Analysis of the *Escherichia coli nusA10*(Cs) Allele: Relating Nucleotide Changes to Phenotypes

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The *Escherichia coli nusA* gene product, known to influence transcription elongation, is essential for both bacterial viability and growth of lambdoid phages. We report the cloning and sequencing of the conditionally lethal *nusA10*(Cs) allele. Changes from *nusA*⁺ were observed at nucleotides 311 and 634. Functional studies showed that both nucleotide changes are necessary for the cold-sensitive phenotype, although bacteria with the change at 634 grew more slowly at 30°C than the *nusA*⁺ controls. The mutant *nusA10*(Cs) gene product is not as active as *nusA*⁺ in supporting transcription antitermination mediated by the *N* regulatory protein of bacteriophage λ . The change at nucleotide 634 is shown to be solely responsible for this phenotype. Four differences were found between the *nusA*⁺ gene that we sequenced and the published *nusA* sequence. These changes alter the reading frame of *nusA* in a functionally important domain [as identified by the *nusA1* and *nusA11*(Ts) mutations], resulting in an arginine-rich region that may be involved with RNA binding.

The product of the *Escherichia coli nusA* gene is a 55-*M_r* protein (14, 27) that influences transcription elongation. *NusA* was first identified by its role in the N-mediated transcription antitermination reaction of bacteriophage λ (8, 9). Subsequent in vitro studies demonstrated that *NusA* binds to RNA polymerase (10, 13) and facilitates pausing (4, 16, 18, 32) and termination (11, 31, 34) of transcription.

NusA is an essential function. Conditionally lethal mutations in *nusA* have been selected and include the *nusA105* amber mutation (25), the *nusA11* heat-sensitive mutation (23), and the *nusA10* cold-sensitive mutation (30). Although the cause(s) for the lethality of these mutations is not known, *E. coli* carrying either the *nusA11*(Ts) or the *nusA10*(Cs) mutation exhibits a reduction in transcription termination at some termination signals (15, 24).

E. coli with the *nusA1* mutation are viable but fail to support the growth of bacteriophage λ at or above 42°C (8, 30). The phage do not grow because of a failure in transcription antitermination catalyzed, in part, by the product of the phage-encoded *N* gene, gpN. gpN, in concert with *E. coli*-encoded Nus proteins, acts through a signal, *nut* (28), to modify RNA polymerase into a termination-resistant form (reviewed in reference 7). The *nut* signals, located downstream of early λ promoters, are read, at least in part, from the RNA (19, 26, 35, 36). The *nusA11*(Ts) and *nusA* amber mutations, which restrict bacterial growth, have little or no effect on N-mediated antitermination (24, 30). The *nusA10*(Cs) mutation, in addition to restricting bacterial growth at low temperature, also causes a slight but observable reduction in the cells' ability to support the N-mediated transcription antitermination reaction. This is manifested by a failure to support the growth of λ bio256sex (30), a λ derivative that has impaired N-mediated antitermination because of a deletion-substitution (*bio256*) that extends into the 3' end of the *N* gene and an uncharacterized mutation (*sex*) located in the *p_L-nut_L* region (1). Collectively, these alterations result in the expression of low levels of a mutant N protein. In comparison, λ derivatives expressing normal levels of N

protein grow in the *nusA10*(Cs) host, although they fail to grow at 42°C in a host with the *nusA1* mutation.

The nucleotide sequence of *nusA*, as well as the nucleotide changes of the *nusA1* and *nusA11* mutations, has been determined (14, 27). Each mutation results from a single-base-pair change. We report here the cloning and sequencing of the *nusA10*(Cs) mutant allele and relate the observed nucleotide changes to the mutant phenotypes. In the course of sequencing the mutant allele and the isogenic wild-type *nusA* gene, we found four differences between our wild-type sequence and the published *nusA* sequence. These changes alter the reading frame of *nusA* in a functionally important domain.

MATERIALS AND METHODS

Bacteria and phages. The bacteria, all of which are *E. coli* K-12 derivatives, are listed in Table 1. The phages used in this study are λ , λ hy100 (12), and λ bio256sex (S. Adhya).

