N activity (unpublished data). Similar results for T4 growth have been found for these mutations by Stitt *et al.* (25) and for the analogous *tabC* mutations by Pulitzer *et al.* (36). These authors suggest that the HDF and TabC phenotype results from abnormal transcription termination, an idea entirely consistent with our findings on phage λ development in these mutants.

Although *rho026* and *tabC* are allelic with *rho*, they differ markedly from *rho15*. The *rho15* mutation is a strong suppressor of bacterial polarity and permits the growth of λ*N*$^-$ phage (22, 37).

N activity has been proposed to involve an interaction, at the *nut* sites, between RNA polymerase and N, NusA, and perhaps other Nus factors, to form a termination-resistant "transcription complex." Our observation that N still suppresses rho-independent terminators in NusD$^-$ strains suggests that the formation of such complexes is unimpaired in these mutants. We imagine, therefore, that the mutant rho protein, unlike wild-type rho, can dissociate the complex at rho-dependent terminators, causing transcription to terminate. It remains to be determined which component of the transcription complex is the target of rho action.

We gratefully acknowledge Sankar Adhya, Don Court, and Douglas Ward for their comments on the manuscript and Annette Kuo for preparation of the manuscript. This work was supported in part by grants from the National Institutes of Health and the American Heart Association to A.D., who is also an Established Investigator of the American Heart Association.

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