

## Requirement for NusG for Transcription Antitermination In Vivo by the $\lambda$ N Protein

Ying Zhou,<sup>1</sup> Joshua J. Filter,<sup>1</sup> Donald L. Court,<sup>2</sup> Max E. Gottesman,<sup>3</sup> and David I. Friedman<sup>1\*</sup>

*Department of Microbiology and Immunology, The University of Michigan, Medical School, Ann Arbor, Michigan 48109-0620<sup>1</sup>; Gene Regulation and Chromosome Biology Laboratory, Division of Basic Sciences, National Cancer Institute at Frederick, Frederick, Maryland 21702<sup>2</sup>; and Institute of Cancer Research, College of Physicians and Surgeons, Columbia University, New York, New York 10032<sup>3</sup>*

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**Transcription antitermination by the bacteriophage  $\lambda$  N protein is stimulated in vitro by the *Escherichia coli* NusG protein. Earlier work suggested that NusG was not required for N activity in vivo. Here we present evidence that NusG also stimulates N-mediated transcription antitermination in intact cells.**

NusG, a 21-kDa essential protein in *Escherichia coli* (8, 20), is an abundant transcription elongation factor that modulates Rho-dependent transcription termination (22, 24, 25). Rho-dependent termination is inhibited by depletion or overexpression of NusG (2, 24). Affinity chromatography experiments showed that NusG associates with Rho or core RNA polymerase (19). It was proposed that NusG facilitates termination by aiding in the recruitment of Rho to the transcription complex (1, 18, 21, 22, 24).

In vitro studies identified NusG as an *E. coli* protein that influences transcription antitermination mediated by the N protein of phage  $\lambda$  (18, 20). Transcription of the  $\lambda$  delayed early genes requires RNA polymerase to read through a number of transcription terminators. N, in complex with the *E. coli* Nus factors, modifies RNA polymerase at specific RNA sites, NUT, to a form that overrides downstream transcription terminators (Fig. 1) (7, 11, 12, 16, 23, 27).

Nus factors were first identified by mutations in *E. coli* that influence N action. Mutations in *nusA* (*nusA1*) (9), *nusB* (a number of mutations) (10, 17), and *rpsJ* (*nusE71*) (14) reduce N activity, particularly at higher temperatures. In contrast, the *nusB101* mutation suppresses this effect of the *nusA1* and *nusE71* alleles on N action (26).

The only genetic evidence for a role of NusG in N antitermination was the isolation of *nusG4*, which has a suppressor phenotype identical to that of the *nusB101* allele (25). However, selection for *nus* mutations that reduce N action did not yield *nusG* mutants. Furthermore, in contrast to its function in vitro, in vivo studies provided evidence that NusG was not required for effective N-mediated transcription antitermination. The in vivo studies employed a strain bearing a *lacZ* reporter fused downstream of the  $\lambda$   $p_L$  promoter, *nutL*, and transcription terminators. Because of the terminators, expression of the *lacZ* reporter is N dependent. NusG was expressed from a plasmid with a temperature-sensitive replicon. NusG depletion was accomplished by shifting the strain from 32 to 42°C. N was expressed from a plasmid under the control of the

*lac* promoter. The experimental design was to supply N after depleting the bacteria of NusG (see below for the method of depletion) by treating the bacteria with IPTG (isopropyl- $\beta$ -D-thiogalactopyranoside). Depleting this strain of NusG did not reduce *lacZ* expression, suggesting that N-mediated antitermination did not require NusG (24). However, more recent experiments show that this result can be explained on the basis of leaky expression of N prior to the depletion of NusG (data not shown). We were concerned because the plasmid supplying N was present during the entire time of growth at 42°C. Since the  $p_L$ -*nutL*- $t_{L1}$ -*lacZ* fusion used to measure N-mediated transcription antitermination is controlled by the temperature-sensitive  $\lambda$  *cI857* repressor, transcription from  $p_L$  would be active during NusG depletion. Thus, a leaky level of N expression during depletion supports read-through of the terminator and permits expression of *lacZ*. We avoided this problem by devising a protocol in which N was supplied only after NusG depletion had been completed.