Media and genetic and nucleic acid techniques. Media and basic genetic techniques were described previously (21, 22). DNA manipulations were performed essentially as described by Maniatis et al. (20). Efficiency of plating experiments were performed as described by Schauer et al. (30). Enzymes, ³⁵S-dATP, and [³²P]dCTP were purchased from commercial suppliers and used according to supplied instructions.

Plasmids and plasmid constructions. The plasmid cloning vectors used were pBR322 (3) and pKB111 (2). Figure 1 shows the relevant restriction sites in *nusA* used to create the following constructs. pNAG3 (9a) contains the 1.1-kb *AatII*-*BalI* *nusA* wild-type DNA fragment inserted into the *AatII*-*BalI* site in pBR322. pNAC10 was constructed by digesting *nusA10*(Cs) genomic DNA isolated from strain K1914 with *KpnI* (*nusA* lies on a 3.3-kb *KpnI* restriction fragment), separating the fragments on a 1% agarose gel, isolating the fragments of 3.0 to 4.0 kb, and ligating them into the *KpnI*-digested and dephosphorylated vector pKB111. The mixture was used to transform competent *E. coli* DH5 α cells. Colony hybridization was used to identify transformants containing *nusA* inserts by using an *AatII*-*PstI* *nusA* DNA fragment isolated from pNAG3 as the probe. pNAC30 was constructed by ligating the 1.2-kb *AatII*-*BglII* *nusA*

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TABLE 1. *E. coli* K-12 strains

Strain	Relevant genotype	Source or reference
K37	<i>nusA</i> ⁺	M. Yarmolinsky
K1914	<i>nusA10</i> (Cs)	30
K4012	<i>nusA11</i> (Ts)	24
K4289	<i>nusA105</i> (Am) <i>supF6</i> (Ts)	Y. Nakamura
K5377	<i>nusA634</i>	This work
DH5 α		2a

fragment from pNAC10 into the 3.9-kb *AatII*-*BamHI* pBR322 fragment. pNAH1 and pGC634 were constructed by replacing, respectively, the *AatII*-*NcoI* and *BclI*-*StuI* *nusA*⁺ fragments from pNAG3 with the analogous *nusA10*(Cs) fragment obtained from pNAC30. Similarly, pCG311 was constructed by replacing the *BclI*-*StuI* fragment from pNAC30 with the *BclI*-*StuI* *nusA*⁺ fragment from pNAG3.

Marker rescue. The various plasmid constructs were transformed into the *nusA11*(Ts) strain K4012. The transformants were grown overnight at 34°C in LB broth supplemented with 32 μ g of ampicillin per ml. Recombinants that had replaced (marker rescued) the chromosomal *nusA11*(Ts) mutation with a *nusA* fragment from the plasmid were selected by spreading diluted aliquots of the overnight culture on TB plates and incubating at 42°C. Bacteria with plasmids containing *nusA* clones survived at a frequency 1,000 times greater than bacteria with a control plasmid.

DNA sequencing. Plasmid DNA was prepared by the alkaline sodium dodecyl sulfate (SDS) lysis procedure and purified by sedimentation through CsCl gradients. The dideoxy chain termination method of Sanger et al. (29) was used to sequence double-stranded plasmid DNA (33) with the Sequenase kit from U.S. Biochemicals. Sequencing reactions were performed as described by the manufacturer with ³⁵S-dATP. Regions of compression were eliminated by sub-

stituting dITP for dGTP in the sequencing reactions (1a). Both strands were sequenced between the *AatII* and *NcoI* sites in *nusA* by using synthetic 18-bp primers. Processing of sequencing reactions was done as described by Schauer et al. (30).

