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The alpha subunit of RNA polymerase and transcription antitermination

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Summary

The *N* gene product of coliphage λ, with a number of host proteins (Nus factors), regulates phage gene expression by modifying RNA polymerase to a form that overrides transcription-termination signals. Mutations in host *nus* genes diminish this N-mediated antitermination. Here, we report the isolation and characterization of the *rpoAD305E* mutation, a single amino acid change in the carboxy terminal domain (CTD) of the α subunit of RNA polymerase, that enhances N-mediated antitermination. A deletion of the 3′ terminus of *rpoA*, resulting in the expression of an α subunit missing the CTD, also enhances N-mediated antitermination and, similar to *rpoAD305E*, suppresses the effect of *nus* mutations. Thus, the N–Nus complex may be affected through contacts with the CTD of the α subunit of RNA polymerase, as is a group of regulatory proteins that influences initiation of transcription. What distinguishes our findings on the N–Nus complex from those of previous studies with transcription proteins is that all of the regulators characterized in those studies bind DNA and influence transcription initiation; whereas the N–Nus complex binds RNA and affects transcription elongation. A screen of some previously identified *rpoA* mutations that influence transcription activators revealed only one other amino acid change, L290H, in the CTD of the α subunit, that influences antitermination. Although our results provide evidence that interactions of the α subunit of RNA polymerase must be considered in forming models of transcription antitermination, they do not provide information as to whether the interactions of α that ultimately influence antitermination occur during initiation or during elongation of transcription.

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Introduction

Programmed regulation of transcription termination is one strategy for control of gene expression that is employed by a wide variety of organisms (Das, 1993). This modulated readthrough of transcription-termination signals, called transcription antitermination, was originally postulated to explain the activation of coliphage λ delayed-early genes by the phage-encoded *N* gene product (Roberts, 1969). Genetic studies of this antitermination process have identified a set of *Escherichia coli* genes, *nus*, whose protein products are required for effective N-mediated antitermination (Friedman *et al.*, 1984a). Some of these Nus proteins serve as transcription-elongation factors for *E. coli* (reviewed by Yager and Von Hippel, 1987) and all are essential for host viability (Friedman and Court, 1995). The participation of a large number of proteins in antitermination raises the obvious question of how the members of such a collection of host and viral proteins interact with each other and, importantly, how the complex itself interacts with RNA polymerase (RNP) to create a termination-resistant transcription complex (Das, 1992; 1993; Friedman, 1988; Roberts, 1992; 1993; Greenblatt *et al.*, 1993).

Central to N-mediated modification of RNA polymerase are the *nut* signals (Rosenberg *et al.*, 1978; Salstrom and Szybalski, 1978a). Located downstream of the early λ promoters, pL and pR, the *nut* sites direct the assembly of the antitermination complex. Transcripts initiating at pL and pR are partially terminated at Rho-dependent terminators tL1 and tR1, respectively (see Fig. 1) (Roberts, 1969; Salstrom and Szybalski, 1978b). The resulting short pL transcript includes the message of the *N* gene, whose product, N, acts with the host Nus proteins at the NUT RNA signals (Rosenberg *et al.*, 1978; Somasekhar *et al.*,

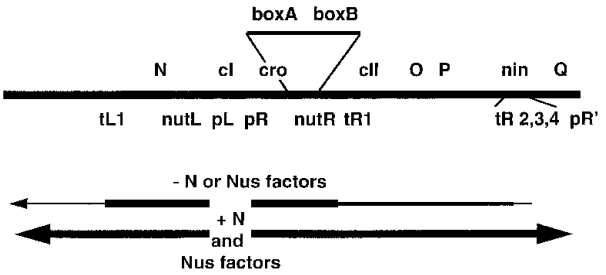

Fig. 1. Schematic genetic map of the early transcribed region of the λ genome. The positions of representative genes are indicated above the bold line, with an expansion of the *nutR* site showing the relative positions of *boxA* and *boxB*. The *nutL* site is positioned downstream of pL in the opposite orientation to *nutR*. Below the line are shown the positions of sequences containing signals (promoters, *nut* sites, and terminators). Lower part of the figure shows the nature of transcription in the absence and presence of effective N–Nus complexes.

1982; Lazinski *et al*., 1989; Salstrom and Szybalski, 1978a,b; Barik *et al*., 1987; Olson *et al*., 1982; 1984; Warren and Das, 1984; Zuber *et al*., 1987; Nodwell and Greenblatt, 1991; Friedman *et al*., 1990; 1984a) to modify the transcribing RNA polymerase. The *nut* regions have been divided by sequence homologies, as well as by function, into two components (Friedman and Olson, 1983; Olson *et al*., 1984; Hasan and Szybalski, 1986; Lazinski *et al*., 1989). The BOX-A component appears to be the recognition site for the NusB and possibly the S10 (NusE) proteins (Friedman *et al*., 1990; Nodwell and Greenblatt, 1993; Patterson *et al*., 1994) and an inhibitory host factor whose existence has been inferred from results of genetic and physiological studies (Patterson *et al*., 1994). The BOX-B component, a region of hyphenated dyad symmetry that forms a hairpin, has been identified as the recognition site for N and NusA (Doelling and Franklin, 1989; Lazinski *et al*., 1989; Das, 1992; Chattopadhyay *et al*., 1995; Mogridge *et al*., 1995). (Note that the following convention is being followed in labelling nucleic acid sites: the DNA site will be in lower-case italics and the RNA site will be capitalized, e.g. the DNA will be referred to as '*nut*', while the RNA will be referred to as 'NUT'.)

Most of the Nus factors were identified by mutations that influence the effectiveness of the N-mediated antitermination process without affecting host viability. Subsequent *in vitro* studies confirmed the essential roles of these products in this antitermination process (Das and Wolska, 1984; Horwitz *et al*., 1987). Two types of mutations were employed in those studies. The first are mutations that cause a failure in N-mediated antitermination at higher temperatures, a phenotype we shall refer to as Nus$^-$ (N undersupplied). Two of these mutations, *nusA1* at minute 69 and *nusB5* at minute 11 on the *E. coli* chromosome, are located in genes that had not previously been identified and thus were defined by their antitermination defect. Three are in genes that were of known function: *rpsJ/nusE*, encodes ribosomal protein S10 and is located at minute 72; *rho/nusD*, encodes the Rho transcription-termination protein and is located at minute 90; and *rpoB*, encodes the β subunit of RNA polymerase and is located at minute 85 (reviewed in: Friedman *et al*., 1984a; Das, 1992; Greenblatt *et al*., 1993; Friedman, 1992). Hosts with any of these *nus* mutations have a reduced ability to support λ growth, solely because of a defect in N-mediated antitermination.

The other type of mutations used to identify host factors involved in N action are second-site suppressors that were isolated on the basis of restoring N antitermination activity in the presence of *nusA1*. Second-site suppressor mutations of this type were identified as *rif*^{r} mutations in the *rpoB* gene (Sparkowski and Das, 1992; Jin *et al*., 1988), the *nusB101* mutation (Ward *et al*., 1983; Court *et al*., 1995) and the *nusG4* mutation in a previously unidentified gene (Downing *et al*., 1990). *In vitro* studies provided evidence that NusG is a component of the N-antitermination complex (Li *et al*., 1992). However, its role in antitermination has not been definitively established, because depleting NusG in the cell does not result in reduced antitermination (Sullivan and Gottesman, 1992). Although other phenotypes associated with mutations in *nus* genes have been identified (Tsugawa *et al*., 1988; Ward *et al*., 1983; Kur *et al*., 1989; Kuroki *et al*., 1982; Miyashita *et al*., 1982; Ward and Gottesman, 1981; Zheng and Friedman, 1994; Court *et al*., 1995), this discussion will be limited to those mutations that influence N action by causing or suppressing a Nus$^-$ phenotype.

The *nusB101* and *nusG4* mutations also suppress the Nus$^-$ phenotype imposed by the *nusE71* mutation (Ward *et al*., 1983; Sullivan *et al*., 1992). Mutations identified in λ that exhibit similar suppression of the *nusA1* and *nusE71* mutations have been located in the *nutR–boxA* sequence (Friedman *et al*., 1990) and in the *N* gene (Schauer *et al*., 1987; Franklin, 1985). These mutations were initially selected because they fostered λ growth in *E. coli nusA1* mutants, but they subsequently were also shown to foster λ growth in *E. coli nusE71* mutants (Schauer *et al*., 1987; Friedman and Olson, 1983). With the exception of *nusB101*, which was not tested for the obvious reason that it is a *nusB* mutation, all of these suppressors, bacterial as well as phage, show relatively poor suppression of the Nus$^-$ phenotype conferred by the *nusB5* mutation (Schauer *et al*., 1987; Ward *et al*., 1983). It has been suggested that suppressor mutations that alter a component of the N–Nus complex suppress because they enhance (one or more) interactions in the complex, compensating for a suboptimal interaction caused by the primary *nus* mutation (Schauer and Friedman, 1985; Das, 1992).

