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Effect of *Escherichia coli nusG* Function on λ N-Mediated Transcription Antitermination

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The *Escherichia coli* Nus factors act in conjunction with the bacteriophage λ N protein to suppress transcription termination on the λ chromosome. NusA binds both N and RNA polymerase and may also interact with other Nus factors. To search for additional components of the N antitermination system, we isolated host revertants that restored N activity in *nusA1* mutants. One revertant, *nusG4*, was mapped to the *rif* region of the *E. coli* chromosome and shown to represent a point mutation near the 3′ end of the *nusG* gene. The *nusG4* mutation also suppressed *nusE71* but not *nusA*$_{Sal}$, *nusB5*, *nusC60* (*rpoB60*), or *nusD026* (*rho026*). However, *nusG*$^+$ expressed from a multicopy plasmid suppressed *nusD026* and related *rho* mutants for both λ and phage T4 growth. These results suggest that NusG may act as a component of the N antitermination complex. In addition, the data imply a role for NusG in Rho-dependent termination.

Modulation of transcription termination is a mechanism of gene regulation in both prokaryotes and eukaryotes (26, 37). The efficiency of termination is determined by nucleic acid sequences and by protein factors that interact with these sequences, with RNA polymerase, or with each other.

The bacteriophage λ *N* antitermination system is the best-characterized example of such regulation (11). Transcription complexes initiating at the λ p_R and p_L early promoters are modified by N protein at *cis*-acting *nut* sequences (30, 31). The modified complexes pass through distal Rho-dependent and Rho-independent terminators.

A set of host factors required for N activity have been defined by mutations (*nus* [N utilization substance]) that inhibit λ growth (10). These include NusA, NusB, and NusE (the small ribosomal subunit S10) (11). Certain mutations in Rho (*nusD*) block N antitermination at Rho-dependent sites (8, 29). The NusA, NusB, and NusE proteins are thought to be components, along with N protein, of the N-modified transcription complex (4, 18).

The activities of the Rho (28) and NusA (6) factors have been extensively studied. NusA is a 69-kDa protein that either stimulates or suppresses termination, depending on the template sequence and the availability of protein cofactors (15, 16, 20, 25, 35, 42). NusA binds to the RNA polymerase core (13) and may generally be associated with elongating polymerase (13, 18). Binding of NusA to N (14), and possibly to Rho (34), has also been demonstrated in vitro.

Mutations in *nusB* suggest a role for this factor in Rho-dependent termination (42) and in the processive transcription of the rRNA operons (36). The role of S10 in termination or antitermination is unclear.

We have isolated host mutations that permitted λ growth on *nusA1* mutants (41). Several suppressors were mapped to *nusB*. With the hope of detecting additional factors that participate in transcription termination and antitermination, we have characterized a *nusA1* suppressor located in the 90-min region of the *Escherichia coli* chromosome. The suppressor is a missense mutation in *nusG* and represents the first mutation identified in that gene.

MATERIALS AND METHODS

Bacterial strains. All bacteria are derivatives of *E. coli* K-12. The genotypes of the basic strains are given in Table 1. Derivatives of these strains were constructed as described below. Standard bacteriological techniques, e.g., P1 transductions, lysogenizations, and use of MacConkey galactose indicator plates, were performed as previously described (22). *nusG* alleles were introduced into strains by cotransduction with one of three closely linked markers, namely, Tet^r, Rif^r, or Kan^r, with selection on Luria-Bertani (LB) plates containing the appropriate antibiotic at 15, 20, or 50 μg/ml, respectively.

The *lacZ* fusion strains, listed in Table 2, were constructed by lysogenization of N7113 with B464, a λ*i*^{21} phage carrying the *trp-lac* fusion W205 (24). The resulting N-dependent transcriptional fusion between *lac*W205 and the p_L promoter of the resident prophage is diagrammed in Fig. 1. To construct isogenic *nus* strains, *nusA1*, *nusA*$_{Sal}$, or *nusE71* alleles linked to Tn*10* were P1 transduced into 7113(B464). Subsequently, *nusG4* linked to Kan^r was transduced into the indicated strains.