RESULTS

Cloning of the *nusA10*(Cs) allele. The complete *nusA10*(Cs) allele, contained in a 3.3-kb *KpnI* restriction fragment, was cloned from strain K1914 into the *KpnI* site of the plasmid vector pKB111. The resulting plasmid, pNAC10, was assumed to contain the *nusA10*(Cs) allele because it complements both the *nusA1* and the *nusA11*(Ts) alleles but does not complement the *nusA10*(Cs) allele (data not shown). A control plasmid with a cloned *nusA* wild-type gene complements all three mutations.

To avoid sequencing the entire mutant *nusA* gene, we adopted the following "marker rescue" strategy for limiting the size of the cloned DNA carrying the *nusA10*(Cs) mutation. A series of plasmids were constructed that contain internal fragments derived from *nusA* DNA (Fig. 1). All of the plasmids used for marker rescue contain a fragment that covers the *nusA11*(Ts) mutation. Since the plasmid constructs contain only part of *nusA*, they are unable to complement *nusA11*(Ts); however, they can replace the *nusA11*(Ts) allele by homologous recombination. If the cloned fragment carries the *nusA10*(Cs) mutation(s), the recombination that rescues the *nusA11*(Ts) mutation should, at some frequency, bring in the *nusA10*(Cs) mutation(s). In this way, the smallest clone that replaces the *nusA11*(Ts) mutation with the *nusA10*(Cs) mutation(s) would be identified and used to determine the sequence change(s) imposed by *nusA10*(Cs).

Two plasmids containing cloned fragments from *nusA10*(Cs) were used in the marker rescue mapping: pNAC30, with a 1.24-kb *AatII*-*BglII* fragment, and pNAH1, with a 0.78-kb *AatII*-*NcoI* fragment (Fig. 1). A control plasmid, pNAG3,

FIG. 1. Restriction map of *nusA* and the fragments used for subcloning. The top line indicates the relevant restriction sites in *nusA* and the locations of the *nusA1*, *nusA11*, *nusA311*, and *nusA634* mutations. Listed below are the pBR322 subclones containing the indicated *nusA* fragment. An open box represents a *nusA* fragment derived from the wild-type gene, while a hatched box represents DNA derived from the *nusA10*(Cs) allele.

TABLE 2. Fine-structure mapping of *nusA10*(Cs)

Plasmid used for marker rescue	<i>nusA</i> fragment in plasmid and size (kb)	No. of marker-rescued colonies tested	No. of colonies with indicated growth phenotype at 30°C		
			Wild type	Slow growth	Cold sensitive
pNAG3	<i>nusA</i> ⁺ (1.1)	16	16	0	0
pNAC30	<i>nusA10</i> (1.2)	24	13	4	7
pNAH1	<i>nusA10</i> (0.78)	15	6	5	4

that contains a 1.1-kb *AatII*-*BclI* wild-type *nusA* fragment was also used. When plasmids pNAC30, pNAH1, and pNAG3 were introduced into the *nusA11*(Ts) strain K4012, heat-resistant recombinants were obtained at a much higher frequency (5×10^{-5}) than were obtained with K4012 containing pBR322 ($<5 \times 10^{-8}$); i.e., the *nusA11*(Ts) allele on the chromosome was replaced by a *nusA* fragment from the plasmid. Screening of the resulting recombinants for cold sensitivity revealed that two plasmids, pNAC30 and pNAH1, yielded cold-sensitive recombinants (Table 2). Since pNAH1 contains a smaller fragment derived from the *nusA10*(Cs) gene than pNAC30, the mutation(s) responsible for the cold sensitive phenotype must be located in the 0.78-kb *AatII*-*NcoI* fragment. For this reason, plasmid pNAH1 was used as the template for determining the sequence changes caused by the *nusA10*(Cs) mutation (see below).