We report that mutations in the *rpoA* gene also suppress defective N–Nus-mediated transcription antitermination

caused by *nus* mutations. These *rpoA* mutations change amino acids within a small segment of the carboxy terminal domain (CTD) of the α subunit of RNA polymerase, indicating that a distinct molecular surface may contribute to the control of the activity of the N–Nus complex.

Results

Selection of rpoAD305E, *a second-site suppressor of* nusE71

The *rpoAD305E* mutation, originally called *sneA16* (Friedman, 1988; Schauer, 1985; Schauer and Friedman, 1985), was isolated by selecting for second-site mutations that suppress the Nus$^-$ phenotype of an *E. coli* strain carrying the *nusE71* mutation. The selection was essentially that devised by Ward *et al.* (1983). The *E. coli* strain (K1953) used in the selection carries the *nusE71* mutation and has the *gal* operon under the control of the λ pL promoter (Fig. 2). Because of intervening transcription terminators, expression of *gal* genes is entirely dependent on N-mediated transcription antitermination. Even though N is expressed, the strain is Gal$^-$, because the *nusE71* mutation does not support N action. Thus, suppression of this *nusE71* defect would result in a Gal$^+$ phenotype. We attempted to direct the mutagenesis to the 72 min region using P1 transduction for localized mutagenesis (see the *Experimental procedures*). This region of the *E. coli* chromosome was chosen as a possible location of suppressor mutations, because it is rich in genes encoding ribosomal proteins. We postulated that if one or more ribosomal proteins interacted with S10 during antitermination, a mutation in one of the genes encoding such a protein might suppress the effect of the *nusE71* mutation. The marker selected in the transduction was a Tn*10* transposon, *zhb-3082*::Tn*10*, located in this region.

The *rpoAD305E* mutation, obtained from this selection, was located on the *E. coli* genetic map using standard genetic techniques (Miller, 1972; Sternberg and Maurer, 1991). These manipulations located the *rpoAD305E* mutation, as expected, in the 72 min region of the *E. coli* chromosome, and finer mapping studies suggested that the mutation is in or closely linked to the *rpoA* gene, which encodes the α subunit of RNA polymerase.

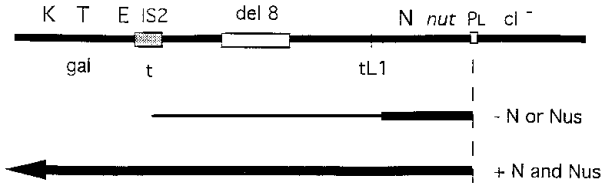

Fig. 2. Fusion for selecting the *rpoAD305E* mutation and for assessing levels of suppression of *nus* mutations (after Ward *et al.* (1983)). The bacterium is a derivative of *E. coli* K-12 in which the bacterial galactose operon (*gal*) is fused to the pL promoter of a defective λ prophage. The essential components of this abbreviated prophage are: a *c*I mutation eliminating repressor, the pL promoter, the *nutL* signal, the *N* gene, and transcription terminators, including a strong termination signal in an IS*2* positioned immediately 5′ of the structural genes of the *gal* operon. In a *nus*$^-$ derivative, N-modified transcription antitermination is not functional and therefore there is no *gal* expression (Gal$^-$). Bacteria with second-site mutations that suppress the *nusE71* mutation were selected as Gal$^+$ mutants.

Complementation analysis and marker rescue

The dominance of the *rpoAD305E* mutation was characterized using a strain with the *rpoAD305E* allele on the chromosome. The effectiveness of N action could be assessed because the bacterium carries the pL–*gal* fusion described above and also in Fig. 2. The bacterium, *E. coli* K6325, carries, in addition to the fusion, the *nusE71* mutation that normally blocks N action, but because K6325 also carries the *rpoAD305E* mutation, this blockage of N action is suppressed. The *gal* operon can be expressed allowing K6325 to metabolize galactose and thus to form red colonies on a MacConkey–galactose plate at 40°C. A derivative of K6325 containing pNO2530 (a plasmid with the wild-type *rpoA* and *rplQ* genes downstream of p*lac*) (Bedwell and Nomura, 1986) forms pink colonies, reflecting a reduction in *gal* operon expression and thus a reduced suppressor phenotype. Note that under these conditions K1953, the starting *nusE71* derivative, forms white colonies (low *gal*-operon expression) and K1947, the *nus*$^+$ derivative, forms red colonies (high *gal*-operon expression). Thus, by this assay, *rpoA*$^+$ expressed from the pNO2530 plasmid is only partially dominant to the single chromosomal copy of the *rpoAD305E* allele.

A plasmid with the *rpoAD305E* allele was obtained from K6325 carrying pNO2530 by identifying rare red colonies on MacConkey–galactose plates containing ampicillin, at 40°C. Presumably these red-colony formers became homozygous for *rpoAD305E* as a result of recombination between the chromosomal and plasmid *rpoA* alleles and subsequent segregation. The details of the screen for the recombinants and the tests confirming the exchange are described in the *Experimental procedures*. When this new *rpoAD305E*-containing plasmid, isolated from these red colonies and named pNO2530*, is transformed into the *rpoA*$^+$ strain K1953, the *nusE71* defect is suppressed (there is effective *gal* operon expression as evidenced by formation of red colonies), indicating the *trans*-dominant nature of the suppressor *rpoAD305E* mutation in multicopy.

Locating the rpoAD305E *mutation to the* rpoA *gene*

The *rpoA* insert in pNO2530 is located immediately downstream of the *lac* promoter (p*lac*). To determine whether *rpoAD305E* was an allele of *rpoA*, we subcloned DNA fragments from pNO2530 and pNO2530* and placed

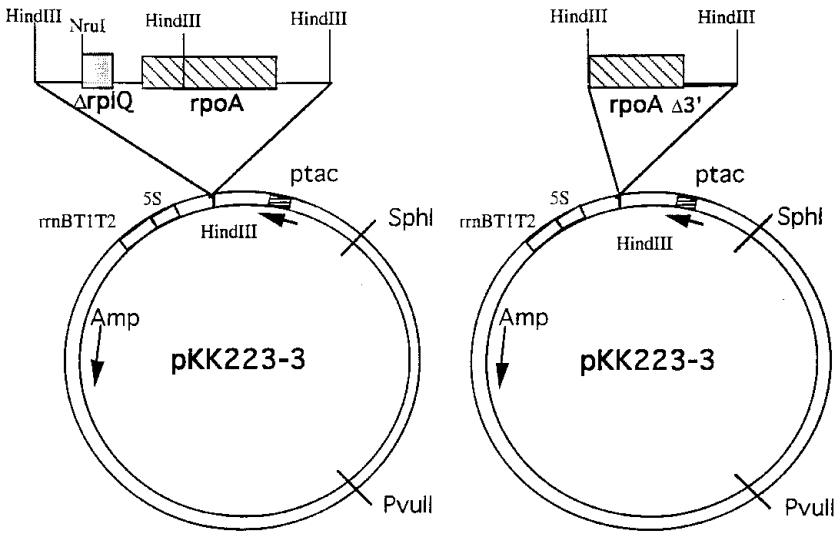

Fig. 3. Derivatives of pKK223-3 (Brosius and Holy, 1984) with cloned *rpoA* genes. The circles show the cloning vector, and the diagrams above show the nature of the cloned fragment in each construct. *rrnB* T1T2 are terminators from the *rrnB* operon. The position of the *amp*^{r} gene is indicated. The cloned *rpoA* genes are oriented so that they are expressed from the p*tac* promoter. On the left is shown the extent of the fragment cloned in both pKKrpoA and pKKrpoA*, and on the right is shown the extent of the fragment cloned in pKKrpoAΔ3′. The region of pKK223-3 between the *Sph*I and *Pvu*II sites was deleted in all of these plasmids.

them in the appropriate orientation for expression, downstream of the *tac* promoter (p*tac*) in a derivative of plasmid pKK223-3 (de Boer, 1984), as shown diagrammatically in Fig. 3. The resulting plasmids were tested for suppression of the *nusE71* mutation. Plasmid pKKrpoA*, which includes the complete *rpoA* gene and the 5′ two-thirds of *rplQ* from pNO2530*, suppresses the *nusE71* mutation, whereas the same region from pNO2530 did not. Another plasmid, which includes only the 3′ end of *rpoA* as well as the 5′ two-thirds of *rplQ* from pNO2530* (our unpublished results), fails to suppress the *nusE71* mutation. Thus, we conclude that *rpoAD305E* is indeed an allele of *rpoA*.

Sequencing the rpoAD305E *mutation*

Comparison of the DNA sequences of the *rpoA* genes in pNO2530 and pNO2530* revealed a single nucleotide difference. The former has GAC at codon position 305 as does the published sequence (Meek and Hayward, 1994), while the latter has GAA at that codon position. The derived amino acid sequence for the mutant α subunit has a glutamic acid at position 305 instead of aspartic acid encoded in the wild-type α subunit at that position; we have sequenced the entire fragment that expresses suppressor activity and find this to be the only change from the wild-type sequence. Additionally, sequencing of the mutant chromosomal allele reveals the same change. Therefore, we conclude that the change from an aspartic to a glutamic acid at amino acid position 305 is responsible for the *rpoAD305E* Nus-suppressor phenotype.