Phage strains. Phages used are listed in Table 3. λ*c*I857 *nusG*$^+$ is a *nin*$^+$ *S*$^+$ derivative of the previously described YU109 (9). To construct a *nusG4* transducing phage, λ*c*I857 *nusG*$^+$ was grown lytically on a strain carrying *nusG4* on the chromosome (N7032). Phages which had acquired the *nusG4* allele by in vivo recombination were selected by plating the lysate on K556 (*nusE71*) at 42°C. Three independent isolates behaved identically and displayed the appropriate *nusG4* suppression pattern (see text). Efficiency of plating on K556 following lytic growth on the *nusG4* strain was 10^{-4}, 50-fold higher than the efficiency of plating following growth on N6871, an isogenic *nusG*$^+$ strain.

Plasmids. Plasmids pSS105 (9), pTUB2 (23), pGA43 (2), and pGA101 (3) were described previously.

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TABLE 1. Bacterial strains

Strain	Genotype	Source
FW329	*bgl rbs ilv met thi nal lacZ319*:IS*1*	B. Stitt
GAG98	N99 *rho026*	NIH[a] collection
K95	N99 *nusA1*	D. Friedman
K450	N99 *nusB5*	D. Friedman
K554	N99 *nusC60* Rif^r	D. Friedman
K556	N99 *nusE71*	D. Friedman
K1102	N99 *nusA* (*Salmonella typhimurium*) (*nusA*$_{Sal}$)	D. Friedman
K1479	N99 *nusA1 nusE71 gal*$^+$	D. Friedman
K3959	N99 *nusA*$_{Sal}$:Tn*10*	D. Friedman
N99	*galK2* Str^r	NIH collection
N5261	SA500 *ilv galE490** (*chlD-blu*)[8] (λ ΔBAM *N*$^+$ *cI14* ΔH1)	NIH collection
N5283	SA500 *ilv galE490** (*chlD-blu*)[8] (λ ΔBAM *N*^{7,53} *cI14* ΔH1)	NIH collection
N6871	N5261 *nusA1*:Tn*10*	NIH collection
N7004	N99 *nusA1*:Tn*10*	NIH collection
N7006	N99 *nusE71*:Tn*10*	NIH collection
N7032	N5261 *nusA1 nusG4*:Tn*10*	This work
N7113	SA500 *ilv galE490*lacZXA21* (λcI857 *bio252* ΔH1)	NIH collection
SA500	*his rpsL*	NIH collection

[a] NIH, National Institutes of Health.

Plasmid pSS106 was constructed by a multistep procedure. First, the *nusG4* allele was transferred to pTUB2 by in vivo recombination to yield plasmid pSS101. To accomplish this transfer, pTUB2 was transformed into N7032. Since *nusG*$^+$ in multicopy complements the *nusG4* phenotype, N7032(pTUB2) is Gal$^-$, i.e., white on MacConkey galactose indicator plates at 42°C. When the *nusG4* allele replaces the plasmid *nusG*$^+$ allele, the strain becomes Gal$^+$. Gal$^+$ papillae were purified, and the plasmid was isolated. When transformed into N6871, the plasmid suppressed the Nus$^-$ phenotype conferred by *nusA1* at 42°C, and the resident N-dependent *gal* fusion was Gal$^+$.

To complete the construction of pSS106, the 2.1-kb *Sma*I-*Eco*RI fragment of pSS101 was ligated to an *Eco*RI linker at the *Sma*I end and inserted into the *Eco*RI site of pBR322. Transformants were selected on LB-ampicillin (50 μg/ml) plates.

Galactokinase assays. The expression of *galK* was monitored by galactokinase assays performed as previously described (1).