Surprisingly, the heat-resistant recombinants resulting from recombination with pNAC30 and pNAH1 fell into three classes of growth at 30°C: the two expected, cold sensitive and wild type, and a third, characterized by slow growth (which we call slow growers). This third class could result either from a separation of two (or more) mutations responsible for the *nusA10* cold sensitivity or from a combination of one or more of the *nusA10*(Cs) mutations with the *nusA11*(Ts) mutation. The second explanation was ruled out by the results of an experiment with plasmid pNAH1 in a marker rescue experiment with K4289, a strain containing the *nusA105*(Am) and *supF6*(Ts) mutations. This strain is not viable at 42°C because *supF6*(Ts) cannot suppress the amber mutation in the *nusA* gene at 42°C (24). Heat-resistant recombinants can be obtained by recombination with pNAH1, replacing *nusA105*(Am) with a corresponding *nusA* region from pNAH1. When these recombinants were examined for their growth characteristics at 30°C, the same three classes were observed as when the *nusA11*(Ts) marker was rescued. Thus, the slow growers are probably not the result of a combination of the *nusA11*(Ts) and *nusA10*(Cs) mutations. This suggests that the *nusA10*(Cs) cold-sensitive phenotype results from multiple changes in the *nusA* gene.

DNA sequencing and analysis. The nucleotide changes resulting from the *nusA10*(Cs) mutation were identified by comparing the mutant and wild-type sequences. The DNA sequence of pNAH1 (mutant) and pNAG3 (wild type) was determined between the *AatII* and *NcoI* restriction sites, the fragment shown by marker rescue to contain the changes both necessary and sufficient for the *nusA10* cold-sensitive phenotype. Comparison of these sequences revealed two changes, both G to A transitions. Since hydroxylamine was used in the isolation of the *nusA10*(Cs) mutant, a G · C to A · T transition was expected (5, 6). The nucleotide changes are located at bp 311 and bp 634 of the coding sequence, resulting, respectively, in changing amino acid 104 from an

TABLE 3. Effect of *nusA311* and *nusA634* on cell growth at 30°C^a

Plasmid used for marker rescue	<i>nusA</i> mutation(s) present on plasmid	No. of marker-rescued colonies tested	No. of colonies with indicated growth phenotype at 30°C		
			Wild type	Slow growth	Cold sensitive
pNAG3	None	16	16	0	0
pNAH1	<i>nusA311</i> , <i>nusA634</i>	32	6	10	16
pCG311	<i>nusA311</i>	67	67	0	0
pGC634	<i>nusA634</i>	54	10	44	0

^a *nusA311* and *nusA634* were crossed from a plasmid onto the chromosome by marker rescue of the *nusA11*(Ts) mutation.

Arg (CGT) to a His (CAT) and amino acid 212 from a Glu (GAA) to a Lys (AAA). We will refer to these two mutations as *nusA311* and *nusA634*, respectively, throughout this article.

In comparing the DNA sequence we obtained for wild-type *nusA* with the published wild-type *nusA* sequence, we found four differences: one was a change of a single base pair and the other three were single-base-pair additions. These differences will be discussed below.

Analysis of the *nusA10*(Cs) component mutations. The *nusA10*(Cs) mutation confers two identifiable phenotypes, cold sensitivity and inability to support the lytic growth of λ derivatives with impaired N-mediated transcription antitermination (30). In order to assess the contribution that each of the component mutations makes to these phenotypes, two plasmids were constructed that separated the *nusA311* and *nusA634* mutations from each other. Plasmid pGC634 contains the *nusA634* mutation, while plasmid pCG311 contains the *nusA311* mutation (Fig. 1).

These two plasmids were used to cross the *nusA311* and *nusA634* mutations onto the chromosome. Recombinants were selected by marker rescue of the *nusA11*(Ts) mutation. The heat-resistant recombinants were tested for both their ability to grow at 30°C and their ability to support the growth of λ derivatives with impaired N antitermination systems.

We first looked at the effect of the component mutations on cell growth at 30°C (Table 3). Control plasmids pNAG3 (which has wild-type *nusA*) and pNAH1 (which contains both the *nusA311* and *nusA634* mutations) yielded the expected classes of recombinants, the former only wild-type and the latter all three classes. Plasmid pCG311, containing the *nusA311* mutation, yielded only one class of recombinants, wild type, while plasmid pGC634, containing the *nusA634* mutation, gave two classes of recombinants, wild type and slow growers. Since the cold-sensitive class was never observed in recombinants obtained by using either plasmid pGC311 or plasmid pGC634 but was obtained with plasmid pNAH1 (which contains both component mutations), we conclude that both the *nusA311* and *nusA634* mutations are necessary for the cold-sensitive phenotype. In addition, the *nusA634* mutation is solely responsible for the slow-growth phenotype because slow growers were obtained from the recombinants with plasmid pGC634 but not from recombinants with plasmid pGC311.