Suppression of other nus *mutations*

To characterize the range of suppression by the *rpoAD305E* mutation, we assessed the effect of *rpoAD305E* on the Nus$^-$ phenotypes imposed by mutations in other *nus* genes. Derivatives of strain K37, each carrying the *rpoAD305E* mutation in addition to one of the *nus* mutations, were tested for their ability to support λ growth at high temperatures using efficiency of plating (EOP) as the assay for λ growth (Table 1).

Results of this assay show that the *rpoAD305E* mutation suppresses the Nus$^-$ phenotypes imposed by all three *nus* mutations. In this and other assays testing Nus activity, different temperatures were used in assessing the Nus$^-$ phenotype: 40°C for those with *nusB5* or *nusE71* mutations, and 42°C for those with the *nusA1* mutation (Friedman *et al.*, 1984a). The two temperatures were used because the *nusA1* mutation is not restrictive for N action at temperatures under 42°C and the *nusB5* and *nusE71* mutations are not suppressed efficiently by *rpoAD305E* at 42°C. Under these specific conditions, derivatives of K37 (*rpoA*$^+$) carrying the respective *nus* mutations fail to support λ growth. The EOP in each case was $<10^{-5}$. The presence of *rpoAD305E* in any

Table 1. Suppression of *nus* mutations by *rpoAD305E*, as measured by EOP of λ.

Strain	Temperature (°C)	Relevant genotype	EOP[a]	Plaque size
K4069	40	*rpoAD305E*	0.7	Large
K556	40	*nusE71*	$<10^{-5}$	
K8248	40	*nusE71 rpoAD305E*	0.7	Small
K95	42	*nusA1*	$<10^{-5}$	
K4047	42	*nusA1 rpoAD305E*	0.7	Tiny
K450	40	*nusB5*	$<10^{-5}$	
K4070	40	*nusB5 rpoAD305E*	0.7	Very tiny

a. EOP is the efficiency of plating. This number is derived from phage titres obtained at the indicated temperatures. EOP is the ratio of the titre on a lawn of the strain with the mutant genotype divided by the titre on a lawn of the parent *E. coli* 'wild-type' strain at the locus being tested. λ*c*I60 was used in this experiment.

Table 2. Suppression of *nus* mutations by *rpoAD305E* as measured from a pL–*nut*-t–*gal* fusion in the presence of N[a].

nus allele in strain	Temperature (°C)	Percent Readthrough[b]	
		rpoA$^+$	*rpoAD305E*
nus$^+$	40	100	100
nusA1	42	13	104
nusE71	40	10	48
nusB5	40	7	27

a. See Fig. 2 for details on the pL–*gal* construct.
b. Percent readthrough equals (GalK level expressed from the fusion in the mutant strain divided by the GalK expressed from the fusion in the parent strain (wild type for relevant markers)) × 100. GalK levels were measured as indicated in the *Experimental procedures*.

one of these *nus*-mutant strains results in an increase of the EOP to nearly 1.

We next employed a more direct assay to assess the degree of suppression by the *rpoAD305E* mutation. Suppression was quantitatively assessed using bacterial derivatives with the previously described pL–*gal* fusion (Fig. 2). As shown in Table 2, hosts carrying any one of the three *nus* mutations (*nusA1*, *nusB5*, or *nusE71*) express *galK* levels that are ~10% of that observed in the K1947 *nus*$^+$ control. In the double *rpoAD305E nus* mutants, *galK* expression was significantly higher, varying from 30–100% of that observed in the *nus*$^+$ control. Although there were differences in the level of suppression, the essential point is that the results of this test, similiar to those from the phage plating, show that *rpoAD305E* suppresses the Nus$^-$ phenotype of the three *nus* mutations.

Suppression by a truncated rpoA *gene*

In vitro studies on the action of transcription activators have shown that RNA polymerase with an α subunit deleted at the carboxy terminus duplicates the effects of point mutations that change specific amino acids in the carboxy terminus of α (Ishihama, 1992). To determine if there was a similar effect on suppression of *nus* mutations, we examined whether the truncated α subunit, expressed from an *rpoA* gene deleted for its 3′ domain, affects the Nus$^-$ phenotype of *E. coli* derivatives with *nus* mutations. *E. coli* strains haploid for an *rpoA* 3′ deletion are not viable, but plasmids expressing the truncated *rpoA* can be maintained in strains that are wild type for *rpoA* (Hayward *et al*., 1991). Based on these findings and our observation that plasmids expressing the *rpoAD305E* allele are *trans*-dominant, we postulated that a 3′ deletion of *rpoA* might suppress the Nus$^-$ phenotype. This hypothesis was tested using a plasmid expressing the 3′-deleted *rpoA* gene. Three plasmids, pKKrpoA, pKKrpoA*, and pKKrpoAΔ3′ (see Fig. 3), were tested in parallel for suppression of *nus* mutations.

Sequence analysis predicts that pKKrpoAΔ3′ should express an α subunit that is truncated at amino acid 229 where an additional 15 amino acids encoded by the vector sequence are added. Western blot analysis, using an anti-α monoclonal antibody, identified a protein corresponding to the size predicted for the truncated polypeptide in bacteria carrying pKKrpoAΔ3′. This protein is not seen in control bacteria that do not contain the plasmid (data not shown).

The suppressor activity of the plasmid-based *rpoA* alleles was examined by assessing the effects on λ growth as measured by EOP. As shown in Table 3, the *rpoAΔ3′* allele, like the *rpoAD305E* allele, suppresses the Nus$^-$ phenotype of both the *nusE71* and *nusA1* mutations. In contrast, when the wild type *rpoA* allele is expressed from a plasmid constructed from the same vector, neither *nus* mutation is suppressed. Although these results clearly demonstrate that the two mutant *rpoA* alleles suppress the Nus$^-$ phenotype, interpretation of the quantitative results is complicated by the necessity of having a wild-type *rpoA* allele for viability. Therefore, the finding that the increase in EOP effected by either the plasmid-based *rpoAD305E* or *rpoAΔ3′* allele is lower in the *nusA1* mutant than in the *nusE71* mutant could suggest a difference in suppression, but also could result from complications owing to the presence of the wild-type *rpoA* allele. The pattern of suppression of the *nusB5* mutation is qualitatively different. By this test the *rpoAΔ3′* mutation, similar to the wild-type allele, fails to suppress the *nusB5* mutation, while the *rpoAD305E* allele does so weakly. We emphasize the essential finding from these experiments that the *rpoAΔ3′* allele, like the *rpoAD305E* allele, suppresses the Nus$^-$ phenotype of some *nus* mutations, while pointing out the difficulty in assessing the significance of the quantitative data because of the possibility of interference with suppression by the wild-type *rpoA* allele.

Table 3. Suppression of *nus* mutations by plasmid-based *rpoA* mutations as measured by EOP of λ.

Strain	Temperature (°C)	Relevant chromosomal genotype	*rpoA* allele on plasmid	EOP of λ[a]
K95	42	*nusA1*	Wild type	$<10^{-5}$
K7565	42	*nusA1*	*rpoAD305E*	0.08
K7625	42	*nusA1*	*rpoAΔ3′*	0.02
K450	40	*nusB5*	Wild type	$<10^{-5}$
K7568	40	*nusB5*	*rpoAD305E*	0.05
K7626	40	*nusB5*	*rpoAΔ3′*	$<10^{-5}$
K556	40	*nusE71*	Wild type	$<10^{-5}$
K7571	40	*nusE71*	*rpoAD305E*	0.8
K7627	40	*nusE71*	*rpoAΔ3′*	0.8

a. For details of EOP see Table 1. λ*c*I60 was used in this experiment.

Effect on transcription initiation and termination

Studies presented in the previous sections show that *rpoA* mutations can suppress the effect of mutations in three *E. coli* genes whose products influence N-mediated transcription antitermination. There are two ways to explain this suppression by an *rpoA* mutation, either by an increase in transcription initiation or by a decrease in transcription termination. Therefore, we assessed the effect of the *rpoAD305E* mutation on transcription initiation and termination in the absence of N. Beta-galactosidase levels were compared between congenic *rpoA*$^+$ and *rpoAD305E* strains carrying a pL–*N*–*lacZ* protein fusion in a defective prophage. There was essentially no difference between the two strains with regard to promoter strength or N expression, because each expressed ~2000 units of the β-galactosidase fused to N. Termination was assessed using three pKL600 derivatives that test the tR2 Rho-independent or tR1 and tR3 Rho-dependent terminators of phage λ (Cheng *et al.*, 1991; 1995; Patterson *et al.*, 1994). Because the transcription terminators are placed upstream of the *galK* gene, expression of *galK* provides a measure of termination. We found that the *rpoAD305E* mutation did not have any significant effect on these terminators (data not shown).