β-Galactosidase assays. Cultures were grown to early logarithmic phase (approximately A_{650} = 0.2) at 32°C in LB media. An aliquot was removed for assay to establish the background level, and the remaining culture was shifted to 42°C. After 60 min, both the 32 and the 42°C samples were

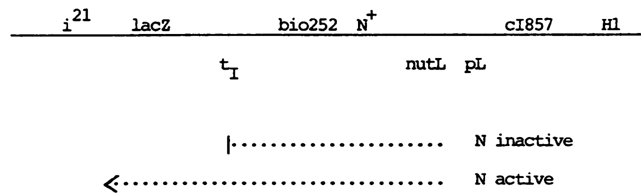

FIG. 1. Diagram of the N-dependent *lacZ* fusion carried by 7113 (B464).

assayed for β-galactosidase activity as previously described (22). The β-galactosidase levels presented in Table 2 are the averaged results of three experiments. Backgrounds ranging between 40 and 60 U have been subtracted.

Sequencing and cloning. DNA sequencing was done by the dideoxynucleotide chain termination method (32). Appropriate DNA fragments were cloned into M13mp18 and M13mp19. M13-specific primers were used to sequence clones by using Sequenase version 2.0 and following the protocol provided by the United States Biochemical Co.

RESULTS

Isolation and mapping of *nusG4*. We selected extragenic suppressors of *nusA1* using strain N6871, which carries a λ p_L-*nutL-N*-IS*2*-*gal* chromosomal fusion (41). Expression of *gal* in N6871 requires functional λ N protein to override the Rho-dependent terminator(s) in IS*2*. The *nusA1* mutation present in N6871 inhibits N antitermination at elevated temperatures and blocks *gal* expression. Suppressors of *nusA1* were selected as Gal$^+$ derivatives of N6871 at 42°C. Mutations that permitted the growth of superinfecting λ and therefore affected transacting functions were further investigated.

One such suppressor of *nusA1*, *nusG4*, was mapped by P1 transduction to the *E. coli nusG* gene (9). A rifampin-resistant wild-type strain was used as a donor to transduce Rif^r into the *nusG4* mutant. Analysis of the transductants for the *nusA1* phenotype indicated that *nusG4* and Rif^r (*rpoB*) were 90% linked; 313 of 347 Rif^r transductants were Gal$^-$. We next demonstrated that *nusG4* constituted a single locus by cotransducing *nusG4* and Rif^r into the parental *nusA1* strain (N6871). Galactokinase enzyme assays indicated that *galK* gene expression in the backcrossed strain was identical to that in the original *nusA1 nusG4* mutant, N7032 (data not shown).

The *nusG4* mutation suppresses *nusA1* rather than bypassing the need for N activity. A *nusG4 nusA1* strain carrying a p_L-*nutL-N*$^-$-IS*2*-*gal* chromosomal fusion (N5283) was Gal$^-$.

Fine-structure mapping was performed by transforming

TABLE 2. Specificity of *nusG4* suppression[a]

Host	β-Galactosidase produced (U)	
	nusG$^+$	*nusG4*
nus$^+$	7,000	5,459
nusA1	12	216
nusA$_{Sal}$	<5	<5
nusE71	17	209

[a] All strains are derivatives of 7113(B464), which carries the N-dependent *lacZ* fusion (Fig. 1). Numbers represent β-galactosidase units produced at 42°C (see Materials and Methods).

TABLE 3. Bacteriophages

Phage	Source
λcI857	NIH[a] collection
λcI857 *nin5*	NIH collection
λcI857 *int6 r32*	NIH collection
λcI857 *nusG*$^+$	This work
λcI857 *nusG4*	This work
λ*i*^{21} *lac*W205 (B464)	NIH collection
T4D	B. Stitt
T4goF1	B. Stitt

[a] NIH, National Institutes of Health.

N7032 (*nusA1 nusG4*) with a series of plasmids carrying markers spanning the *rpoB* region (Fig. 2). Complementation of *nusG4* was scored on MacConkey galactose plates at 42°C. N7032 carrying pTUB2 was converted to Gal^-, whereas strains bearing plasmids pGA43 and pGA101 remained Gal^+. An operon composed of two genes, *secE* and *nusG*, is present uniquely on pTUB2. The *secE* gene encodes a 127-amino-acid transmembrane protein involved in protein secretion (9, 33). The product of the *nusG* gene is a 181-amino-acid protein (9).