The heat-resistant recombinants were next tested for the other phenotype conferred by the *nusA10*(Cs) mutation, failure to support the lytic growth of λ derivatives with impaired N systems. Two λ derivatives were used to test the effect of the *nusA10*(Cs) mutations on N-mediated antiter-

TABLE 4. Effect of *nusA10*(Cs) and *nusA634* on growth of lambdoid phages with impaired N antitermination systems

Strain	<i>nusA</i> allele	pBB1 ^a present	Growth temp (°C)	Efficiency of plating ^b		
				λ	λ bio256sex	λ hy100
K1914	<i>nusA10</i> (Cs)	—	30	—	—	—
			40	0.8	<10 ⁻⁶	<10 ⁻⁶
K5377	<i>nusA634</i>	—	30	1.0	<10 ⁻⁶	<10 ⁻⁶
			40	0.7	<10 ⁻⁶	<10 ⁻⁶
K4660	<i>nusA</i> ⁺	+	30	1.0	1.0	1.0
			40	1.0	1.0	1.0
K3731	<i>nusA10</i> (Cs)	+	30	1.0	<10 ⁻⁶	<10 ⁻⁶
			40	0.8	<10 ⁻⁶	<10 ⁻⁶

^a pBB1 expresses the *B. subtilis orfE* gene, which allows the *nusA10*(Cs) strain to grow at 30°C.

^b Efficiency of plating was determined by dividing the titer of the phage on the indicated bacterial lawn by the titer obtained on a lawn of K37 (*nusA*⁺). —, No bacterial lawn was formed.

mination, λ bio256sex and λ hy100. We have previously reported that λ bio256sex is unable to grow in hosts with the *nusA10*(Cs) mutation because it expresses low levels of a mutant N protein (30). We also found that another λ derivative, λ hy100 (12), fails to grow in the *nusA10*(Cs) host. λ hy100 has the *N* gene of lambdoid phage P22 and the *nutR* target signal from phage 21. This results in a heterologous N-*nutR* interaction that is relatively ineffective in forming an antitermination complex, as evidenced by the failure of λ hy100 to grow in *nus* mutants under conditions in which the parental phages as well as λ grow (12; unpublished experiments). The marker-rescued *nusA11*(Ts) recombinants that exhibited wild-type growth at 30°C could support the growth of λ bio256sex and λ hy100, while recombinants with either the slow-growth or cold-sensitive phenotype did not support growth of these phages (data not shown). Since *nusA634* is responsible for the slow-growth phenotype and slow growers fail to support the growth of λ bio256sex and λ hy100, the *nusA634* mutation must also be responsible for the failure to support the growth of these phages with impaired N antitermination systems.

Effect of *nusA10*(Cs) and *nusA634* on N-mediated antitermination at 30°C. We next examined the effect of the *nusA10*(Cs) mutation on λ growth at 30°C. Although this temperature is nonpermissive for growth of bacteria carrying the *nusA10*(Cs) mutation, we have isolated a gene from *Bacillus subtilis*, *orfE*, that, when expressed from a plasmid, allows the *nusA10*(Cs) mutant bacterium to grow at 30°C. *orfE* is not the *B. subtilis* analog of *nusA*, as there is no sequence identity between *nusA* and *orfE*, nor does *orfE* complement any of the other *nusA* mutations (unpublished data).