Effect of other rpoA *alleles*

We employed a λ variant carrying the *cro62* mutation to screen other mutations in the carboxy terminus of *rpoA* for this suppressor phenotype. The *cro62* mutation results from a deletion of a single base pair (bp) in the 3′ terminus of the λ *cro* gene (Olson *et al.*, 1982) that causes a shift in the *cro* reading frame, resulting in translation extending four nucleotides downstream of the usual UAA termination codon to a UAA codon in the new reading frame. This extension of translation moves the terminating ribosomes closer to the 5′ junction of NUT-R (three nucleotides away instead of seven nucleotides away) and causes a failure in N-mediated antitermination (Olson *et al.*, 1982). Presumably, the shift downstream of the terminating ribosomes interferes with the assembly of the antitermination complex at NUT-R (Olson *et al.*, 1984). This defect in antitermination results in the failure of λ*cro62* to grow in K37 which is wild type for *nus* alleles; however, λ*cro62* grows in K4069, which is a K37 derivative carrying the *rpoAD305E* mutation. λ*cro62* exhibits a four-log-greater EOP on a lawn formed from K4069 than from a lawn formed from K37 and also shows a similar high EOP on a lawn formed from a K37 derivative carrying a plasmid with the *rpoA*Δ3′ allele.

We exploited our observation that the *rpoAD305E* mutation converts *E. coli* from being non-permissive to being permissive for λ*cro62* growth, to screen other *rpoA* mutations for this suppressor phenotype. A number of *rpoA* mutations constructed in *E. coli* C (Ayers *et al.*, 1994) were tested for their ability to enhance antitermination by examining λ*cro62* growth (Table 4). Two control experiments confirmed that we could use this set of otherwise isogenic mutants for this screen. First, λ*cro62* fails to form plaques on a lawn formed from the parental *E. coli* C strain containing the wild-type *rpoA* and, second, λ*cro62* forms plaques on a derivative of this *E. coli* C that carries the *rpoAD305E* mutation.

Table 4. Effect of *rpoA* alleles on λ*cro62* growth.

Strain[a]	Change in α subunit	Phage tested[b] λ	Phage tested[b] λ*cro62*
C1	None	+	–
C4540	L290H	+	+
C4618	T323S	+	–
C4622	P323L	+	–
C4624	P322S	+	–
C4630	K271E	+	–
C4679	A272T	+	–
C4680	E215K	+	–
DC721	D305E	+	+

a. All strains, with one exception, are derivatives of the *rpoA*$^+$ *E. coli* C strain C1.
b. Phage growth was assessed by a spot test. A lawn of the indicated bacterium was poured onto a Luria–Bertani (LB) plate. A dilution of λ sufficient to give single plaques was spotted onto the plate with the seeded lawn. Plates were incubated at 37°C. +, phage growth; –, no phage growth. λ was λ*c*I857 and λ*cro62* was λ*c*I857*cro62*.

Table 4 lists the seven *rpoA E. coli* C derivatives tested, and specifies the amino acid change in the encoded α subunit of each strain. This test identified only one other strain, C4540, which had an *rpoA* mutation that suppresses the antitermination defect. The *rpoA* mutation in this strain results in a Leu to His change at amino acid position 290.

Discussion

The α subunit of *E. coli* RNA polymerase has been identified as the probable site for interaction of a number of transcription factors (Ishihama, 1992; Russo and Silhavy, 1992; Ebright and Busby, 1995; Giladi *et al.*, 1992). Mutations influencing the action of such transcription factors are usually found in non-overlapping clusters in the 3′ region of the *rpoA* gene. Proteolytic treatment of the 329-amino-acid α subunit revealed that the carboxy terminal 85 amino acids form a domain (CTD) that dimerizes and binds to DNA (Blatter *et al.*, 1994). The CTD includes the amino acids changed by the mutations that interfere with the action of transcription activators. Clusters of mutations are located at the following amino acid positions: (i) 265–270, influencing the action of the cAMP-binding protein at the *lac* P1 promoter (Zou *et al.*, 1992); (ii) 289–290, influencing the Ogr protein of phage P2

(Ayers *et al*., 1994; Sunshine and Sauer, 1975); (iii) 311–317, influencing the action of Fnr (an activator of anaerobically expressed genes) (Lombardo *et al*., 1991); and (iv) 322 or 323, influencing the action of OmpR and EnvZ (which are regulators of porin expression) (Slauch *et al*., 1991).

We have identified an *rpoA* mutation, *rpoAD305E*, that changes amino acid 305 in the CTD of α. This altered α subunit suppresses a variety of mutations, including those in *nus* genes, that reduce the effectiveness of the N-mediated transcription antitermination system of phage λ. A screen of seven other *rpoA* mutations that alter the α CTD and affect transcription-initiation factors (Ayers *et al*., 1994) identified one additional mutation that exhibits suppression of a defect in N-mediated antitermination. This mutation, *rpoA109*, changes amino acid 290 of the α subunit and was originally identified because it interferes with the action of the phage-encoded Ogr transcription-activation protein (Sunshine and Sauer, 1975). Those changes in the CTD of α that had no effect on N-mediated antitermination altered amino acids are at positions 215, 271, 272, 322, and 323. These mutations are all thought to affect transcription initiation, while the *rpoAD305E* mutation influences transcription elongation. Moreover, selections that yielded *rpoA* mutations that affect the actions of other transcription factors have not yielded, to our knowledge, any mutations in codon 305. Thus, amino acid position 305 appears to have specificity, in this case, for an interaction influencing transcription elongation. Preliminary studies suggest that *rpoAD305E*, unlike *rpoA109* (*L290H*), does not affect P2 Ogr function (G. Christie, personal communication). Thus, there appears to be overlap, but not identity, of amino acid patches used by the Ogr initiation activator of P2 and the N antitermination system of λ.

RNA polymerase reconstituted with an α subunit truncated in its CTD also fails to interact with, and is not stimulated by, activator proteins (Ishihama, 1992). This led to the idea that the point mutations cause changes in the α subunit that block interaction with the activators. To determine if something similar is occurring with *rpoAD305E*, we examined the effect of a 3′-truncated *rpoA* gene on N-mediated antitermination and found that the truncated gene product, like the product of the *rpoAD305E* allele, suppresses the defect in antitermination caused by *nus* mutations. These observations raise questions regarding the nature of the interaction influenced by *rpoAD305E*. If the results of only the experiments with *rpoAD305E* were to be considered, a simple explanation for our observations would be that the *rpoAD305E* mutation enhances the interaction of one, or more, of the components of the N–Nus complex with RNA polymerase. Accordingly, this enhanced interaction could compensate for an impaired interaction of another component. The observation that a variant α subunit missing its carboxy terminus also suppresses some Nus$^-$ phenotypes calls into question enhanced interactions of the α CTD as the cause for suppression by the *rpoAD305E* mutation. Because the truncated α subunit is missing amino acids that include amino acid 305, suppression cannot be mediated by facilitation of an interaction with amino acids at and near 305 in the carboxy terminus, but, instead, it probably results from a failure to interact with amino acids in this region. This means that *rpoAD305E* is not *sui generis*, but, like the other *rpoA* mutations, is affecting regulatory activity by blocking an interaction with the α subunit. We suggest that antitermination is restored for one of two reasons: either *rpoAD305E* and *rpoAΔ3′* reduce transcription termination or they enhance antitermination. Our experiments with tester systems designed to measure terminator activity appear to rule out the first explanation, because the *rpoAD305E* mutation has no effect on several λ transcription terminators in the absence of N.

We therefore favour the latter explanation, namely that the *rpoA* mutations stimulate antitermination. This stimulation is unlikely to be caused by overexpression of N or any of the Nus factors. First, overexpression of N, NusA, NusE, or NusG fails to duplicate the suppressive effects observed with the *rpoAD305E* mutation (our unpublished results). Second, the *rpoAD305E* mutation partially suppresses a *nusB* null mutation for λ growth (M. Neeley, D. Court, and D. Friedman, unpublished), showing that NusB is not necessary for the suppression activity of *rpoAD305E*.

Models explaining how mutations in the α subunit enhance impaired N-mediated transcription elongation would necessarily depend on interactions of α that might occur either at initiation or during elongation of transcription. The former would involve DNA and/or proteins at the promoter in a manner similar to interactions previously described for transcription activators, while the latter would involve interactions downstream of the promoter with the RNA, DNA, and/or a protein(s) composing the antitermination complex. Regardless of the site of the interaction, under conditions of impaired N action, the final signal to RNA polymerase must result in a failure in N-mediated antitermination that can be relieved by mutations in *rpoA*. Although we have shown that transcription levels from the λ pL promoter are not affected by the *rpoAD305E* mutation, this does not rule out the possibility that α activity at the pL promoter affects transcription antitermination, but in an indirect manner.