To verify that the *secE-nusG* operon was sufficient to complement strain N7032, the operon was subcloned into pBR322 on a 2.1-kb fragment. The resultant plasmid, pSS105, fully complemented N7032.

Finally, the *nusG4* mutation was crossed into pTUB2 by in vivo recombination, yielding pSS101, and was subsequently cloned on a 2.1-kb fragment into pBR322, yielding pSS106 (see Materials and Methods). Plasmid pSS106 suppressed the *nusA1* phenotype, i.e., restored *gal* expression in strain N6871. Thus, both *nusG4* and the wild-type allele are dominant when expressed from a multicopy plasmid.

Sequence of the *nusG4* mutation. The sequence of the *secE-nusG4* mutant operon genes carried by pSS106 was compared with that of the wild type (9). A single C-to-A transition at position 487 near the 3′ end of *nusG* was detected. The resultant serine-to-phenylalanine change at residue 163 has the potential to alter significantly the structure of NusG (see Discussion).

Suppression of other *nus* mutations by *nusG4*. The specificity of suppression by *nusG4* was determined with strain N7113. N7113 carries a chromosomal λ p_L-*lacZ* fusion, which is negatively controlled by the λcI857 repressor. The fusion carries *N*, a Rho-independent terminator (tI), as well as Rho-dependent terminators. Expression of *lacZ* in N7113 is entirely N dependent.

We constructed a series of isogenic fusion strains carrying $nusG^+$ or *nusG4* in conjunction with nus^+, *nusA1*, *nusE71*, or $nusA_{Sal}$. All three *nus* mutations blocked the expression of *lacZ* (Table 2). As expected, *nusG4* suppressed the *nusA1* mutation, although the effect was incomplete (Table 2). In contrast to *nusA1*, the $nusA_{Sal}$ allele was not suppressed by *nusG4*, indicating that *nusG4* does not bypass *nusA* for N function. The *nusE71* (S10) mutation was suppressed by *nusG4* to about the same extent as *nusA1* (Table 2). Although *nusG4* did not fully suppress either *nusA1* or *nusE71*, λ plated with unit efficiency on both *nusA1 nusG4* and *nusE71 nusG4* mutants (data not shown).

In a second assay, we determined the efficiency of plating of λ$nusG^+$ or λ*nusG4* transducing phage on an isogenic set of wild-type and *nus* mutant hosts (Table 4). The *nusG4* allele carried by the infecting phage suppressed *nusA1* and permitted phage growth. Consistent with the results of the p_L-*lacZ* fusion assays, λ*nusG4* formed plaques on *nusE71* hosts but not on strains carrying the $nusA_{Sal}$ allele. The pattern of λ*nusG4* growth (Table 4) also shows that *nusG4* does not suppress *nusC60*, *nusB5*, or *rho026* mutations. The failure of λ*nusG4* to grow on the double-mutant *nusA1 nusE71* strain probably reflects the additive effects of the two host mutations. Recall that *nusG4* only partially suppressed the *nusA1* and *nusE71* mutations individually.

NusG overexpression suppresses certain *rho* mutations. During the course of our complementation studies, we noted that pSS105, a multicopy plasmid that expresses $nusG^+$, suppressed a *rho026* mutant for λ growth. Other *nus* mutants, *nusA1*, *nusB5*, *nusC60*, and *nusE71*, were not suppressed (data not shown). To determine whether the suppression of *rho026* was allele specific, isogenic *rho* mutants were transformed with pSS105 and screened for the plating of λ and phage T4 (Table 5). The λ*r32* variant used in this assay carries an IS*2* insert promoter-proximal to gene *O*. Unlike in wild-type λ, the expression of genes *O* and *P* and the replication of phage DNA are N dependent in λ*r32*. The variant phage is thus exquisitely sensitive to reduced N activity and fails to propagate on *nus* mutants even at 32°C. Our results (Table 5) demonstrate that of the three *rho* mutations that block λ*r32* plating, two (*rho026* and *rho4008*) were suppressed when $nusG^+$ was expressed from a multicopy plasmid. A multicopy plasmid that expressed *secE* but not *nusG* did not restore phage plating on *rho* mutants (data not shown).