The results of an efficiency of plating experiment (Table 4) indicated that there was no difference in the growth of lambdoid phages on the *nusA10*(Cs) strain containing the *B. subtilis orfE* plasmid (pBB1) at high (permissive for bacterial growth) or low (nonpermissive for bacterial growth) temperatures. We were also able to directly test phage growth on the strain carrying the single *nusA634* mutation at the low temperature, since this mutant does grow at 30°C. As in the case of the *nusA10*(Cs) mutant with the *orfE* plasmid, the *nusA634* mutant supported the growth of λ at both high and low temperatures and failed to support growth of λ bio256sex and λ hy100 at both high and low temperatures.

Variations from the reported *nusA* sequence. There are four differences between the DNA sequence we found for the

nusA gene in the region defined by the *Aat*II and *Nco*I restriction sites and the published *nusA* sequence. These differences were also found in the sequence of the *nusA10*(Cs) allele. There are three separate, single-base-pair additions: a T · A following bp 499, a G · C following bp 566, and a C · G following bp 568 (Fig. 2). These additions, which alter the reading frame, would obviously change the amino acid sequence of the protein, adding an amino acid and changing 24 of the deduced amino acids between codons 167 and 190 (numbers corresponding to the published sequence). The fourth difference that we found is a T · A instead of a C · G at bp 517 of the coding sequence (corresponding to the published sequence). This change eliminates an *Xmn*I restriction site identified on the published sequence, explaining why we were unable to obtain cleavage by that enzyme at this site.

As an independent means of determining whether these corrections to the published sequence represent changes peculiar to our strain or changes of general significance, we examined the sequence of the analogous region from the *nusA* gene of *Salmonella typhimurium*. DNA sequence analysis reveals that these two genes have significant similarities at the amino acid level (unpublished data). Any matches with the *S. typhimurium* sequence would imply that the conserved amino acid sequence is unlikely to be a polymorphism or the result of a sequencing artifact. We found that the sequence of the *S. typhimurium nusA* gene in the region containing the additions is identical to the sequence that we have determined for the *E. coli nusA* gene (data not shown). These differences from the published sequence have been confirmed by Ito et al. (accompanying paper [14a]) and Robledo et al. (26a).

The region of nucleotide addition described above includes the nucleotides changed by the *nusA1* and *nusA11* point mutations. We therefore examined the amino acid changes resulting from these point mutations in the context of our revised sequence. The *nusA1* mutation changes amino acid 183 from Leu (CTC) to Arg (CGC), and the *nusA11* mutation changes amino acid 181 from Gly (GGC) to Asp (GAC).

DISCUSSION

In this study we identified two single-base-pair changes in the *nusA10*(Cs) allele of the *E. coli nusA* gene and related each to the previously identified phenotypes conferred by the mutant allele. These phenotypes are cold sensitivity, as evidenced by a failure of the mutant bacterium to grow at 30°C (growth phenotype), and reduction in support of the N-mediated transcription antitermination reaction of lambdoid phages, as evidenced by the inability of λ bio256sex and λ hy100 to form plaques (Nus phenotype). The component mutations, called *nusA311* and *nusA634*, change, respectively, codon 104 from an Arg (CGT) to a His (CAT) and codon 212 from a Glu (GAA) to a Lys (AAA).

The identification of two widely spaced amino acid changes in the mutant protein suggested that each of its phenotypes, growth and Nus, could be ascribed to one of the component mutations. To test this idea, the two mutations were separated and each was crossed back to the chromosome. Neither cross yielded cold-sensitive bacteria. However, the cross with the *nusA634* mutation generated recombinants that grew very slowly at 30°C, while the cross with the *nusA311* mutation failed to generate any recombinants with an altered phenotype. Provided that the cross with *nusA311* did place the mutation on the chromosome and that

FIG. 2. Variations from the published *nusA* sequence. The upper two lines are the deduced amino acid and nucleotide sequences published for the *nusA* *E. coli* gene for codons 165 to 190 (14, 27). The lower two lines are the nucleotide and amino acid sequences based on the sequencing results presented in this work. The three extra nucleotides that were identified are in boldface type, and the single nucleotide difference is underlined. The amino acid changes caused by the *nusA1* and *nusA11* mutations are indicated for each of the sequences.

the *nusA311* mutation by itself is not lethal, these results suggest that the *nusA634* change is the major cause of cold sensitivity, with the *nusA311* change serving to enhance the effect of *nusA634* on bacterial viability at low temperature.