The method of Sullivan and Gottesman was used to produce bacteria depleted of NusG (24). Briefly, a conditionally lethal *E. coli* strain, K9683, was constructed by disrupting the chromosomal *nusG* allele with an insertion of a gene conferring resistance to kanamycin, *kanR*. The *nusG* defect is complemented with an expressible wild-type *nusG* gene on pSS119, a plasmid that has a thermosensitive defect in replication. As shown previously, the NusG protein in the *E. coli* cells can be depleted by growing the cells at 42°C in Luria-Bertani broth (15) for 6 h. The cultures were diluted every hour into fresh prewarmed Luria-Bertani broth to maintain logarithmic growth. Because the plasmid fails to replicate under these conditions, cell division segregates cells that lack the *nusG* plasmid and eventually the NusG protein. The control strain, K9435, carries the disrupted chromosomal *nusG* allele, but the *nusG* plasmid, pSS120, is replication proficient at high temperature. Depletion was confirmed by Western blotting. As shown in Fig. 2, K9683 and K9435 grown at 32°C contained similar quantities of NusG protein. However, when the bacteria were grown at 42°C, NusG was undetectable in strain K9683, which carries the temperature-sensitive *nusG* plasmid, whereas no decrease in NusG concentration was observed in the control strain, K9435.

We assessed the role of NusG in N antitermination by in-

\* Corresponding author. Mailing address: Department of Microbiology and Immunology, University of Michigan, 5641 Medical Science Bldg. II, Ann Arbor, MI 48109-0620. Phone: (734) 769-0181. Fax: (734) 764-3562. E-mail: davidfri@umich.edu.