The *rho* mutations tested in Table 5 were originally isolated as conferring resistance to phage T4 (27, 39).

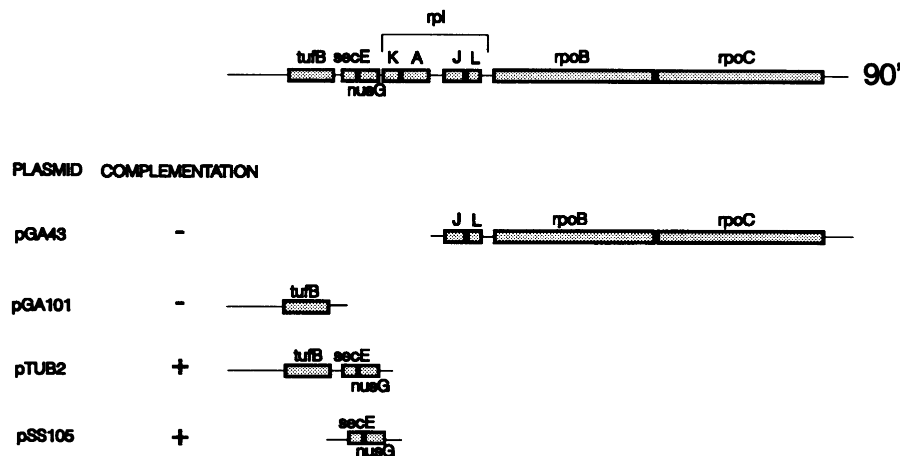

FIG. 2. Structures of the *rif* region of the *E. coli* chromosome and the plasmids used for complementation analysis for mapping of *nusG4*. Only the intact genes carried by each plasmid are shown. The left side of the figure indicates the results of the complementation test performed by transforming N7032 with each of the different plasmids and scoring the Gal phenotype at 42°C. The N-dependent fusion in N7032 is Gal^+ because of the suppression of *nusA1* by *nusG4*. Since $nusG^+$ in multicopy overrides the *nusG4* phenotype, N7032 transformed with a complementing plasmid is Gal^-.

TABLE 4. Plating characteristics of *nusG* transducing phage[a]

Phage	Efficiency of plating on the indicated host:							
	nus^+	*nusA1*	*nusE71*	*nusB5*	*nusC60*	*rho026*	$nusA_{Sal}$	*nusA1 nusE71*
λ	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
λ*nin5*	1	1	1	1	1	<0.01	<0.4[b]	0.6[b]
λ$nusG^+$	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
λ*nusG4*	1	0.8	0.7	<0.01	<0.01	<0.01	<0.01	<0.01

[a] After overnight incubation at 32°C (*nusC60*, $nusA_{Sal}$, and *nusA1 nusE71*) or 42°C (nus^+, *nusA1*, *nusE71*, *nusB5*, and *rho026*), efficiencies of plating were determined relative to that of wild-type control. All phages carry the *c*I857 allele. All strains are derivatives of N99 and listed in Table 1.
[b] Very small to minute plaque size.

Expression of $nusG^+$ from plasmid pSS105 fully restored the growth of T4 on all the *rho* mutants (Table 5).

DISCUSSION

To define the interacting host components in the λ N transcription antitermination system, we isolated suppressors that restored N function in *E. coli nusA1* mutants. One suppressor was mapped to the *nusG* gene. The *nusG* gene is linked to *rif* and lies in a region of the *E. coli* chromosome that includes a cluster of genes encoding the large ribosomal subunit proteins L7/12, L10, L1, and L11, as well as EFtu. Although we have not excluded the possibility that NusG, like NusE (S10), plays a role both in transcription and in translation, our recent experiments indicate that the primary effect of NusG depletion is on transcription termination (40). We also note that *nusG* is cotranscribed with *secE* (9), which encodes an integral membrane protein involved in protein secretion (33). The biological relevance of this association remains to be established.

nusG encodes a 181-amino-acid protein that is essential for cell viability (9). The *nusG4* mutation represents a serine-to-phenylalanine amino acid substitution at position 163. Computer-based protein modelling (7) predicts that this mutation alters the secondary structure of amino acids 160 to 167 from a random coil to a β-sheet conformation. Since this region of NusG connects two well-predicted α-helices, this more ordered structure is likely to alter the alignment of these helices and to cause a shift in the position of the carboxyl end of the polypeptide. The magnitude of this shift would depend upon the constraints imposed by the three-dimensional structure of the whole protein.