As opposed to action at the promoter with a subsequent effect on elongation, α could affect the elongation process *per se*. For example, an interaction between α and the DNA, similar to that observed at the *rrn* promoter (Ross *et al*., 1993) but in this case during elongation, might render polymerase less susceptible to modification by N

and Nus factors. Alternatively, an interaction between α and the NUT RNA might hinder formation of the antitermination complex directly, either by interfering with complex formation or by facilitating action of an inhibitor of N-mediated antitermination-complex formation. Liu and Hannah (1995) have shown that, *in vitro*, the α CTD interacts with the nascent RNA during transcription and this interaction is not observed in the presence of NusA. We have proposed an inhibitor to explain the results of our genetic and physiological studies (Patterson *et al*., 1994), and an inhibitor was also suggested to explain NusB-independence for processive N-mediated antitermination *in vitro* with purified protein components (DeVito and Das, 1994). The inhibitor could conceivably prevent antitermination, in part, by interacting with the α subunit of RNA polymerase. According to this model, the suppression observed with *rpoAD305E* and *rpoAΔ3′* mutations (and presumably *rpoAL290H*) results from a reduction in the interaction between α and the inhibitor. This loss of inhibitor action, in turn, would permit a more unencumbered assembly of the N antitermination complex. Under conditions where all components are functioning effectively, the action of the inhibitor would be effectively blocked. However, under conditions less optimal for formation of antitermination complexes (e.g., in the presence of *nus* mutations), the presence of the inhibitor would interfere with complex formation, leading to a failure in antitermination. Thus, loss of the inhibitor activity would allow for effective complex formation under the suboptimal conditions and result in effective antitermination.

We are unable to propose a definitive model to explain how the *rpoAD305E* and *rpoAΔ3′* mutations suppress the inhibitory effect on λ growth caused by the *cro62* frameshift mutation, i.e., why λ*cro62* fails to grow in a *nus*$^+$ host, K37, but grows in a derivative of K37 that carries copies of either the *rpoAD305E* or *rpoAΔ3′* alleles. However, we offer the following hypothesis to explain this observation. The *cro62* frameshift results in an incursion of translating ribosomes into the NUT region (Olson *et al*., 1984) that appears to compete with the efficient entry of N and/or Nus factors to the NUT site. We suspect that the enhancement of N action mediated by the α mutations tilts this competition in favour of N and thus allows growth of λ*cro62*.

In vitro studies by Liu *et al*. (1996) found that RNA polymerase containing α subunits missing the carboxy terminal domain can be modified by N and Nus factors into effective antitermination complexes. This indicates that the carboxy terminus of α is not required to assemble the antitermination complex and is consistent with the arguments we have advanced regarding the requirement for the carboxy terminal domain of the α subunit and the putative inhibitory factor.

A role for an inhibitor of transcription elongation has been described in humans. A 213-amino-acid protein encoded by the *VHL* tumour-suppressor gene has been proposed to modulate transcription by inhibiting action of the SIII transcription-elongation factor (Latif *et al*., 1993; Duan *et al*., 1995). Interestingly, it has been pointed out that portions of the p15 subunit of SIII share sequence similarity with the *E. coli* NusB and Rho transcription-elongation proteins (Garrett *et al*., 1994).

In summary, we have presented evidence showing that an interaction that promotes transcription elongation appears to be regulated through interactions with the α subunit of RNA polymerase. As many other studies have identified a role for the α subunit in initiation of transcription, our studies identify an expanded role for this subunit in the transcription-elongation process.

Experimental procedures

Bacteria

Relevant genotypes and sources of bacteria used in these studies are listed in Table 5.

Media

The media used in these experiments have been described previously (Miller and Friedman, 1980).

Plasmids

pNO2530, pNO2341, pNO2685, and pNO2695 were supplied by M. Nomura (Cerretti *et al*., 1983). pNO2530* was obtained by recombining the *rpoAD305E* mutation from the chromosome with pNO2530. pKKrpoA (Fig. 3) was constructed by isolating a 1318 bp *Hin*dIII–*Nru*I fragment, which contained all of *rpoA* and a 5′ portion of the adjacent *rplQ* gene from pNO2530, and ligating it into the *Hin*dIII site of pKK223-3 (Brosius and Holy, 1984). The resulting plasmid was deleted of 1503 nucleotides of pKK223-3 located between the *Sph*I and *Pvu*II sites. The identical procedure was followed to construct pKKrpoA*, except that pNO2530* was used as the donor of the *Hin*dIII–*Nru*I fragment. pKKrpoAΔ3′ was constructed by cloning the 711 bp *Hin*dIII fragment from pNO2530, containing a truncated *rpoA* gene deleted of its 3′-terminal 300 nucleotides, into the *Hin*dIII site of pKK223-3 (also deleted for the 1503 nucleotides between the *Sph*I and *Pvu*II sites). pKL600, supplied by K. McKenney, is a terminator-tester plasmid that has a polycloning site between p*lac* and a *galK* reporter gene (McKenney *et al*., 1981). pKLtR2 and pKLtR3 are derivatives of pKL600 that have the tR2 (Rho-independent) or tR3 (Rho-dependent) terminators, respectively, of λ cloned in the proper orientation in the polycloning site (Cheng *et al*., 1991; 1995).

Phage and source

λ*c*I60 and λ*c*I857 were obtained from the NIH collection; λ*c*I857*cro62* is from these laboratories.

Table 5. Bacterial strains used in this study.

Strain	Parent strain	Relevant genotype	Source
AB1133		*thr1 leu6 thi1 supE44 lacY1 galK2 ara14 xyl5 mtl1 proA2 his4 argE3 str31 sipA1*	L. S. Baron
K37		*galK*, Str^R	This laboratory
K450	K37	*nusB5*	This laboratory
K556	K37	*nusE71*	This laboratory
K1947	N5261	*gal490* (chl–blu)$_{del8}$ Bam$_{del}$ N$^+$ cI14 H1$_{del}$*	M. Gottesman
K1953	K1947	*gal490* (chl–blu)$_{del8}$ Bam$_{del}$ N$^+$ cI14 H1$_{del}$ nusE71 zhb-3082*::Tn*10*	This work
K2090	K1953	*gal490* (chl–blu)$_{del8}$ Bam$_{del}$ N$^+$ cI14 H1$_{del}$ nusE71 rpoAD305E zhb-3082*::Tn*10*	This work
K4069	K37	*rpoAD305E*	This work
K6325	K2090	*gal490* (chl–blu)$_{del8}$ Bam$_{del}$ N$^+$ cI14 H1$_{del}$ nusE71 rpoAD305E zhb-3082*::Tn*10kan*	This work
K6953	AB1133	*nusA1 rpoAD305E*	This work
K6995	K6953	K6953 + pNO2530	This work
K7004	K6953	K6953 + pNO2530*	This work
K7005	K1953	K1953 + pNO2530*	This work
K7006	K1953	K1953 + pNO2530	This work
K4047	K4069	*nusA1 rpoAD305E*	This work
K4090	K4069	*nusB5 rpoAD305E*	This work
K7257	K37	K37 + pKKrpoA	This work
K7254	K37	K37 + pKKrpoA*	This work
K7258	K37	K37 + pKKrpoAΔ3′	This work
K4195	AB1133	*nusA1 rpoAD305E nusE71 zhb-3082*::Tn*10*	This work
K8248	K37	*nusE71 rpoAD305E zhb-3082*::Tn*10*	This work
NC221		*lacZu169$_{del}$ gal490*(chl–blu)$_{del8}$ Bam$_{del}$ N–lacZ cI857 (cro–bioA)$_{del}$ zhb-3082*::Tn*10*	This work
NC222		*lacZu169$_{del}$ gal490*(chl–blu)$_{del8}$ Bam$_{del}$ N–lacZ cI857 (cro–bioA)$_{del}$ zhb3082*::Tn*10 rpoAD305E*	This work

Str^R, streptomycin resistant.

Phage techniques

Efficiency of phage plating (EOP) was determined as described previously (Friedman *et al.*, 1984b). Dilution spot tests used to obtain a semi-quantitative evaluation of phage growth were performed by spotting drops of serial dilutions of phage on lawns formed from the indicated bacterium and scoring growth according to the dilutions that showed phage growth.

Nucleic acid and protein procedures

DNA preparation, restriction enzyme digestions, ligations, and transformations were performed essentially as described by Sambrook *et al.* (1989). Protein purification and two-dimensional electrophoresis of ribosomal proteins have previously been described (Friedman *et al.*, 1981). Western analysis was performed as previously described (Craven *et al.*, 1994).

DNA sequencing

Sequenase version 2.0 dideoxy sequencing system (United States Biochemical) was employed using an [α-^{35}S]-dATP (Amersham) label. Derivatives of M13mp18 or M13mp19 (Yanisch-Perron *et al.*, 1985) carrying the desired cloned fragments served as single-stranded DNA templates for sequencing. The sequencing reactions were run on a six or eight percent polyacrylamide gel for varying lengths of time. Both strands of the DNA were sequenced.

Galactokinase assay

Enzyme levels were measured according to the published method (Adhya and Miller, 1979).

Beta-galactosidase assay

Enzyme levels were measured according to Miller (1992).

Genetic techniques

P1 transduction and Hfr crosses were performed in the standard manner (Miller, 1972; Sternberg and Maurer, 1991).