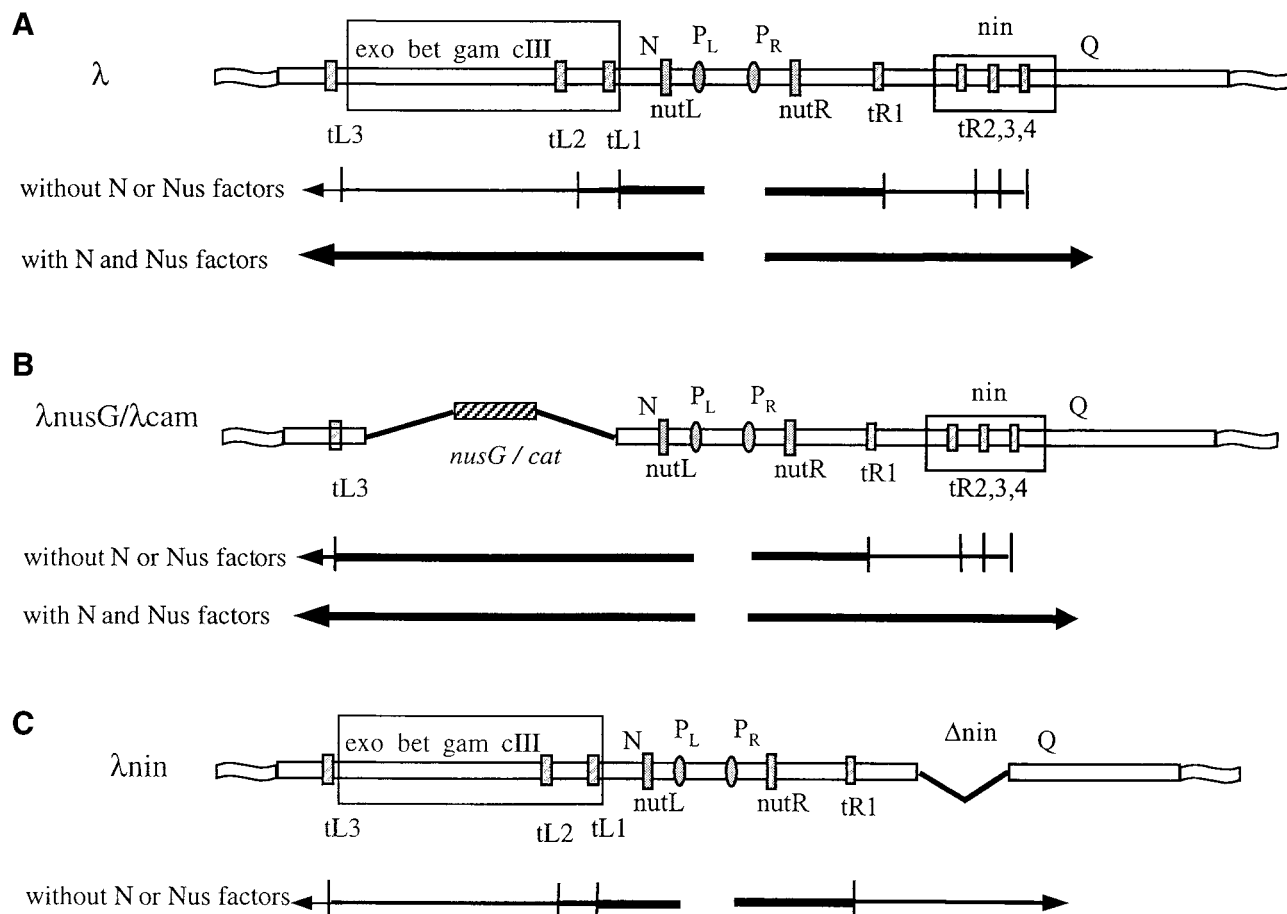


FIG. 1. Map of relevant region of the  $\lambda$  genome showing location of promoters (P), terminators (t), *nut* sites, and representative genes. Also shown are the regions of the *nin* deletion and the gene replacements. The  $\lambda$  recombination system (28) was used to replace  $\lambda$  sequences from nucleotides 31,351 to 34,700 in the standard  $\lambda$  sequence (6) with either the *nusG* or *cat* gene. (A)  $\lambda$  wild-type; (B)  $\lambda$ *nusG* and  $\lambda$ *cam*; (C)  $\lambda$ *nin*. Below each genetic map are the patterns of transcription in both the presence and absence of N or Nus factors (A and B) or only in the absence of N or Nus factors (C).

fecting K9435 and K9863 with four  $\lambda$  variants.  $\lambda$ ,  $\lambda$ *nusG*, and  $\lambda$ *cam* all require N; they fail to grow in *E. coli* carrying the *nusA1*, *nusB5*, or *nusE71* mutation at the nonpermissive temperature (reference 14 and data not shown).  $\lambda$ *nin* has a deletion of a critical set of transcription terminators (Fig. 1) and thus can grow independently of N (3, 4). As expected,  $\lambda$ *nin* grows in the three *nus* mutant strains (reference 14 and data not shown).  $\lambda$ *nusG* is identical to  $\lambda$ *cam* but carries a *nusG* gene instead of *cat*.  $\lambda$ *nusG* is expected to grow in an *E. coli* strain depleted of NusG even if NusG is required for N action.  $\lambda$ *cam* serves as a control for  $\lambda$ *nusG* (see the legend to Fig. 1). Phage growth was determined by measuring burst size (phage produced per infected bacterium) 90 min after infection (13). The results of these experiments are shown in Table 1. The burst sizes of  $\lambda$  and  $\lambda$ *nin* at 42°C are the same in the control strain, K9435, where NusG is present. However, the burst size of  $\lambda$  is ~30-fold lower than that of  $\lambda$ *nin* in strain K9683, which is depleted of NusG at 42°C. Note that the burst size of  $\lambda$ *nin* is reduced in K9683 relative to that produced in K9435, likely reflecting the unfavorable environment provided by a bacterium growing in the near absence of NusG.  $\lambda$ *nin*, then, pro-