The suppression pattern of *nusG4* indicates that the mutation is not a bypass of the different Nus proteins or of N function. Unlike *nusA1*, the $nusA_{Sal}$ allele still restricted λ growth in *nusG4* mutants. The temperature sensitivity of *nusAts11* was also not suppressed by *nusG4* (data not shown). Among other *nus* mutations, we found that *nusG4* suppressed *nusE71* but not *nusC60* (*rpoB60*), *nusB5*, or *nusD026* (*rho026*). To explain the suppression pattern of *nusG4*, we favor a holistic model in which the components of the antitermination complex make multiple contacts with each other. These protein-protein and protein-RNA interactions contribute to the overall stability of the complex. We suggest that NusG4 forms more stable contacts with a complex component(s), compensating for the weakened interaction of NusA1 or NusE71. A similar model has been proposed to explain the cosuppression of *nusA1* and *nusE71* by mutations in *N* and *nusB* (11).

The overproduction of NusG from a multicopy plasmid suppressed members of the *nusD* class of *rho* mutations; other *nus* mutations remained restrictive for λ growth. This result suggests that high levels of NusG affect antitermination by a mechanism different from that of NusG4. Recall that *nusD026* was not suppressed by *nusG4*. Furthermore, *nusG4* restored N-mediated antitermination of Rho-independent (λtI) as well as Rho-dependent (IS*2*) terminators in *nusA1* mutants. We propose that NusG interacts directly with Rho, altering its activity. This proposal, based on genetic evidence, is directly supported by the in vitro observation that Rho is retained on NusG affinity columns (12).

Overproduction of NusG allows phage T4 to propagate on all *nusD* mutants tested. T4 is reported to regulate the expression of its genome by a mechanism of termination-antitermination that utilizes sequences homologous to the λ *nut* sites (5, 17, 19, 38). Our results are consistent with the idea that the host components of the T4 antitermination system are similar or identical to those of λ. However, NusG may directly affect Rho activity in a reaction that does not involve an antitermination complex.

The demonstration that *nusG4* suppressed *nusA1* and *nusE71* for N antitermination does not prove that NusG is required for this reaction in wild-type cells. Greenblatt and his collaborators isolated NusG from *E. coli* extracts as a factor that enhanced N activity in a purified in vitro system (12). In addition, he has detected NusG in the N antitermination complex along with RNA polymerase, NusA, NusB, and NusE (21). Although his results suggest an essential role

TABLE 5. Multicopy *nusG* suppression of *rho* mutations[a]

rho allele	Presence or absence of pSS105	Plating efficiency[b] of:	
		λ*r32*	T4
+	−	1	1
	+	1	1
026	−	<0.01	0.5[c]
	+	1	1
125	−	<0.01	<0.01
	+	<0.01	1
4008	−	<0.01	<0.01
	+	1	1
4406	−	1	<0.01
	+	1	1

[a] All strains are derivatives of FW329 P1 transduced with the indicated *rho* alleles by linkage to ilv^+ and then transformed with the $nusG^+$ plasmid, pSS105, or the parental control, pBR322.
[b] Values refer to efficiencies of plating at 32°C for λ*c*I857*r32* or at 42°C for phage T4.
[c] Minute, poorly defined plaques.

for NusG in the N reaction, we find that the efficiency of N antitermination is identical in wild-type and NusG-depleted cells (40). To explain these findings, we propose that NusG enhances the stability of the antitermination complex. Although not required in wild-type complexes formed in vivo, NusG may be essential for the formation of stable complexes under in vitro conditions.

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