Selection for second-site suppressors of nusE71

This scheme, devised by Ward *et al.* (1983), uses derivatives of an *E. coli* K-12 strain, K1947, that has a fusion of the bacterial *gal* operon to the pL promoter of a defective λ prophage. The abbreviated prophage also has the associated *nutL* signal, *N* gene, and a defective *c*I allele (Fig. 2). Additionally, there are at least two transcription-termination signals located between pL–*nut* and the *gal* operon. One of the terminators is located in an IS*2* positioned in the leader region of the *gal* operon. This Rho-dependent terminator is very efficient and, therefore, in this construct, transcription initiating at p*gal* should terminate upstream of the *gal* genes. However, the prophage in K1947 does not express an active repressor and constitutively expresses N from the pL promoter. The expressed N, in turn, modifies the RNA polymerase transcribing the pL operon, allowing transcription to transcend all downstream terminators and read into the *gal* operon, producing a Gal^+ phenotype.

In our adaptation of the selection, a derivative of K1947, K1953, carries the *nusE71* mutation, causing N to be ineffective. Hence, the bacterium is Gal^- even though N expression is constitutive. Suppressors of the Nus^- phenotype should result in the bacteria being Gal^+ because N action would be supported. Gal^+ colonies were obtained (at a frequency of ~10^{-8}) and ~80% of these supported λ growth and, therefore, supported N action more effectively than did the starting parental recipient. We attempted to limit the mutant search to the 72 min region of the *E. coli* chromosome which has a large number of genes encoding ribosomal proteins (Nomura *et al.*, 1977). Localized mutagenesis (Hong and Ames,

1971) was employed using hydroxylamine treatment of a P1 lysate grown on a *nusE71* host, K2016. This bacterium has a Tn*10* insertion (Kleckner *et al.*, 1991) (*zhb-3082*::Tn*10*, conferring resistance to tetracycline (Tet^R)) linked to the 72 min region (Singer *et al.*, 1989). Because P1 transducing particles can package 100 kb (2%) of the *E. coli* chromosome (Sternberg and Maurer, 1991), it was expected that by using this lysate to transduce for Tet^R, we would enrich for mutations in the 72 min region. Second-site suppressors were distinguished from true revertants using two-dimensional gel electrophoresis to determine if the S10 protein maintained the altered mobility caused by the *nusE71* mutation (Friedman *et al.*, 1981). One mutant, K2090, identified as having a second-site suppressor, was chosen for further characterization. The second-site mutation, *rpoAD305E*, was located to the 72 min region of the *E. coli* genetic map by cotransduction with *zhb-3082*::Tn*10*. As the predominant change caused by hydroxylamine mutagenesis is C to T (Budowsky, 1976), we assume that the *rpoAD305E* mutation did not result from the hydroxylamine treatment but, instead, from a spontaneous mutation. The selection of an apparent spontaneous mutation following *in vitro* hydroxylamine mutagenesis of phage is not without precedent (Min Jou *et al.*, 1972).

Marker rescue of rpoAD305E

K6325 (a derivative of K2090 with a substitution of a kan^r marker for the tet^r marker of *zhb-3082*::Tn*10*) carrying pNO2530 was employed to transfer the *rpoAD305E* mutation to the plasmid by recombination between the chromosome and the cloned genes of the plasmid. Colonies containing such candidate recombinant plasmids were identified on MacConkey–galactose plates that contained ampicillin (30 $\mu g\ ml^{-1}$) to maintain selective pressure for the plasmids. The starting strain has the $rpoA^+$ allele on the plasmid and the *rpoAD305E* mutation on the chromosome. Because of partial dominance of *rpoAD305E*, K6325 with pNO2530 forms pink colonies on these indicator plates, unlike K6325, which forms dark-red colonies. However, dark-red colonies were occasionally observed (~1/100) when K6325 harbouring pNO2530 was plated on MacConkey–galactose–ampicillin plates. When these colonies were restreaked on the indicator plates containing ampicillin, dark-red colonies could be isolated. These bacteria obviously expressed higher levels of *gal* enzymes than those forming the pink colonies. We assumed that the *rpoAD305E* allele had recombined from the chromosome to the plasmid and the bacteria had become homogenotized for the mutant allele.

Plasmid DNA was isolated from a dark-red colony and this plasmid, named pNO2530*, was shown to have an insert that was seemingly identical to pNO2530. However, unlike pNO2530, pNO2530* suppressed the Nus^- phenotype; when pNO2530* was transformed into the *nusE71* parent strain, K1953, dark-red colonies were formed on MacConkey–galactose plates. The suppressor activity of pNO2530* was also assessed by phage plating. At 40°C, λ will not form plaques on a lawn of K1953, because N-mediated antitermination is inoperative. λ also fails to form plaques on a lawn of a derivative of K1953 carrying pNO2530, but does form plaques on a lawn of a derivative of K1953 carrying pNO2530* (data not shown). These results lead us to conclude that the *rpoAD305E* mutation had recombined from the chromosome to the plasmid, and that pNO2530* carries the *rpoAD305E* mutation.

Crossing rpoAD305E *away from* nusE71

A triple *nusA1 nusE71 rpoAD305E* mutant is thermosensitive, growing only at temperatures below 42°C. Previous studies had shown that a *nusA1 nusE71* double mutant grows at 42°C but fails to support growth of N-dependent λ phage (Friedman *et al.*, 1983). Thus, if the *rpoAD305E* mutation suppresses the Nus^- phenotype imposed by the *nusA1* mutation, we suspected that it would be possible to select and screen for a *nusA1 rpoAD305E* double mutant following P1 transduction from the *nusE71 rpoAD305E zhb-3082*::Tn*10* donor to a *nusA1* recipient. This could be accomplished by selecting for Tet^R colonies at 42°C and screening the survivors for their Nus phenotype. Because *nusE71, rpoAD305E*, and *zhb-3082*::Tn*10* are linked, one expected class of recombinants, triple *nusA1 nusE71 rpoAD305E* mutants, should be selected against by growing at the high temperature. Surviving bacteria that do not have *rpoAD305E,* but carry *nusA1* only, or *nusA1* and *nusE71*, should not support λ growth at 42°C. We expected that the *nusA1 rpoAD305E* double mutant might grow and support λ growth at 42°C. Recombinant progeny of such a cross were obtained at 42°C and screened for support of λ growth at 42°C; 4% of the Tet^R bacteria had this phenotype. One such recombinant, K4047, was chosen for further study. Genetic outcrosses confirmed that this recombinant contains the *nusA1* and *rpoAD305E* mutations (data not shown).

A nus^+ recombinant, K4069, that has the *rpoAD305E* mutation free of any *nus* mutations was constructed by P1 transduction using K4047, the *nusA1 rpoAD305E* strain, as the donor. The recipient was the K37 nus^+ strain and the selective marker was the *zhb-3082*::Tn*10* located near *rpoAD305E*. We could not ascribe any phenotype to the *rpoAD305E* mutation *per se*; therefore, potential *rpoAD305E* and *zhb-3082*::Tn*10* recombinants were backcrossed to the *nusA1* mutant, K95. Because the Tn*10* is near *rpoA*, we assumed that if a donor harboured the *rpoAD305E* mutation then some of the Tet^R recombinants generated in the backcross would also acquire the *rpoAD305E* mutation. When one Tet^R K37 derivative, K4069, was the donor in the backcross to K95, 14/30 Tet^R transductants scored as having suppressors of the Nus^- phenotype, demonstrating that the donor K4069 strain contains the *rpoAD305E* allele.

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References

Adhya, S., and Miller, W. (1979) Modulation of the two promoters of the galactose operon of *Escherichia coli. Nature* **279:** 492–494.

Ayers, D.J., Sunshine, M.G., Six, E.W., and Christie, G.E. (1994) Mutations affecting two adjacent amino acid residues in the alpha subunit of RNA polymerase block transcriptional activation by the P2 Ogr protein. *J Bacteriol* **176:** 7430–7438.

Barik, S., Ghosh, B., Whalen, W., Lazinski, D., and Das, A. (1987) An antitermination protein engages the elongating transcription apparatus at a promoter-proximal recognition site. *Cell* **50:** 885–899.

Bedwell, D.M., and Nomura, M. (1986) Feedback regulation of RNA polymerase subunit synthesis after the conditional overproduction of RNA polymerase in *Escherichia coli. Mol Gen Genet* **204:** 17–23.

Blatter, E.E., Ross, W., Tang, H., Gourse, R.L., and Ebright, R.H. (1994) Domain organization of RNA polymerase a subunit: C-terminal 85 amino acids constitute a domain capable of dimerization and DNA binding. *Cell* **78:** 889–896.

de Boer, H.A. (1984) A versatile plasmid system for the study of prokaryotic transcription signals in *Escherichia coli. Gene* **30:** 251–255.

Brosius, J., and Holy, A. (1984) Regulation of ribosomal RNA promoters with a synthetic *lac* operator. *Proc Natl Acad Sci USA* **81:** 6929–6933.

Budowsky, E.I. (1976) The mechanism of the mutagenic action of hydroxylamines. *Prog Nucl Acids Res Mol Biol* **16:** 125–188.

Cerretti, D.P., Dean, D., Davis, G.R., Bedwell, D.M., and Nomura, M. (1983) The *spc* ribosomal protein operon of *Escherichia coli*: sequence and cotranscription of the ribosomal protein genes and a protein export gene. *Nucl Acids Res* **11:** 2599–2616.