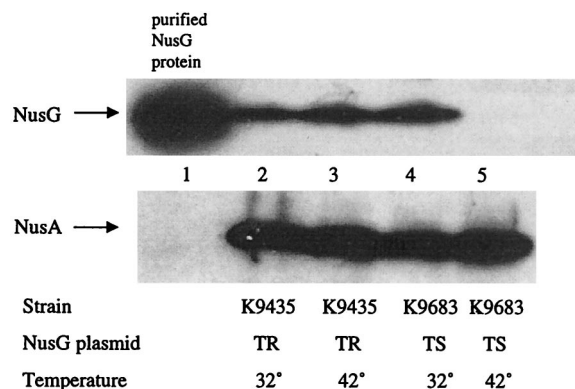


FIG. 2. Western blot to assess levels of NusG. Samples from logarithmically grown bacteria were isolated, prepared, and analyzed as described previously (5). The upper panels were blotted with anti-NusG, and the lower panels were blotted with anti-NusA. Lane 1 was loaded with purified NusG (24). Other lanes were loaded with extracts prepared from the indicated strains. TR, the plasmid in the strain replicates at high temperature; TS, the plasmid in the strain fails to replicate at high temperature. The last line shows the temperature at which the bacterial cultures were grown prior to preparation of extracts.

TABLE 1. Growth of  $\lambda$  derivatives in the absence of NusG

Phage <sup>c</sup>	Burst size <sup>a</sup> at 42°C	
	NusG present (K9435)	NusG absent <sup>b</sup> (K9683)
$\lambda$	22 ± 7	0.1 ± 0.1
$\lambda nin$	22 ± 9	3 ± 2
$\lambda nusG$	72 ± 17	6 ± 4
$\lambda cam$	68 ± 35	0.2 ± 0.1

<sup>a</sup> Burst size is calculated as the number of phage produced per infected bacterium.

<sup>b</sup> NusG was depleted as described in the text.

<sup>c</sup> All of these phages have the *cI857* allele, which encodes a temperature-sensitive repressor. Since the phenotype imposed by this mutation has no bearing on the results of our studies, we have not included this genotype in the nomenclature of the various phages in the text.

vides a way to assess the effectiveness of bacteria depleted of NusG in supporting growth of  $\lambda$  independently of the requirement for N-mediated antitermination; i.e., the *nin* phage is the positive control. In summary, these data suggest that poorer growth of  $\lambda$  in NusG-depleted *E. coli* is due to a failure of N-mediated transcription antitermination over and above that caused by the failing physiological environment.

To show that N would be effective if NusG were present in a NusG-depleted bacterium, we compared growth of  $\lambda nusG$  and  $\lambda cam$  in the absence of NusG. Since these phages are essentially identical except for the ability of the former to express NusG, support of growth of  $\lambda nusG$  but not  $\lambda cam$  would be evidence that NusG is required for  $\lambda$  growth. The bursts of these two phages were the same when grown in the host K9435, which expresses NusG at 42°C. However, the burst of  $\lambda cam$  is ~30-fold lower than the burst of  $\lambda nusG$  in K9683 depleted of NusG by prior growth at 42°C.

Thus, the defect in N activity in K9683 can be corrected by expressing NusG. The 10-fold-lower burst of  $\lambda nusG$  in K9683 relative to K9435 is not unexpected, since NusG is supplied to the depleted bacterium only during the short time of infection.

Taken together, these observations indicate that NusG is required for N action. First, the low burst of  $\lambda$  relative to  $\lambda nin$  in K9683 provides evidence that N-mediated transcription antitermination is reduced in vivo in the absence of NusG. Second, the ability of  $\lambda nusG$  to grow in K9683 shows that the absence of NusG is responsible for the failure of N antitermination. We conclude, therefore, that NusG is required for N-mediated antitermination in vivo as it is in vitro.

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