The *nusA634* mutation appears to be solely responsible for the Nus phenotype of the *nusA10*(Cs) mutation, since there is no apparent difference in growth of the tester phages in the single *nusA634* mutant and the double *nusA10*(Cs) mutant. The reduction in N-mediated antitermination observed in hosts carrying either the *nusA634* or the double *nusA10*(Cs) mutation was less than that observed with the *nusA1* mutation. A reduction in N-mediated antitermination is only observable in the *nusA634* and *nusA10*(Cs) mutants with phages that have impaired N antitermination reactions either because they express low levels of an altered N protein (*λbio256sex*) or have a heterologous N-*nutR* combination (*λhy100*). This reduction is also seen at 30°C in the *nusA634* or in the *nusA10*(Cs) strain when a plasmid containing the *B. subtilis orfE* gene is present. Wild-type lambda appears to grow normally in *nusA10*(Cs) and *nusA634* mutants at both high and low temperatures.

The *nusA634* mutation, then, results in a protein that functions suboptimally both in supporting an activity required for bacterial growth and in *λ* N-mediated transcription antitermination. Two *nusA* mutations that have been extensively characterized, *nusA1* and *nusA11*(Ts), primarily affect only one or the other phenotype. The *nusA1* mutation exerts a strong effect on N-mediated antitermination, while the *nusA11*(Ts) mutation influences cell viability. Interestingly, these mutations are closely linked, located, respectively, at nucleotides 548 and 542 of the coding sequence (based on our sequence data). The fact that these mutations are so closely linked (the encoded amino acids are separated by one amino acid) suggests that the altered amino acids lie in an active domain of NusA that presumably functions both in supporting the host activity and in *λ* N-mediated antitermination. In other studies we have found that the *nusA634* mutation is solely responsible for an observed instability of pUC plasmids in the *nusA10*(Cs) host (unpublished observation).

Assuming that the *nusA634* mutation is primarily responsible for both the growth and Nus phenotypes of *nusA10*(Cs) strains, the fact that the resulting amino acid change is located 30 amino acids away from the *nusA1* and *nusA11*(Ts) changes does not necessarily preclude the idea of an active pocket in the region defined by the *nusA1* and *nusA11*(Ts) mutations. The protein might fold in such a way that the region defined by *nusA634* is brought into proximity to the *nusA1* and *nusA11* region. *nusA634* could decrease the effectiveness of this pocket. Alternatively, *nusA634* may be located far from the *nusA1* and *nusA11* region, and its effect could be manifested by adversely altering the tertiary structure or stability of NusA.

Our sequencing results revealed differences from the published sequence of *nusA*. Although the differences might be explained by polymorphisms, we believe that this is not the case, given the fact that the *S. typhimurium nusA* sequence is identical to our wild-type *E. coli nusA* sequence in this region. In addition, the deduced amino acid composition of our *nusA* sequence more closely matches the empirically derived composition than does the published sequence (14, 17, 27). The difference between these two sequences is a change of 24 amino acids in the region of the *nusA1* and *nusA11* mutations. In the revised sequence, this region no longer has a significant hydrophobic profile but, interestingly, contains six arginines in a run of 22 amino

acids (amino acids 170 to 191). Two features of arginine bear on any consideration of the functional role of this region: the density of arginines in this small region differs significantly from that in the rest of the protein (27 versus 5%), and arginine-rich regions are known to be regions for binding of proteins to nucleic acids (19).

In this light, it is plausible to consider this arginine-dense region of NusA, which contains *nusA1* and *nusA11*(Ts), as a site for binding to nucleic acid. This binding could occur in either a sequence-specific or nonspecific manner. The *nusA1* mutation (which changes a leucine to an arginine) and the *nusA311* mutation (which changes an arginine to a histidine) may exert their effect by influencing the binding of NusA to nucleic acid. Alternatively, these changes might influence a protein-protein interaction.

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