Chattopadhyay, S., Garcia-Mena, J., DeVito, J., Wolska, K., and Das, A. (1995) Bipartite function of a small RNA hairpin in transcription antitermination in bacteriophage lambda. *Proc Natl Acad Sci USA* **92:** 4061–4065.

Cheng, S.C., Lynch, E.C., Leason, K.R., Court, D.L., Shapiro, B.A., and Friedman, D.I. (1991) Functional importance of sequence in the stem-loop of a transcription terminator. *Science* **254:** 1205–1207.

Cheng, S.C., Court, D.L., and Friedman, D.I. (1995) Transcription termination signals in the nin region of bacteriophage lambda: Identification of Rho-dependent termination regions. *Genetics* **140:** 875–887.

Court, D.L., Patterson, T.A., Baker, N., Costantino, N., Mao, C., and Friedman, D.I. (1995) Structural and functional analysis of the transcription-translation proteins NusB and NusE. *J Bacteriol* **177:** 2589–2591.

Craven, M.G., Granston, A.E., Schauer, A.T., Zheng, C., Gray, T.A., and Friedman, D.I. (1994) *Escherichia coli–Salmonella typhimurium* hybrid *nusA* genes: Identification of a short motif required for action of the λ N transcription antitermination protein. *J Bacteriol* **176:** 1394–1404.

Das, A. (1992) How the phage lambda N gene product suppresses transcription termination: communication of RNA polymerase with regulatory proteins mediated by signals in nascent RNA. *J Bacteriol* **174:** 6711–6716.

Das, A. (1993) Control of transcription termination by RNA-binding proteins. *Annu Rev Biochem* **63:** 893–930.

Das, A., and Wolska, K. (1984) Transcription antitermination *in vitro* by lambda N gene product: requirement for a phage *nut* site and the products of host *nusA*, *nusB*, and *nusE* genes. *Cell* **38:** 165–173.

DeVito, J., and Das, A. (1994) Control of transcription processivity in phage lambda: Nus factors strengthen the termination-resistant state of RNA polymerase induced by N antiterminator. *Proc Natl Acad Sci USA* **91:** 8660–8664.

Doelling, J.H., and Franklin, N.C. (1989) Effects of all single base substitutions in the loop of *boxB* on antitermination of transcription by bacteriophage lambda's N protein. *Nucl Acids Res* **17:** 5565–5577.

Downing, W.L., Sullivan, S.L., Gottesman, M.E., and Dennis, P.P. (1990) Sequence and transcriptional pattern of the essential *Escherichia coli secE–nusG* operon. *J Bacteriol* **172:** 1621–1627.

Duan, D.R., Pause, A., Burgess, W.H., Aso, T., Chen, D.Y., Garrett, K.P., Conaway, R.C., Conaway, J.W., Linehan, W.M., and Klausner, R.D. (1995) Inhibition of transcription elongation by the VHL tumor suppressor protein. *Science* **269:** 1402–1406.

Ebright, R.H., and Busby, S. (1995) The *Escherichia coli* RNA polymerase a subunit: structure and function. *Curr Op Genet Devel* **5:** 197–203.

Franklin, N.C. (1985) 'N' transcription antitermination proteins of bacteriophages lambda, phi 21 and P22. *J Mol Biol* **181:** 85–91.

Friedman, D.I. (1988) Regulation of phage gene expression by termination and antitermination of transcription. In *The Bacteriophages 2.* Calendar, R. (ed.). New York: Plenum Press, pp. 263–319.

Friedman, D.I. (1992) Interaction between bacteriophage lambda and its *Escherichia coli* host. *Curr Op Genet Dev* **2:** 727–738.

Friedman, D.I., and Court, D.L. (1995) Transcription antitermination: The lambda paradigm updated. *Mol Microbiol* **18:** 191–200.

Friedman, D.I., and Olson, E.R. (1983) Evidence that a nucleotide sequence, 'boxA', is involved in the action of the NusA protein. *Cell* **34:** 143–149.

Friedman, D.I., Schauer, A.T., Baumann, M.R., Baron, L.S., and Adhya, S.L. (1981) Evidence that ribosomal protein S10 participates in control of transcription termination. *Proc Natl Acad Sci USA* **78:** 1115–1118.

Friedman, D.I., Schauer, A.T., Mashni, E.J., Olson, E.R., and Baumann, M.F. (1983) *Escherichia coli* factors involved in the action of the λ gene N antitermination function. In

Microbiology. Schlessinger, D. (ed.). Washington, DC: American Society for Microbiology, pp. 39–42.

Friedman, D.I., Olson, E.R., Georgopoulos, C., Tilly, K., Herskowitz, I., and Banuett, F. (1984a) Interactions of bacteriophage and host macromolecules in the growth of bacteriophage lambda. *Microbiol Rev* **48:** 299–325.

Friedman, D.I., Plantefaber, L.C., Olson, E.J., Carver, D., O'Dea, M.H., and Gellert, M. (1984b) Mutations in the DNA *gyrB* gene that are temperature sensitive for lambda site-specific recombination, Mu growth, and plasmid maintenance. *J Bacteriol* **157:** 490–497.

Friedman, D.I., Olson, E.R., Johnson, L.L., Alessi, D., and Craven, M.G. (1990) Transcription-dependent competition for a host factor: the function and optimal sequence of the phage lambda boxA transcription antitermination signal. *Genes Dev* **4:** 2210–2222.

Garrett, K.P., Tan, S., Bradsher, J.N., Lane, W.S., Conaway, J.W., and Conaway, R.C. (1994) Molecular cloning of an essential subunit of RNA polymerase II elongation factor SIII. *Proc Natl Acad Sci USA* **91:** 5237–5241.

Giladi, H., Igarashi, K., Ishihama, A., and Oppenheim, A.B. (1992) Stimulation of the phage lambda pL promoter by integration host factor requires the carboxy terminus of the alpha-subunit of RNA polymerase. *J Mol Biol* **227:** 985–990.

Greenblatt, J., Nodwell, J.R., and Mason, S.W. (1993) Transcriptional antitermination. *Nature* **364:** 401–406.

Hasan, N., and Szybalski, W. (1986) Boundaries of the *nutL* antiterminator of coliphage lambda and effects of mutations in the spacer region between *boxA* and *boxB. Gene* **50:** 87–96.

Hayward, R.S., Igarashi, K., and Ishihama, A. (1991) Functional specialization within the alpha-subunit of *Escherichia coli* RNA polymerase. *J Mol Biol* **221:** 23–29.

Hong, J.S., and Ames, B.N. (1971) Localized mutagenesis of any specific small region of the bacterial chromosome. *Proc Natl Acad Sci USA* **68:** 3158–3162.

Horwitz, R.J., Li, J., and Greenblatt, J. (1987) An elongation control particle containing the N gene transcriptional antitermination protein of bacteriophage lambda. *Cell* **51:** 631–641.

Ishihama, A. (1992) Role of the RNA polymerase alpha subunit in transcription activation. *Mol Microbiol* **6:** 3283–3288.

Jin, D.J., Cashel, M., Friedman, D.I., Nakamura, Y., Walter, W.A., and Gross, C.A. (1988) Effects of rifampicin resistant *rpoB* mutations on antitermination and interaction with *nusA* in *Escherichia coli. J Mol Biol* **204:** 247–261.

Kleckner, N., Bender, J., and Gottesman, S. (1991) Uses of transposons with emphasis on Tn*10. Meth Enzymol* **204:** 139–180.

Kur, J., Hasan, N., and Szybalski, W. (1989) Repression of transcription from the b2-att region of coliphage lambda by integration host factor. *Virology* **168:** 236–244.

Kuroki, K., Ishii, S., Kano, Y., Miyashita, T., Nishi, K., and Imamoto, F. (1982) Involvement of the *nusB* gene products in transcription of *Escherichia coli* tryptophan operon *in vitro. Mol Gen Genet* **185:** 369–371.

Latif, F., Tory, K., Gnarra, J., Yao, M., Duh, F.M., Orcutt, M.L., Stackhouse, T., Kuzmin, I., Modi, W., and Geil, L. (1993) Identification of the von Hippel–Lindau disease tumor suppressor gene. *Science* **260:** 1317–1320.

Lazinski, D., Grzadzielska, E., and Das, A. (1989) Sequence-specific recognition of RNA hairpins by bacteriophage antiterminators requires a conserved arginine-rich motif. *Cell* **59:** 207–218.

Li, J., Horwitz, R., McCracken, S., and Greenblatt, J. (1992) NusG, a new *Escherichia coli* elongation factor involved in transcriptional antitermination by the N protein of phage lambda. *J Biol Chem* **267:** 6012–6019.

Liu, K., and Hanna, M.M. (1995) NusA interferes with interactions between the nascent RNA and the C-terminal domain of the alpha subunit of RNA polymerase in *Escherichia coli* transcription complexes. *Proc Natl Acad Sci USA* **92:** 5012–5016.

Liu, K., Zhang, Y., Severinov, K., Das, A., and Hannah, M.M. (1996) Role of *Escherichia coli* RNA polymerase alpha subunit in modulation of pausing, termination and anti-termination by the transcription elongation factor NusA. *EMBO J* **15:** 150–161.

Lombardo, M.J., Bagga, D., and Miller, C.G. (1991) Mutations in *rpoA* affect expression of anaerobically regulated genes in *Salmonella typhimurium. J Bacteriol* **173:** 7511–7518.

McKenney, K., Shimatake, H., Court, D., Schmeissner, U., Brady, C., and Rosenberg, M. (1981) A system to study promoter and terminator signals recognized by *Escherichia coli* RNA polymerase. *Gene Amplif Anal* **2:** 383–415.

Meek, D.W., and Hayward, R.S. (1984) Nucleotide sequence of the *rpoA–rplQ* DNA of *Escherichia coli*: a second regulatory binding site for protein S5? *Nucl Acids Res* **12:** 13–21.

Miller, J.H. (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Miller, J.H. (1992) *A Short Course in Bacterial Genetics. A Laboratory Manual for* Escherichia coli *and Related Bacteria*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Miller, H.I., and Friedman, D.I. (1980) An *E. coli* gene product required for lambda site-specific recombination. *Cell* **20:** 711–719.

Min Jou, W., Haegeman, G., Ysebaert, M., and Fiers, W. (1972) Nucleotide sequence of the gene coding for the bacteriophage MS2 coat protein. *Nature* **237:** 82–88.

Miyashita, T., Kano, Y., Kuroki, K., Ishii, S., and Imamoto, F. (1982) *In vivo* evidence for *nusA* and *nusB* gene function in general transcription of the *Escherichia coli* genome. *Biken J* **25:** 121–130.

Mogridge, J., Mah, T.-F., and Greenblatt, J. (1995) A protein–RNA interaction network facilitates the template-independent cooperative assembly on RNA polymerase of a stable antitermination complex containing the l N protein. *Genes Dev* **9:** 2831–2844.

Nodwell, J.R., and Greenblatt, J. (1991) The nut site of bacteriophage lambda is made of RNA and is bound by transcription antitermination factors on the surface of RNA polymerase. *Genes Dev* **5:** 2141–2151.

Nodwell, J.R., and Greenblatt, J. (1993) Recognition of *boxA* antiterminator RNA by the *E. coli* antitermination factors NusB and ribosomal protein S10. *Cell* **72:** 261–268.

Nomura, M., Morgan, E.A., and Jaskunas, S.R. (1977) Genetics of bacterial ribosomes. *Annu Rev Genet* **11:** 297–347.

Olson, E.R., Flamm, E.L., and Friedman, D.I. (1982) Analysis of *nutR*: a region of phage lambda required for antitermination of transcription. *Cell* **31:** 61–70.

Olson, E.R., Tomich, C.S., and Friedman, D.I. (1984) The *nusA* recognition site. Alteration in its sequence or position relative to upstream translation interferes with the action of the N antitermination function of phage lambda. *J Mol Biol* **180:** 1053–1063.

Patterson, T.A., Zhang, Z., Baker, T., Johnson, L.L., Friedman, D.I., and Court, D.L. (1994) Bacteriophage lambda N-dependent transcription antitermination. Competition for an RNA site may regulate antitermination. *J Mol Biol* **236:** 217–228.

Roberts, J. (1992) Antitermination and the control of transcription elongation. In *Transcriptional Regulation.* S.L. McKnight, and K.R. Yamamoto (eds). Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, pp. 389–406.

Roberts, J.W. (1969) Termination factor for RNA synthesis. *Nature* **224:** 1168–1174.

Roberts, J.W. (1993) RNA and protein elements of *E. coli* and lambda transcription antitermination complexes. *Cell* **72:** 653–655.

Rosenberg, M., Court, D., Shimatake, H., Brady, C., and Wulff, D.L. (1978) The relationship between function and DNA sequence in an intercistronic regulatory region in phage lambda. *Nature* **272:** 414–423.

Ross, W., Gosink, K.K., Salomon, J., Igarashi, K., Zou, C., Ishihama, A., Severinov, K., and Gourse, R.L. (1993) A third recognition element in bacterial promoters: DNA binding by the alpha subunit of RNA polymerase. *Science* **262:** 1407–1413.

Russo, F.D., and Silhavy, T.J. (1992) Alpha: the Cinderella subunit of RNA polymerase. *J Biol Chem* **267:** 14515–14518.

Salstrom, J.S., and Szybalski, W. (1978a) Coliphage lambda *nutL*: a unique class of mutants defective in the site of gene N product utilization for antitermination of leftward transcription. *J Mol Biol* **124:** 195–221.

Salstrom, J.S., and Szybalski, W. (1978b) Transcription termination sites in the major leftward operon of coliphage lambda. *Virology* **88:** 252–262.

Sambrook, J., Fritsch, E., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Schauer, A.T. (1985) Proteins involved in phage lambda transcription antitermination. PhD thesis. University of Michigan, Ann Arbor, Michigan, USA.

Schauer, A.T., and Friedman, D.I. (1985) Gene expression in coliphage λ: Proteins and sites involved in transcription antitermination. In *Sequence Specificity in Transcription and Translation.* Calendar, R., and Gold, L. (eds). New York: Alan R. Liss Inc., pp. 171–184.

Schauer, A.T., Carver, D.L., Bigelow, B., Baron, L.S., and Friedman, D.I. (1987) Lambda N antitermination system: functional analysis of phage interactions with the host NusA protein. *J Mol Biol* **194:** 679–690.

Singer, M., Baker, T.A., Schnitzler, G., Deischel, S.M., Goel, M., Dove, W., Jaacks, K.J., Grossman, A.D., Erickson, J.W., and Gross, C.A. (1989) A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli. Microbiol Rev* **53:** 1–24.

Slauch, J.M., Russo, F.D., and Silhavy, T.J. (1991) Suppressor mutations in *rpoA* suggest that OmpR controls transcription by direct interaction with the alpha subunit of RNA polymerase. *J Bacteriol* **173:** 7501–7510.

Somasekhar, G., Drahos, D., Salstrom, J.S., and Szybalski, W. (1982) Sequence changes in coliphage lambda mutants affecting the *nutL* antitermination site and termination by tL1 and tL2. *Gene* **20:** 477–480.

Sparkowski, J., and Das, A. (1992) Simultaneous gain and loss of functions caused by a single amino acid substitution in the beta subunit of *Escherichia coli* RNA polymerase: suppression of *nusA* and *rho* mutations and conditional lethality. *Genetics* **130:** 411–428.

Sternberg, N.L., and Maurer, R. (1991) Bacteriophage-mediated generalized transduction in *Escherichia coli* and *Salmonella typhimurium. Meth Enzymol* **204:** 18–43.

Sullivan, S.L., and Gottesman, M.E. (1992) Requirement for *E. coli* NusG protein in factor-dependent transcription termination. *Cell* **68:** 989–994.

Sullivan, S.L., Ward, D.F., and Gottesman, M.E. (1992) Effect of *Escherichia coli nusG* function on lambda N-mediated transcription antitermination. *J Bacteriol* **174:** 1339–1344.

Sunshine, M.G., and Sauer, B. (1975) A bacterial mutation blocking P2 phage late gene expression. *Proc Natl Acad Sci USA* **72:** 2770–2774.

Tsugawa, A., Saito, M., Court, D.L., and Nakamura, Y. (1988) *nusA* amber mutation that causes temperature-sensitive growth of *Escherichia coli. J Bacteriol* **170:** 908–915.

Ward, D.F., and Gottesman, M.E. (1981) The *nus* mutations affect transcription termination in *Escherichia coli. Nature* **292:** 212–215.

Ward, D.F., DeLong, A., and Gottesman, M.E. (1983) *Escherichia coli nusB* mutations that suppress *nusA1* exhibit lambda N specificity. *J Mol Biol* **168:** 73–85.

Warren, F., and Das, A. (1984) Formation of termination-resistant transcription complex at phage lambda *nut* locus: effects of altered translation and a ribosomal mutation. *Proc Natl Acad Sci USA* **81:** 3612–3616.

Yager, T.D., and von Hippel, P.H. (1987) Transcript elongation and termination in *Escherichia coli.* In Escherichia coli *and* Salmonella typhimurium: *Cellular and Molecular Biology.* F.C. Neidhardt *et al.* (eds). Washington, DC: ASM, pp. 1241–1275.

Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33:** 103–119.

Zheng, C., and Friedman, D.I. (1994) Reduced Rho-dependent transcription termination permits NusA-independent growth of *Escherichia coli. Proc Natl Acad Sci USA* **91:** 7543–7547.

Zou, C., Fujita, N., Igarashi, K., and Ishihama, A. (1992) Mapping the cAMP receptor protein contact site on the alpha subunit of *Escherichia coli* RNA polymerase. *Mol Microbiol* **6:** 2599–2605.

Zuber, M., Patterson, T.A., and Court, D.L. (1987) Analysis of *nutR*, a site required for transcription antitermination in phage lambda. *Proc Natl Acad Sci USA* **84:** 4514–4518.